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Contents

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Contents

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OPINION REVIEW

Challenges involved in the application of artificial intelligence in gastroenterology: The race is on!

Chrysanthos D Christou, Georgios Tsoulfas

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Abstract

Gastroenterology is a particularly data-rich field, generating vast repositories of data that are a fruitful ground for artificial intelligence (AI) and machine learning (ML) applications. In this opinion review, we initially elaborate on the current status of the application of AI/ML-based software in gastroenterology. Currently, AI/ML-based models have been developed in the following applications: Models integrated into the clinical setting following real-time patient data flagging patients at high risk for developing a gastrointestinal disease, models employing non-invasive parameters that provide accurate diagnoses aiming to either replace, minimize, or refine the indications of endoscopy, models utilizing genomic data to diagnose various gastrointestinal diseases, computer-aided diagnosis systems facilitating the interpretation of endoscopy images, models to facilitate treatment allocation and predict the response to treatment, and finally, models in prognosis predicting complications, recurrence following treatment, and overall survival. Then, we elaborate on several challenges and how they may negatively impact the widespread application of AI in healthcare and gastroenterology. Specifically, we elaborate on concerns regarding accuracy, cost-effectiveness, cybersecurity, interpretability, oversight, and liability. While AI is unlikely to replace physicians, it will transform the skillset demanded by future physicians to practice. Thus, physicians are expected to engage with AI to avoid becoming obsolete.

Key Words: Artificial intelligence; Machine learning; Gastroenterology; Cost-effectiveness; Interpretability; Accuracy

Core Tip: Currently, artificial intelligence (AI) and machine learning (ML) have several applications in the prevention, diagnosis, treatment, and prognosis of various gastrointestinal diseases, including gastroesophageal reflux disease, esophageal cancer, gastric cancer, gastrointestinal bleeding, inflammatory bowel diseases, polyps, colorectal cancer, and others. Despite their promising results, AI/ML applications in gastroenterology are hindered by several challenges, including accuracy, cost-effectiveness, cybersecurity, interpretability, oversight, and liability concerns. In this opinion review, we elaborate on these challenges and present different ways to overcome them.

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INTRODUCTION

Gastroenterology is a particularly data-rich field, with data being omnipresent. Several gastroenterology norms, including patient electronic records, imaging, endoscopy images and videos, capsule endoscopy videos, genomic analyses, microbiome data, and histopathology images, generate vast data repositories[1-3]. Conforming with big data features, these repositories provide a fruitful ground for artificial intelligence (AI) applications and, more specifically, AI's subfield, machine learning (ML)[4]. By learning from these data, ML algorithms are able to predict future data points [5]. AI/ML applications have been described in many studies as the potential solution to long-standing healthcare challenges, including the need for cost reduction, enhancing diagnostic accuracy, facilitating individualized and evidence-based care, and more importantly, significantly ameliorating the morbidity and mortality associated with various diseases[6-8].

In this opinion review, we first present the current status of the application of AI in the field of gastroenterology. Then, we pose a series of challenges that these applications face and explore ways to overcome them. Specifically, we elaborate on the following challenges: Accuracy concerns, cost-effectiveness, cybersecurity, interpretability, oversight, and liability.

CURRENT STATUS

Physicians use experience, intuition, and quantifiable and non-quantifiable variables for decision-making. ML algorithms, on the other hand, perform a series of precise calculations of all the quantifiable variables to perform a certain task. Computer systems, in general, and AI/ML tools, particularly, surpass, by far, physicians in their ability to quantify multiple correlations, even in fields where the physicians hold in-depth expertise. Thus, the application of ML tools is particularly useful for performing tasks requiring extensive analytical skills such as unraveling correlations in datasets, following laboratory results trends for long periods of time, and recognizing patterns in various imaging modalities. AI classifiers commonly employed in ML tools in gastroenterology are support vector machines (SVMs), artificial neural networks (ANNs), and convolutional neural networks (CNNs). SVMs are used in supervised learning. Data points of the instances are mapped in a high-dimensional space, where the hyperplane that separates the instances based on their assigned label, retaining the highest performance, is selected[9]. SVMs can be used for linear and non-linear problems using kernel functions[10]. ANNs are inspired by the human brain and consist of an input and output layer with at least one hidden layer in between[11]. As the architectures of ANNs became more sophisticated with the addition of multiple hidden layers and even more layer connections, the concept of deep neural networks (DNNs) emerged[12,13]. A particular type of DNN, the CNN, has found profound application in gastroenterology since the demonstrated high performance in image interpretation. CNNs are based on convolution, where the image is processed using a series of filters or kernels to detect patterns within the image, such as edges and textures[14].

PREVENTION

Table 1 presents a series of studies where AI/ML models have been applied in the field of gastroenterology. These models could be integrated into the clinical setting and follow real-time patient data trends. Then, these models could flag patients at high risk of developing a plethora of gastrointestinal diseases. A notable example is ColonFlag, an ML algorithm used to stratify the risk of developing colorectal cancer. Impressively, the tool has been shown to identify patients with colorectal cancer who would otherwise have avoided screening and to identify patients at the early stages of the disease 18 to 24 mo prior to the usual diagnosis time[15,16]. Other recent studies focus on the stratification of gastric cancer development by employing electronic health care records[17,18]. These models demonstrate how AI/ML models

Table 1 Studies employing artificial intelligence/machine learning tools in the field of gastroenterology

Ref.	Parameters employed/study design	AI classifier	Sample size, control group, validation	Outcomes	Performance[1]
Prevention					
Goshen *et al*[15]	EHR data/prospective validation	LR, DT, GB	688 flagged patients, NA, NA	High risk of CRC development	19 (7.5%) CRCs were found in 254 colonoscopies
Holt *et al*[16]	EHR data/case-control study	LR, DT, GB	1893641 patients, 15 controls matched to 1 case, NA	Early detection of CRC	0.536-0.624$^{c\text{-index}}$
Huang *et al*[17]	EHR data/case-control study	LR, LASSO, SVM, KNN, RF	1035 patients, 3 controls matched to 1 NCGC case, 10-fold CV	Early detection of NCGC	LR: 72.4^{acc}, 79.3^{sen}, 70.4^{spe}. LASSO: 75.1^{acc}, 80^{sen}, 73.6^{spe}. SVM: 75.1^{acc}, 76.3^{sen}, 74.7^{spe}. KNN: 78.1^{acc}, 68.9^{sen}, 80.8^{spe}. RF: 72.2^{acc}, 77.8^{sen}, 70.6^{spe}
Briggs *et al*[18]	EHR data/case-control study	RF, SVM, LR, NB, EGBDT	40348, 7471 cases/32877 controls), hold-out validation (75:25), 5-fold CV	Early detection of oesophago-gastric cancer	87-89^{acc}, 0.81-0.87$^{c\text{-index}}$
Diagnosis					
Manandhar *et al*[20]	Gut microbiome data (fecal 16S metagenomic data)/case-control study	RF, SVM, DT, ANN	1429 patients, 729 IBD and 700 non-IBD patients, hold-out validation	Diagnosis of IBD	77-84^{acc}, 0.41-0.82^{sen}, 77-84^{sen}, 46-64^{spe}
Papa *et al*[21]	Gut microbiome data/case-control study	SVM, RF	105 children and young adults, 91 with IBD and 24 controls, 10-fold CV	Diagnosis of IBD in child population	0.83-0.91$^{c\text{-index}}$
Pace *et al*[22]	Laboratory results, clinicopathological parameters/retrospective cohort study	ANN	159 patients, 103 with gastroesophageal reflux and 56 controls, 20-fold CV	Diagnosis of gastroesophageal reflux disease	78-100^{acc}
Yepes *et al*[25]	Expression levels of 1046/315 microRNAs in gastric samples/retrospective cohort study	SVM, RF	648 gastric samples, 479 cancer and 169 controls, leave-one-out CV	Classification of non-tumor mucosa *vs* tumor sample	SVM: 94^{acc}, RF: 89.3^{acc}
Huang *et al*[26]	Serum expression levels of miR-21-5p, miR-22-3p, and miR-29c-3p/retrospective cohort study	Several	192, 122 cancer samples and 70 controls, leave-one-out CV	Identification of patients with gastric cancer	56-93^{acc}
Alizadeh *et al*[27]	Serum expression levels of miR-663a, miR-1469, miR-92a-2-5p, miR-125b-3p, and miR-532-5p/retrospective cohort study	ANN	671, NR, 5-fold CV	Identification of patients with pancreatic ductal adenocarcinoma	93^{acc}, 93^{sen}, 92^{spe}
Afshar *et al*[28]	Serum expression levels of miR-6726-5p, miR-7111-5p, miR-1247-3p, and miR-614/retrospective cohort study	ANN	200, 50 with colorectal cancer and 150 healthy controls, hold-out validation (70:15:15)	Identification of patients with colorectal cancer	100^{acc}, 1$^{c\text{-index}}$, 100^{sen,spe}
Duttagupta *et al*[29]	Expression levels of 847 microRNAs in the peripheral blood/case-control study	SVM	40, 20 patients with ulcerative colitis and 20 controls, 10-fold CV	Identification of patients with ulcerative colitis	92.3-92.8^{acc}, 87.8-89.5^{sen}, 96.2-96.8^{spe}
Treatment					
Morilla *et al*[30]	Colonic microRNA profiles (9 microRNAs) and five clinical factors/retrospective cohort study	DNN	76 patients, 22 responders and 54 non-responders, hold-out validation (47:29)	Prediction of response to treatment of patients with active severe ulcerative colitis	80-93[1], 0.80-0.91$^{c\text{-index}}$
Takiyama *et al*[31]	Esophago-gastro-duodenoscopy imaging/retrospective cohort study	CNN	17080 images, 363 larynx/2142 esophagus/13048 stomach/1528 duodenum, hold-out validation	Anatomical classification among the larynx, esophagus, stomach, and duodenum	0.99-1.00$^{c\text{-index}}$
Rogers *et al*[35]	Data from baseline impedance, nocturnal baseline impedance, and acid exposure time/retrospective cohort study	DT	335 patients, 210 with gastroesophageal reflux and 115 controls, NR	Prediction of response to treatment with proton pump inhibitors for patients with gastroeso-	0.31-0.938$^{c\text{-index}}$

				phageal reflux disease	
Takayama *et al* [36]	Clinicopathological parameters, treatment data/retrospective cohort study	ANN	90 patients, 32 non-responders and 58 remission-effect, hold-out validation (54:36)	Prediction of the need for operation for UC patients treated with cytoapheresis	96^{sen}, 97^{spe}
Das *et al*[37]	Laboratory results, clinicopathological parameters/retrospective cohort study	ANN	587 patients, 246 patients with major stigmata-emergent endoscopy to 162 patients, hold-out validation (194:193:200)	Prediction of major stigmata of recent hemorrhage	89^{acc}, 89-96^{sen}, 63-89^{spe}
				Prediction of the need for emergent endoscopy	61-81^{acc}, 61-94^{sen}, 48-82^{spe}
Chu *et al*[38]	Laboratory results, clinicopathological parameters/retrospective cohort study	Several	189 patients, NR, hold-out validation (122:67)	Prediction of the source of GIB	69.7-94.3^{acc}, 0.658-0.999$^{c\text{-}index}$, 90.1-98.0^{sen}, 89-100^{spe}
				Prediction of the need for blood resuscitation	64.7-94.1^{acc}, 0.381-0.993$^{c\text{-}index}$, 90.3-93.9^{sen}, 18.4-95.5^{spe}
				Prediction of the need for emergent endoscopy	62.7-83.3^{acc}, 0.404-0.913$^{c\text{-}index}$, 80.1-89.1^{sen}, 13.8-85.7^{spe}
				Prediction of disposition	58.4-89.7^{acc}, 0.324-0.972$^{c\text{-}index}$, 81.9-92.9^{sen}, 18.4-90.9^{spe}
Loftus *et al*[39]	Laboratory results, clinicopathological parameters/retrospective cohort study	ANN	147 patients, 41% of patients with severe lower GIB and 13 patients needed surgical intervention, hold-out validation (103:44)	Prediction of severe lower GIB	0.979$^{c\text{-}index}$
				Prediction of the need for surgical intervention	0.954$^{c\text{-}index}$
Ayaru *et al*[40]	Laboratory results, clinicopathological parameters/retrospective cohort study	GB	300 patients, 88 with severe bleeding, 53 with recurrent bleeding, and 35 requiring intervention, hold-out validation (170:130)	Prediction of severe lower GIB	78-83^{acc}
				Prediction of recurrent bleeding	88-88^{acc}
				Prediction of the need for intervention	88-91^{acc}
Prognosis					
Sato *et al*[41]	Laboratory results, clinicopathological parameters, tumor characteristics/retrospective cohort study	ANN	395 patients, 281 alive at 1-year-89 alive at 5 years, hold-out validation (53:27:20)	1-yr and 5-yr survival of patients with esophageal cancer following surgery	0.883-0.884$^{c\text{-}index}$, 78.1-80.7^{sen}, 84.7-86.5^{spe}
Shung *et al*[42]	Laboratory results, clinicopathological parameters/retrospective cohort study	GB	2357 patients, 1109 requiring either transfusion or hemostatic intervention, hold-out validation (1958:399)	Stratification of risk of patients with gastrointestinal bleeding	0.91$^{c\text{-}index}$
Zhou *et al*[43]	Laboratory results, clinicopathological parameters, tumor characteristics/retrospective cohort study	Several	2012 patients, 405 patients with recurrence, hold-out validation (80:20)	Recurrence of gastric cancer following surgery	0.77-0.80$^{c\text{-}index}$

[1]Test set results are reported.

Acc: Accuracy (%); c-index: under the receiver operating curve or C-index; Sen: Sensitivity (%); Spe: Specificity (%); ANN: Artificial neural network; CD: Chron's disease; CNN: Convolutional neural network; CRC: Colorectal cancer; CV: Cross-validation; DNN: Deep neural network; DT: Decision tree; EGBDT: Extreme gradient boosting decision tree; EHR: Electronic healthcare record; GB: Gradient boosting; IBD: Inflammatory bowel disease; KNN: K-nearest neighbor; LASSO: Least absolute shrinkage and selection operator; LR: Logistic regression; NA: Not applicable; NB: Naïve Bayes; NBI: Narrow-band imaging; NCGC: Noncardia gastric cancer; NPV: Negative predictive value; NR: Not recorded; RF: Random forest; SVM: Support vector machine; UC: Ulcerative colitis.

can be used at the primary care level to lessen the burden of a plethora of gastrointestinal diseases significantly.

DIAGNOSIS

Currently, endoscopy constitutes the gold standard in the diagnosis of various gastrointestinal diseases. Nevertheless, endoscopy is an unpleasant intervention for the patient, has a significant cost, and is related to a series of potential complications[19]. AI/ML models utilizing non-invasive parameters that provide comparably accurate diagnoses could

either replace, minimize, or refine the indications of endoscopy. Two studies have used data from the microbiota and microbiome to identify patients with inflammatory bowel disease (IBD)[20,21]. Although these models could not replace endoscopy in the diagnosis of IBD, they can be used to identify patients requiring endoscopic evaluation. However, in the diagnosis of gastroesophageal reflux disease, the employment of ML tools could substitute the use of endoscopy for patients at low risk of developing cancer[22]. These efforts demonstrate how ML tools could be used to significantly reduce the cost and complications related to endoscopy, avoiding, at the same time, an unpleasant experience for the patient.

The introduction of next-generation sequencing has widened our capabilities regarding detecting host genetic factors related to certain diseases and allowed us to unveil the complex hereditary background of several diseases by clarifying the cascade of gene interactions and the impact of the environment[23,24]. This progress allowed for the emergence of novel biomarkers, including microRNAs. In gastroenterology, various studies have employed microRNA profiling data to develop tools able to diagnose various gastrointestinal diseases. Specifically, tools have been developed that classify non-tumor from tumor mucosa from gastric samples[25]. In addition, by employing microRNA profiling, data from serum models have been developed that identify patients with gastric cancer[26], pancreatic ductal adenocarcinoma[27], colorectal cancer[28], and ulcerative colitis[29]. Finally, a model has been developed using microRNA profiling from colonic samples to predict the response to treatment of patients with active severe ulcerative colitis[30]. These models again demonstrate how AI/ML tools can provide a non-invasive endoscopy alternative.

Except for replacing endoscopy, AI/ML tools could significantly enhance the endoscopy's clinical outcomes. Such models could be used for developing Computer Aided Detection or Diagnosis systems (CAD systems) to facilitate the interpretation of endoscopy images. First, these CAD systems could aid the endoscopist to navigate in the gastrointestinal tract. Existing models have demonstrated outstanding performance[31]. These CAD systems could be used as a "third eye" for the endoscopist, an "eye" that does not get tired, is not susceptible to any distractions, and can identify lesions missed by the endoscopist. Following the identification of lesions, these models could be used to classify the lesions, for example, as benign or malignant. In capsule endoscopy, CAD systems could automatically identify and then classify lesions from capsule endoscopy videos and then provide the images to the gastroenterologist for review. Such models could significantly decrease the cost associated with the labor hours required to review the capsule endoscopy videos. Several models have been developed for a plethora of gastrointestinal diseases, including IBD, gastric cancer, small bowel lesions (ulcerative/hemorrhagic), colon cancer, and others[6,32]. Another application of CAD systems is proposing an ideal site for biopsy when needed. CAD systems also find a particular application in colonoscopy in classifying benign from malignant polyps[33]. By avoiding the removal of benign polyps and correctly removing all malignant polyps with the assistance of CAD systems, the cost-efficiency of coloscopy significantly increases. Notably, regarding polyp classification, the reported negative predictive value in the literature of CAD systems is above the 90% recommended by the American Society of Gastrointestinal Endoscopy for adenoma detection[34].

TREATMENT

In addition to diagnosis, AI/ML-based tools can be employed to facilitate treatment allocation for various gastrointestinal diseases. Data regarding impedance and acid exposure time were employed in a study to develop an ML tool predicting treatment response[35]. Other studies focusing on IBD have developed tools that predict treatment response to specific drugs or the need for operation[35,36]. A different group of studies, which focused on gastrointestinal bleeding management, achieved the development of models identifying patients in need of a transfusion, emergent endoscopy, emergent surgery, and intensive care unit triage[37-40]. These studies demonstrate how AI/ML tools could be used to create frameworks for individualized, evidence-based treatment allocations.

PROGNOSIS

AI/ML algorithms have also been employed in the development of predictive tools able to predict complications, recurrence following treatment, and overall survival. Examples of such studies include a study developing an ML tool to predict overall survival in patients with esophageal cancer[41], a model predicting in-hospital death for patients with gastrointestinal bleeding[42], and an ML model predicting the chance of recurrence in operated gastric cancer patients [43]. Such models could be used for family and patient counseling.

ACCURACY CONCERNS

The accuracy of a developed AI/ML tool largely depends on the quality of the training data. The notion "garbage in, garbage out" is particularly true in ML models. Flaws and weaknesses of the datasets, such as wrongly labeled instances, low image quality, small dataset size and variability of instances, and discrepancies in the data collection processes, will be inadvertently built into the end model. For example, capsule endoscopy has significantly lower image resolution compared with other types of endoscopy images, and thus, a model trained from capsule endoscopy imaging is expected to have lower classification accuracy. Standardization of data collection methods is essential to acquire datasets of clean, high-quality data that are representative of a diverse patient population. A main challenge encountered in ML

development is class imbalance. Most of the time, normal findings represent the majority class, while patients with a disease are usually the minority class. This problem is amplified in multi-class situations where accurate classification of rare classes is particularly challenging. A series of techniques to undersample the majority class and oversample the minority class in medical datasets have been proposed that, to some extent, resolve this issue[44]. In addition, in a study, researchers developed a model able to add polyps in the endoscopic images, which can then be used for training ML models[45]. Such methodologies of creating synthetic instances could again resolve the imbalance challenge. A different challenge in ML model development is underfitting, which can be defined as the incapability of the model to capture the variability of the data[46]. On the other hand, overfitting can be described as the inability of the model to retain its performance in datasets other than the training dataset[47]. In other words, the model's performance is significantly superior in the training set and drops in new datasets. CNNs, which are widely used in gastroenterology, are vulnerable to overfitting[14]. There is a series of proposed methodologies to resolve underfitting and overfitting challenges, including early stopping/dropout, penalty method/regularization, adjusting the learning rate and the optimizer, weight matrix initialization, data augmentation, and batch normalization[46,48]. Another intriguing point is that ML models are trained from datasets not practically available in the clinical setting. The different training variables are derived at various time points during the treatment of the patient and are not readily available at a given time. These missing parameters could become a shortcoming anchoring the use of ML in the clinical setting. All the above challenges have a common "true" solution. Physicians should be included in the development of ML models from the early stages of the procedure. Effective communication among physicians, the developing team, and the investors could address and resolve any potential shortcomings of these models. The key is to involve physicians in all the stages of the process. Physicians should be involved in problem identification to ensure that the developed tool addresses actual needs and in data collection and annotation to ensure that the data are labelled correctly, which is crucial in supervised learning. Additionally, we believe that the following steps should be followed during model development to avoid potential drawbacks: Physicians should provide a detailed description of the problem at hand to the developing team, explain what is requested by the model (the endpoint), describe the features thoroughly, cooperate to identify and engineer features of high predictive value, discuss the sample size needed based on the nature of the task (binary classification, multi-class classification, regression), ensure that the patients included in the developing procedure are a representative sample of the targeted group, conduct a rigorous clinical validation before the clinical application of the model, provide input on how the tool can be seamlessly be integrated into the existing clinical workflows without causing disruption, discuss the prospects of real-time learning, where the model continues to learn and improves following clinical application, and provide insights into ethical considerations including ensuring patient privacy and avoiding potential biases. Even, following the model's application in the clinical setting is essential to establish a framework of continuous monitoring and feedback from healthcare professionals to address model shortcomings and improve the model's efficiency. Finally, when applying AI/ML-based tools in the clinical setting is crucial to collect data on the impact of the use of such tools on the clinical outcome, the well-being of the patient, and the patient-physician relation. Failing to cooperate efficiently and to communicate what exactly is expected from the model could result in an end product that does not meet the expectations of the physicians and jeopardizes the safety of the patients.

COST-EFFECTIVENESS

Unfortunately, resources are not infinite. Especially in an era of technological advancement, with other cutting-edge technologies, such as three-dimensional printing and bioprinting, virtual and augmented reality, regenerative medicine, and robotics that also promise to facilitate patient care, AI must prove that it is worth investing in. In reality, AI, which has been at the forefront for many decades, has not yet been able to provide the expected results in healthcare. This is evident during AI winters, where investors demonstrate their frustration by cutting funding[4]. The expectations are often placed extremely high, and from their perspective, AI overpromises and constantly underdelivers. As mentioned above, effective communication among the physicians, the developing team, and the investors is crucial in agreeing on the expectations and avoiding disappointment in the end product. Nevertheless, even if physicians, developers, and investors are satisfied, the true success of the model should be assessed in association with the clinical outcome. Thus, conducting a series of cost-effective analyses is crucial to determine whether AI applications' benefits in healthcare are worth the associated cost. The costing of new technologies is generally challenging due to the lack of pre-existing data, and it involves bottom-up costing approaches, which typically require more resources and are time-consuming[49]. The cost associated with new technologies tends to drop over time; thus, cost estimation studies will need to be updated throughout the lifecycle of the AI application. Despite its importance, the topic of the cost-effectiveness of the application of AI in medicine is poorly investigated, mainly due to the lack of real AI applications in the clinical setting. There are several ways to improve the cost-effectiveness of implementing AI/ML-based technologies in healthcare. First, a series of models has been developed and patented with a high performance in performing several tasks, such as CAD systems that classify benign from malignant polyps. Thus, employing existing or pre-trained models rather than developing new tools from scratch could significantly decrease the cost of adopting these technologies in clinical practice. When, however, healthcare institutions decide to invest in developing AI/ML-based tools from scratch, a plethora of approaches could be adopted to improve the cost-effectiveness, including the utilization of collaborative platforms and open-source tools, running models on existing hardware when possible to reduce the need of specialized hardware, use of existing datasets when available to avoid data acquisition costs, and finally focus on developing tools that allow for the automatization of routine, time-consuming tasks while prioritizing high-incidence conditions with the potential for significant cost savings. One of the few studies addressing this issue is a recent study in the field of gastroenterology, where researchers

performed a Markov model microsimulation of AI application in screening colonoscopy and concluded that at the United States population level, AI application could prevent more than seven thousand colorectal cancer cases and more than two thousand related deaths, and provide a yearly saving of 290 million dollars[50]. These results are very promising, and as soon as AI models are widely applied in the clinical setting, studies proving their cost-effectiveness must be conducted.

CYBERSECURITY

Cybersecurity is a challenge not exclusive to AI but to the tendency of digitalization in general. Nevertheless, AI introduces further concerns since it requires a wholly electronic tracking of patient records. This raises concerns that a huge amount of sensitive data is vulnerable to massive disclosures[51]. For example, a transfer of 1.6 million patient records to Google DeepMind was ruled illegal in the United Kingdom[52]. Additionally, due to the sensitive nature of data (including financial data), healthcare facilities are essentially attractive targets for cyberattacks. The introduction of AI in healthcare allows for the emergence of new vulnerabilities. Imagine an intrusion into an ML, which is now manipulated into making wrong decisions or even introducing malevolent data. For example, in a study, the researchers demonstrated how a DNN was used to add or remove lung tumors from computed tomography scans at a level of accuracy that remained undetected by specialized radiologists[53]. Imagine a similar intrusion that will introduce colorectal cancer images during real-time colonoscopy. Such incidents could severely undermine any trust in AI in healthcare and have severe consequences on patient outcomes. How could patients and physicians be confident about any AI model suggestions, knowing that they can be manipulated? Evidently, the information technology infrastructure will need to be expanded in order to accommodate AI applications and shield these models from potential threats.

INTERPRETABILITY

Interpretability in ML refers to the degree of explainability and transparency of the model[54]. In other words, whether the logic behind the model's decisions is understandable or not. Imagine interpretability as a spectrum, where at the "white" end, we find "white-box" models, where their inner workings and decision-making processes are fully explainable and interpretable. At the other end of the spectrum, we find "black-box" models, which are characterized by a highly complex architecture to the extent that we are unable to comprehend their inner mechanisms and decision-making processes. In simple tasks, for example, when the features and target variable have a linear relation, white box models demonstrate a sufficiently high performance, rendering the use of black box models redundant. However, in complex tasks with high-dimensional data, black boxes exhibit superior performance to white boxes due to their ability to capture intricate patterns and relationships within the data. However, the application of black box models in healthcare introduces a series of challenges, including undermining the trust towards AI, inability to oversight any biases or prejudiced decisions towards minority groups, failure to justify our AI-assisted decision to patients, and violation of fundamental ethical tenets that physicians should hold a basic comprehension of the devices that they use. Avoiding using "black-box" models due to the above challenges could significantly handicap the potential of AI applications in healthcare. Thus, we should aim for methodologies that allow for a level of reasoning of "black-box" model predictions. Introducing a level of interpretability in black boxes applied in healthcare is crucial to enhance patient and physician trust in these tools and facilitate clinical decision-making. Interpretability can be divided into global and local. Global interpretability offers a level of explainability of the model as a whole[55]. Ways to improve global interpretability include feature importance analysis, surrogate models, and interactive visualization tools developed to allow users to explore how the various features influence the model's predictions[55-58]. For example, a model stratifying the risk of colorectal cancer development could provide its prediction along with a notification that the prediction is based mainly on the patient's sex, age, and blood count. On the other hand, local interpretability provides the reasoning behind individual predictions[55]. Methodologies to enhance local interpretability include surrogate models, Shapley values, saliency regions, and visualization techniques[55-58]. Shapley values could be used to demonstrate how each value of each feature contributes to the model's prediction, while saliency maps provide a *post-hoc* visualization to comprehend which parts of the input were used by the model to reach its decision and are particularly helpful for CNNs to visualize which parts of the image were used in the model's interpretation. For example, a saliency map would highlight exactly which parts of a biopsy that the model focused on to reach its diagnosis. Providing a level of explainability for "black-box" models is key to gaining the trust of both physicians and patients and will play a pivotal role in determining which models will dominate the industry.

OVERSIGHT AND LIABILITY CONCERNS

As acknowledged by the Food and Drug Administration (FDA), applications regarding AI/ML-based software marketing in healthcare have increased exponentially[59,60]. In early 2021, the FDA published the Artificial Intelligence/Machine Learning (AI/ML)-Based Software as a Medical Device (SaMD) Action Plan, while in 2023, it published the Marketing Submission Recommendations for a Predetermined Change Control Plan for Artificial Intelligence/Machine Learning (AI/ML)-Enabled Device Software Functions[59,61]. Similarly, the European Union has announced its first regulation on

AI applications, the EU AI Act[62]. Evidently, governments feel the need for AI oversight and regulation. Regarding law and regulation, the true challenge for the future is to find the golden mean between too little oversight, which may have devastating and severe consequences for the well-being of our patients, and too strict regulation, which will handicap innovation. AI/ML-based tools are unique in the way that they constantly evolve following the initial audit and marketing. Thus, as the model encompasses more data and is retrained, it diverges from the original product to the extent that it can be considered an entirely different product at different time points. Who ensures the safety and credibility of the product at different time points? Evidently, a lifecycle regulatory framework is needed that provides post-marketing oversight, or alternative time-limited authorizations should be granted with periodic audits when several modifications have been made to the initial product[63].

Liability is the main physician-related concern that could undermine the widespread application of AI in healthcare. Even if the research succeeds in providing highly accurate, reliable AI models, for AI to impact the clinical outcome, physicians will need to accept and adopt these models in their everyday lives. Liability could be the main concern that discourages physicians from doing so. To evade malpractice liability, a physician is expected to provide care at the same level as a competent, same-specialty physician, considering the available resources[64]. However, the introduction of AI makes liability a far more complicated matter. An intriguing concept to consider is the potential legal binding of AI's decisions in the future. As AI/ML-based software continues to advance and grow, it is reasonable to assume that they may eventually outperform physicians, at least in certain tasks. In such a scenario, how can physicians justify ignoring the decisions presented by AI, especially when AI's decisions are solely data-driven and lack any subjective bias?

Furthermore, who should be held liable if following the AI model's decision leads to patient harm? Currently, no legal precedent clarifies liability in cases where a patient suffers an injury due to an inaccurate output generated by AI/ML-based software[65]. In a published legal analysis, the authors present a series of scenarios based on the various combinations of whether the physicians follow the AI recommendation, whether the AI recommendation is correct, and whether a patient injury occurs. The authors' conclusion highlights that since existing laws protect physicians from liability when adhering to standard care, it incentivizes them to downplay AI's actual usefulness, potentially turning AI into a mere confirmatory tool instead of a tool to enhance the quality of care[65]. Until a comprehensive legal framework regarding liability is established, healthcare facilities have valid concerns and may hesitate to adopt AI technologies due to fears and uncertainties about potential liabilities that they may expose the facility and its staff to.

CONCLUSION

In this opinion review, we initially elaborate on the current status of the application of AI/ML-based software in gastroenterology. Currently, AI/ML-based models have been developed in the following applications: Models integrated into the clinical setting following real-time patient data flagging patients at high risk for developing a gastrointestinal disease, models employing non-invasive parameters that provide accurate diagnoses aiming to either replace, minimize, or refine the indications of endoscopy, models utilizing genomic data to diagnose various gastrointestinal diseases, CAD systems facilitating the interpretation of endoscopy images, models to facilitate treatment allocation and predict the response to treatment, and finally, models in prognosis predicting complications, recurrence following treatment, and overall survival that can be used for family and patient counseling. Then, we elaborate on several challenges and how they may negatively impact the widespread application of AI in healthcare and gastroenterology, offering possible ways to overcome them. Specifically, we have elaborated on concerns regarding accuracy, cost-effectiveness, cybersecurity, interpretability, oversight, and liability.

It is worth noting that the vast majority of AI research originates from high-income countries[66]. Despite being described as the solution that could narrow the gap in quality of care between high and low-income countries, AI may become one more brick in the wall of inequality. To avoid that, future AI initiatives should include middle and low-income countries. In any healthcare system where AI/ML-based software will be integrated, it is essential to simultaneously integrate equally sophisticated oversight mechanisms to ensure the safety of patients; otherwise, physicians may encounter unintended ramifications.

As mentioned before, AI/ML models' decision-making is based on quantifiable parameters. However, clinicians rely on non-quantifiable parameters for decision-making. It is crucial to state that overreliance on AI could impair our critical thinking and reasoning. Thus, the ultimate goal is not AI-driven but AI-assisted clinical practice. AI could be the means to augment our intelligence by embracing the inherent complexity of medicine. Envision the hospital of the future where hospitals are fully robotic, AI and robots conduct the majority of procedures, 3D printers are used to print almost everything, from medical equipment to artificial parts, telemedicine allows patients to stay at home, virtual and augmented reality will assist physicians at all procedures, and biosensors are placed at birth diagnosing all diseases at an early stage and alerting physicians[67-69]. It is unlikely that AI will replace physicians. Nevertheless, it is also unlikely that the skillset demanded by future physicians will be in any way similar to the present. In this upcoming transformation of healthcare with the integration of cutting-edge technologies, AI is expected to be at the center. Thus, physicians are expected to engage with AI to avoid becoming obsolete.

FOOTNOTES

Author contributions: Christou CD and Tsoulfas G performed the screening of articles for eligibility; Christou CD drafted the manuscript; Tsoulfas G edited the manuscript.

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REVIEW

Gene targeted and immune therapies for nodal and gastrointestinal follicular lymphomas

Takuya Watanabe

Specialty type: Gastroenterology & hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

Follicular lymphoma (FL) is the most common indolent B-cell lymphoma (BCL) globally. Recently, its incidence has increased in Europe, the United States, and Asia, with the number of gastrointestinal FL cases expected to increase. Genetic abnormalities related to t(14;18) translocation, BCL2 overexpression, NF-κB pathway-related factors, histone acetylases, and histone methyltransferases cause FL and enhance its proliferation. Meanwhile, microRNAs are commonly used in diagnosing FL and predicting patient prognosis. Many clinical trials on novel therapeutics targeting these genetic abnormalities and immunomodulatory mechanisms have been conducted, resulting in a marked improvement in therapeutic outcomes for FL. Although developing these innovative therapeutic agents targeting specific genetic mutations and immune pathways has provided hope for curative options, FL treatment has become more complex, requiring combinatorial therapeutic regimens. However, optimal treatment combinations have not yet been achieved, highlighting the importance of a complete understanding regarding the pathogenesis of gastrointestinal FL. Accordingly, this article reviews key research on the molecular pathogenesis of nodal FL and novel therapies targeting the causative genetic mutations. Moreover, the results of clinical trials are summarized, with a particular focus on treating nodal and gastrointestinal FLs.

Key Words: Gastrointestinal follicular lymphoma; Genetic mutation analysis using next-generation sequencing; MicroRNA; Gene targeted therapy; Immune therapy

Core Tip: Recently, the incidence of follicular lymphoma (FL) has increased in Asia, Europe, and the United States, with the number of gastrointestinal FL cases expected to increase. This article reviews the recent literature on the molecular origins of, innovative treatments for, and clinical trial findings on FL, focusing on gastrointestinal FL. Genetic factors [*e.g.*, t(14;18) translocation and B-cell lymphoma 2 overexpression] drive FL growth, while microRNAs aid in its diagnosis and prognosis. Although recent trials have reported enhanced treatment outcomes, curative therapies remain elusive, and combinatorial regimens have not been optimized. Hence, gastroenterologists require a more comprehensive understanding of gastrointestinal FL pathogenesis to facilitate improved therapeutic outcomes.

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INTRODUCTION

Follicular lymphoma (FL) is the most common form of indolent B-cell lymphoma (BCL)[1]. In recent years, FL incidence has rapidly increased in Europe, the United States, and Asia[2], and the number of advanced-stage gastrointestinal FL cases is expected to increase in the near future. Although it responds well to treatment, it is prone to relapse and is refractory; hence, FL is regarded as an incurable disease with a poor prognosis. The NF-κB pathway is an intracellular signaling pathway activated by cell surface receptor stimuli in B-cell receptor (BCR)- and Toll-like receptor (TLR)-expressing cells. Indeed, the constant activation of this pathway contributes to lymphoma development. Histone methylation and acetylation of gene promoters regulate gene expression; abnormalities in gene expression can influence lymphoma development and proliferation. In addition, the t(14;18) translocation, found in most FLs, causes the overexpression of BCL2, eliciting an anti-apoptotic effect that mediates cell immortalization. Recently, genome-wide association studies (GWAS) have identified several loci involved in FL development.

Approximately 150 microRNAs (miRNAs) participate in the development of FL *via* enhanced cell proliferation. These include the miR-17-92 cluster, the importance of which has been evaluated in FL diagnosis and prognosis.

Rituximab, a monoclonal antibody against the CD20 surface antigen expressed in more than 90% of BCL-expressing cells, was developed approximately 20 years ago, and its combination with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) represents a standard chemotherapy that continues to be the mainstay of treatment. Nevertheless, the past decade has witnessed considerable advancements in developing new FL therapeutic agents targeting various oncogenes.

This article reviews the latest findings in nodal FL, particularly related to molecular genetic analysis and diagnosis while summarizing the progress and future perspectives of novel FL therapeutics targeting various genetic abnormalities. The article concludes with a personal view of gastroenterologists' role in treating advanced-stage FL of gastrointestinal origin.

INCIDENCE AND EPIDEMIOLOGY

FL is the leading indolent (low-grade) BCL, accounting for 10%-20% of all non-Hodgkin lymphomas[1]. Its incidence is increasing rapidly in Western and Asian countries[2]. In particular, the incidence of FL in Japan is increasing[3]. Gastrointestinal FL (GI-FL) occurs in the lymphoid tissue of the gastrointestinal tract and is derived from the FL of B-cell lymphocytes and primarily arises from the lymph follicles (lymphoid apparatus) in the submucosa of the gastrointestinal tract. Histologically, GI-FL contains a folliculocentric lymphoma cell population; FL is categorized histopathologically as grade 1, 2, 3a, or 3b, with grade 3b usually identified and treated as an aggressive medium- or high-grade lymphoma. Most patients with grade 3b FL present with enlarged lymph nodes, and approximately 70%-85% are diagnosed with an advanced-stage III/IV disease, often associated with significant bone marrow involvement. Although the incidence of GI-FL is low and relatively rare compared with that of other lymphomas, it is more common in middle-aged and older adults, with no apparent difference in incidence between men and women; however, the peak incidence in certain age groups is not reported.

SYMPTOMS

The symptoms of gastrointestinal FL vary among individuals, ranging from asymptomatic with very mild symptoms (most common) to well-defined symptoms, depending on the stage of the disease, degree of disease progression, and tumor location. The symptoms of GI-FL include: Discomfort or pain in the upper abdomen, often worsening after meals or at night; gastrointestinal symptoms: Nausea, vomiting, loss of appetite, bloating, upset stomach, diarrhea, and constipation; weight loss due to anorexia and impaired nutrient absorption; anemia due to intestinal bleeding and tumor

growth (anemia-induced fatigue and shortness of breath may occur); and edema due to the compression on lymphatic vessels and blood vessels, particularly in the abdomen and lower limbs.

Among the 249 GI-FL cases reported in a previous study, 150 with clinical symptoms were asymptomatic at onset (43.4%), 9.3% presented with vague gastrointestinal symptoms, including abdominal discomfort or heartburn, 28.7% had abdominal pain, 8.0% had symptoms of bowel obstruction, such as nausea and vomiting, and 6.0% had melena, such as tar-like black stools, a symptom of upper gastrointestinal bleeding[4].

DIAGNOSIS OF GI-FL

GI-FLs are diagnosed using a combination of methods. The following section details those most commonly used.

Endoscopy

Endoscopy represents the most important diagnostic test for GI-FL as it is useful for gross diagnosis and facilitates the extraction of biopsies for pathological examination and histological analysis. Gastric malignant lymphomas are divided into several gross types[5]. Colorectal malignant lymphomas often present with characteristic polyp-like endoscopic findings, termed multiple lymphomatous polyposis (MLP)[6], which are useful for diagnosis. The frequency of GI-FL detection has increased rapidly in Japan owing to the widespread and generalized use of small bowel endoscopy, advances in endoscopic equipment, improved endoscopist techniques, and increased opportunities for health screening [7].

Histopathological examination

Tissue samples obtained from biopsies are assessed pathologically using a microscope. GI-FL is diagnosed based on the presence of folliculocentric lymphoma cell populations and other histological features characteristic of FLs. Moreover, testing for specific proteins through immunostaining is performed. The following is a list of parameters used in the pathological diagnosis of FL.

Follicular-like structures: FLs are characterized by the formation of follicular-like structures within the tumor that are B-cell aggregates forming follicles within tumors. Additional abnormal follicular structures can be present in FL.

Grade: FLs are classified into three grades (1-3) by assessing the degree of formation of follicular-like structures within the tumor and the atypia (morphological abnormalities) of lymphoma cells.

Immunohistopathological examinations: Immunostaining findings showing CD20 and CD10 positivity and BCL2 overexpression are characteristic of FL and essential for the histological diagnosis of FL.

In FLs, lymphoma cells express the CD20 surface protein, a specific B-cell marker and an important indicator for diagnosing FL. CD10 is also expressed by FL cells. BCL2 is a protein rarely expressed in normal lymphoid tissues but is overexpressed in FLs. It is involved in the regulation of apoptosis (cell death) and is a characteristic immunohistochemical marker in FLs.

Chromosomal abnormalities: The t(14;18) translocation is frequently observed in FLs, causing the fusion of *BCL2* with the immunoglobulin H chain (*IGH*) gene, leading to overexpression of BCL2. Pathologists confirm IGH reconstruction as a basis for a definitive FL diagnosis.

Molecular genetic analyses

Molecular genetic analyses provide important information regarding the pathogenesis and prognosis of FL. This section details the molecular genetic analyses of FLs.

Detection of t(14;18) translocation: As mentioned in the section on pathological diagnosis above (section 1.3), t(14;18) translocation is a key component of FL diagnosis.

Detection of gene rearrangements using polymerase chain reaction: In FLs, there are rearrangements of *IGH* and other genes. These can be detected *via* polymerase chain reaction, allowing for the evaluation of the presence and frequency of specific gene rearrangements.

Genetic mutation analysis using next-generation sequencing: In FL, specific genetic mutations are associated with disease progression and prognosis. Next-generation sequencing (NGS) enables rapid and large-scale DNA sequencing to identify such mutations and assess their significance in FL. In particular, numerous genetic abnormalities have been characterized for BCL using NGS. These abnormalities in BCL impact proteins involved in tumorigenesis, resulting in: (1) Constant activation of the NF-κB signaling pathway; (2) Abnormal functioning of epigenome-related enzymes; (3) Abnormal expression of apoptosis-related factors; and (4) Immune system evasion (Figure 1)[8].

Constant activation of the NF-κB signaling pathway: This activation is involved in lymphoma development. Mutations in NF-κB pathway-related factors such as CD79B, MYD88, A20/tumor necrosis factor alpha-induced protein 3 (TNFAIP3), and CARD11 occur in active B-cell (ABC)-type diffuse large BCL (DLBCL).

Functional abnormalities in epigenome-related enzymes: Genetic abnormalities related to histone methyltransferases (such as KMT2D and EZH2) and histone acetylases (such as EP300 and CREBBP) occur in FL- and germinal center B-cell (GCB)-type DLBCL.

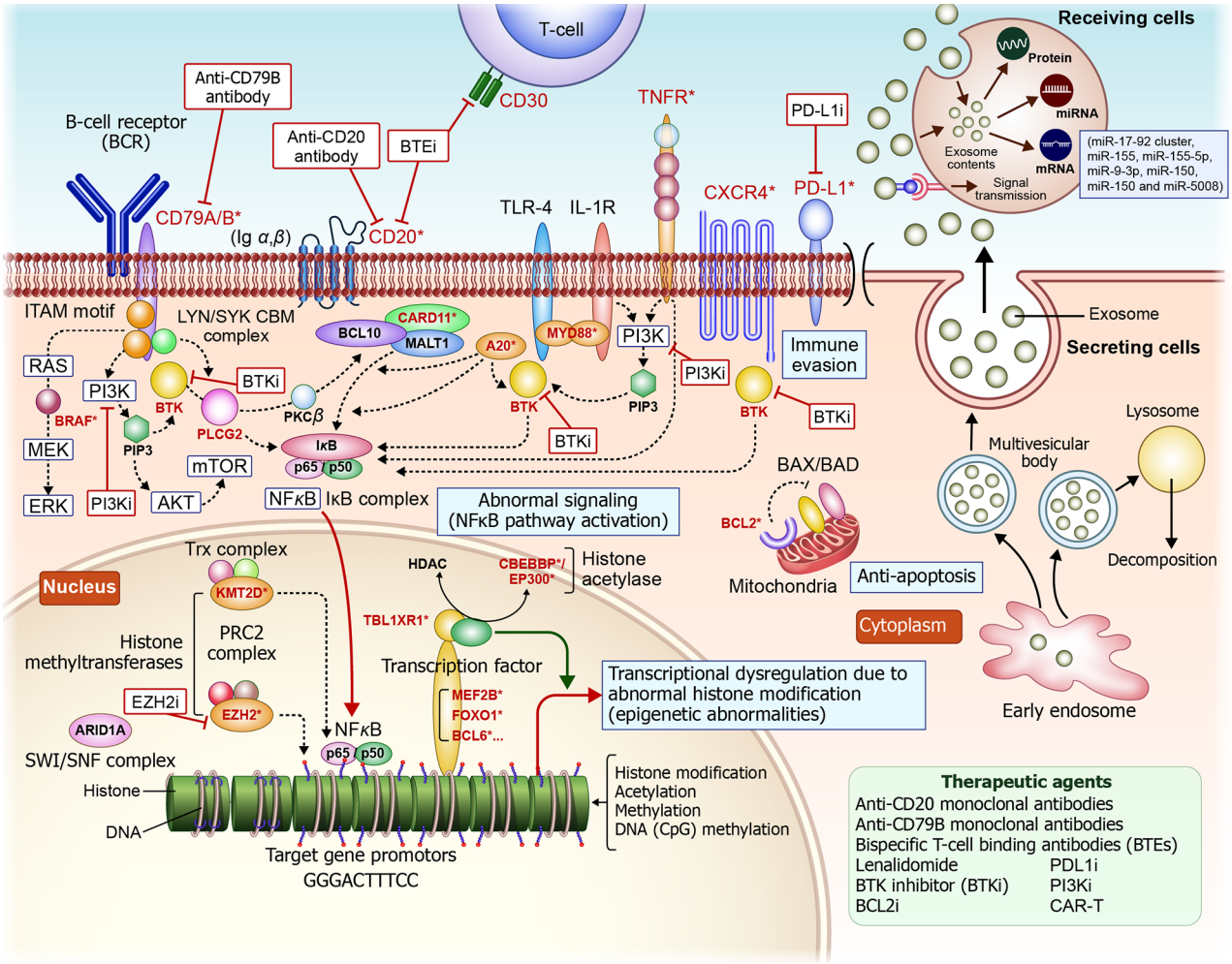

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Figure 1 Follicular lymphoma-related genes involved in follicular lymphoma pathogenesis and proliferation, and the action of novel therapeutic agents targeting them. MicroRNAs are secreted from the follicular lymphoma cytoplasm as exosomes, taken up by recipient cells, and regulate gene expression (right side of the figure).

Abnormalities in apoptosis-related factors: Chromosome t(14;18) reciprocal translocations, found in most FLs and 20%-30% of DLBCLs, promote the expression of BCL with anti-apoptotic properties and are involved in cell immortalization.

Immune system evasion: Abnormalities in genes related to immune evasion, such as PDL1/L2, are detected in DCLBL-like forms, Hodgkin lymphomas, and adult T-cell lymphomas. These abnormalities possibly aid lymphoma cells in evading immune attacks. The following findings have recently been reported for each NGS-discovered BCL-related gene involved in FL development and proliferation:

Polatuzumab vedotin is a first-in-class anti-CD79B antibody-drug conjugate (ADC) that combines an anti-CD79B monoclonal antibody with a tubulin polymerization inhibitor. CD79B is specifically expressed in B-cells. Polatuzumab vedotin binds to CD79B to deliver the chemotherapeutic agents and destroy B-cells. Three major clinical trials have investigated the efficacy and safety of polatuzumab vedotin. The G029376 trial demonstrated its efficacy in relapsed/refractory (r/r) FL and DLBCL[9]. The GO29365 trial tested the efficacy of polatuzumab vedotin + bendamustine and rituximab (BR) or bendamustine and obinutuzumab therapy in patients with r/r FL or DLBCL[10]. The JO40762 (P-DRIVE) study evaluated the complete response rate in patients with r/r DLBCL treated with polatuzumab vedotin + BR therapy[11]. Comprehensive genetic analyses classified DLBCL into ABC-type DLBCL and GCB-type DLBCL. *MYD88* and *CD79B* mutations are highly prevalent in ABC-type DLBCL and contribute to the constant activation of the NF-κB pathway, resulting in lymphoma development. In addition, ABC types with mutations in *MYD88* and *CD79B* in BCLs have a poorer prognosis[12]. These classifications are important for predicting prognosis and treatment response.

A20/TNFAIP3 is a cytoplasmic zinc finger protein that inhibits NF-κB activity mediated by tumor necrosis factor (TNF). AIP3 deficiency is observed in ABC-type BCL, MALT lymphoma, and Hodgkin lymphoma. A20, also known as TNFAIP3, is involved in the negative regulation of the NF-κB pathway. A20 induces a suppressive inflammatory response and exhibits anti-apoptotic effects while affecting immune homeostasis. Mutations and deletions in *A20* are frequent in non-GCB-typey DLBCL and DLBCL. *A20* gene abnormalities are also reported in certain DLBCLs[13]. Deletions, inactivating mutations, and methylation of the *A20* promoter at 6q23 inactivate A20 in various lymphomas. A20 inactivation is a key feature of lymphomas, and loss of A20 expression occurs in FLs[14]. Meanwhile, BCL10 is an essential regulator of NF-κB activation and is implicated in B-cell proliferation[15].

KMT2D is a histone lysine methyltransferase important in suppressing BCLs. Deletion of KMT2D increases the number of germinal center B-cells and promotes B-cell proliferation in mice. The overexpression of BCL2 combined with KMT2D deletion increases the incidence of lymphomas derived from germinal center B-cells. Therefore, KMT2D can function as a tumor suppressor and may be involved in reconstructing epigenetics that promote lymphoma development. Accordingly, targeting KMT2D is an effective approach for alleviating the early stages of tumorigenesis[16]. Tazemetostat is an orally administered EZH2 inhibitor that selectively inhibits a specific epigenetic-related protein, EZH2, to regulate the expression of cancer-related genes and inhibit cancer cell growth[17]. Tazemetostat was approved in Japan based on the results of clinical trials in patients with r/r B-cell non-Hodgkin lymphoma with EZH2 mutations[18,19]. Mutations in EZH2 occur in 7%-27% of FLs[20,21]; these patients may be eligible for treatment with tazemetostat. However, considering that FL is a refractory disease with frequent relapses, new treatment strategies are required. CREBBP, EZH2, MEF2B, and EP300 mutations occur in 33%, 27%, 15%, and 9% of FLs, respectively. Genome-wide analysis *via* NGS has shown that *EZH2, CREBBP*, and *MLL2*—histone-modifying genes—are frequently mutated in FL with essential roles in lymphomagenesis. *IGH-BCL2* translocations and CREBBP mutations are early events, whereas MLL2 and TNFSFR14 mutations are late events during disease evolution. In the 2008 WHO classification, three new variants were included: (1) Pediatric FL; (2) Primary intestinal FL; and (3) *In situ* FL[22].

MiRNAs

MiRNAs are non-coding RNAs involved in regulating gene expression. The aberrant expression of specific miRNAs is reported in FL. Hence, analyses of miRNAs provide essential information for predicting disease mechanisms and prognoses.

Relationship between exosomes and miRNAs: Exosomes are small vesicles released by cells containing various biomolecules (proteins, ribonucleic acids, and lipids) with myriad biological roles, including transmitting information between cells and expulsing unwanted intracellular components. MiRNAs are produced intracellularly and regulate gene expression by binding to mRNAs and regulating their stability and translation efficiency. The miRNAs produced inside cells can be wrapped in exosomes and released to the outside of the cell, facilitating the transport of miRNAs to other cells in remote areas, where they participate in information transfer and cell–cell interactions. This interaction is an important mechanism of information transfer and disease progression. The transport of miRNAs by exosomes is a form of extracellular signal transduction. When a sender cell receives a specific signal or is subjected to pathological conditions, the miRNA profile of the released exosomes changes, influencing the effect on recipient cells. Therefore, miRNA transduction by exosomes is important in regulating disease progression and cell–cell interactions (Figure 1).

MiRNAs associated with FL: Among the miRNAs related to FL are miR-17, miR-18a, miR19-a, miR-20a, miR-19b-1, and miR92a of the miR-17-92 cluster, and others are miR-155, miR-21, miR-155-5p, miR-150, miR-9-3p, miR-29 family, miR-21, miR-5008, miR-7e-5p, miR-451, miR-338-5p, miR-142, miR-376 cluster, and approximately 150 others (Table 1).

The miR-17-92 cluster is overexpressed in FLs and contains several miRNAs, including miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a. These are involved in cancer cell proliferation, cell cycle regulation, and apoptosis inhibition. The miRNA-17-92 cluster was first identified in malignant BCLs and has important roles in the immune system, heart and lung development, and oncogenic events[23]. Overexpression of the miR-17-92 cluster in MCL negatively regulates the expression of various BCR suppressor molecules, resulting in the overactivation of BCR signaling [24]. In a genetic analysis of 33 cases of small intestinal DLBCL and gastrointestinal MALT lymphomas, miRNA levels were reduced by more than two-thirds compared with those in normal tissues (13 species in the decreased group) and increased by more than 1.5-fold (15 species in the increased group), indicating the existence of miRNAs that were upregulated and downregulated[25], in association with FL development and tumor tissue growth. In patients with B-cell non-Hodgkin lymphoma (B-NHL), members of the miR-17-92 cluster are differentially expressed among the B-NHL subtypes, with the most prominent differences between FL and germinal center B-cell-like (GBC) subtypes[26]. MiR-18, miR-19a, and miR-92a were overexpressed in patients with B-NHL with better overall survival (OS), and high levels of miR-19a and miR-92a significantly reduced event-free survival (EFS). Meanwhile, miR-17-92 overexpression reduces the incubation period required for xenograft tumor visualization, and its knockout reduces tumor formation. MiR-17-92 expression in FL differs significantly from that in GBC, and miR-19a could have marked effects on OS and EFS in patients with B-NHL[26].

MiR-155 is a miRNA encoded by the *MIR155* host gene or *MIR155HG* in humans. MiR-155 is involved in various physiological and pathological processes[27,28]. *In vivo* regulation of miR-155 expression from external molecular sources might suppress malignant tumor growth[29] and viral infections[30] while encouraging the advancement of cardiovascular diseases[31]. *MIR155HG* was initially identified as a gene that is transcriptionally activated *via* promoter insertion into a retroviral integration site commonly found in BCLs, earlier known as the B-cell integration cluster. RNA polymerase II carries out the transcription of *MIR155HG*. The resultant RNA is approximately 1500 nucleotides long and is capped and polyadenylated; single-stranded miR-155—a 23-nucleotide segment—is retained in exon 3 and undergoes processing from the parent RNA molecule[32]. Sequence analysis of small RNA clone libraries compared with those from other organ systems shows that miR-155-5p is one of five miRNAs (miR-142, miR-144, miR-150, miR-155, and miR-223) specific to hematopoietic cells, including B-cells, T-cells, monocytes, and granulocytes[33].

MiR-155 is transferred from leukemic cells to healthy B-cells *via* gap junctions and promotes their transformation into tumor-like cells. In addition, immature B-cells deficient in miR-155 evade apoptosis due to elevated BCL2 protein levels. This protein is involved in B-cell malignancy and is regulated by miR-155. Lawrie *et al*[34] reported that miR-155, miR-210, miR-106A, miR-149, and miR-139 are differentially regulated in non-neoplastic lymph nodes and DLBCL/FL. Meanwhile, Roehle *et al*[35] reported that miR-155, miR-21, and miR-221 are more highly expressed in ABC- than GCB-

Table 1 MicroRNAs involved in follicular lymphoma development and proliferation

MicroRNA(s)	Role in FL development	Sequence (5' to 3')	Ref.
miR-17	Involved in cell proliferation and apoptosis	UAAAGUGCUUACAGUGCAGGUAG	[23-26]
miR-18a	Role in angiogenesis and tumorigenesis	UAAGGUGCAUCUAGUGCAGAUAG	[26]
miR-19a	Promotes cell survival	UGUGCAAAUCUAUGCAAAACUGA	[26]
miR-20a	Involved in cell cycle regulation	UAAAGUGCUCAUAGUGCAGGUAG	
miR-19b-1	Promotes cell survival	UGUGCAAAUCCAUGCAAAUCUGA	
miR-92a	Role in angiogenesis	UAUUGCACUUGUCCCGGCCUGU	[26]
miR-155	B-cell transformation in follicular lymphoma	UUAAUGCUAAUUGUGAUAGGGGU	[27-35]
miR-21	Overexpressed and promotes tumor progression	UAGCUUAUCAGACUGAUGUUGA	[35]
miR-155-5p	Role for diagnosis of FL	UUAAUGCUAAUCGUGAUAGGGGU	[33,36]
miR-150	Influences B-cell differentiation	UCUCACAUUGGUCUACAAUCU	[33,36,41]
miR-9-3p	Role for diagnosis of FL and in B-cell malignancies	UCUUUGGUUAUCUAGCUGUAUGA	[36-40]
miR-29 family	Epigenetic regulation and tumorigenesis	Varies by family member	[42]
miR-5008	Regulation of endogenous mRNA levels	Sequence is not available	[43]
miR-7e-5p	Downregulation by c-MYC over-expression arise poor prognosis of FL	UGAGGUAGGAGGUUGUAUAGUU	[44]
miR-451	Involved in cell cycle progression	AAACCGUUACCAUUACUGAGUU	[45]
miR-338-5p	Possible role in cellular proliferation	UAAUGACUGCACUGACCUUUGA	[45]
miR-142	Role in hematopoiesis and B-cell function	UGUAGUGUUUCCUACUUUAUGGA	[46]
miR-376 cluster	Involvement in cellular differentiation	Varies by cluster member	[42]

FL: Follicular lymphoma.

type cell lines. Notably, the expression levels of miR-155 and miR-21 were higher in non-malignant ABC cells than in GCB cells. Moreover, Arzuaga-Mendez *et al*[36] reported that miR-155 expression is a valuable biomarker for the diagnosis and differentiation of lymphoma subtypes, specifically miR-155-5p and miR-9-3p for the diagnosis of FL, and miR-150 and miR-17-92 cluster for the differential diagnosis of FL and DLBCL. Additionally, miR-9-3p is associated with tumorigenesis, functioning as an oncogene in papillary thyroid cancer[37,38] and a tumor suppressor in gastric cancer[38,39]. It also modulates breast cancer by influencing growth and drug resistance[38,40]. Lower levels of miR-150 and higher levels of its target, FOXP1, are associated with reduced OS, indicating that miR-150 might be an effective biomarker that can be quantified in formalin-fixed paraffin-embedded tissue. In addition, the MYC/miR-150/FOXP1 axis in malignant B-cells helps determine the aggressiveness and high-grade transformation of FL[41].

An array-based assay including 76 non-Hodgkin B-cell mixed lymphomas was performed to investigate the etiology of different BCLs using miRNAs. The miR-29 family and miR-17-92 cluster are regulated by *MYC* genes associated with MYC proteins in BCLs. A network analysis was used to demonstrate that a small set of differentially expressed miRNAs associated with MYC can either upregulate or downregulate a select group of hub proteins, including BCL2, CDK6, MYB, ZEB1, CTNNB1, BAX, and XBP1 in BCLs[42]. MiR-21 is associated with cancer progression and metastasis; it is also highly expressed in FLs and has been implicated in the regulation of cell proliferation and apoptosis.

Larrea *et al*[43] identified 21 genes with altered miRNA-binding sites in transformed FL (tFL), that is, FL that is transformed to have more aggressive tumor cells. More than 40% of these mutations are present only in patients with tFL. Mutations in BCL2 and EZH2 reduce the binding efficiency of miR-5008 and miR-5008, regulating endogenous mRNA levels[43].

Additionally, the downregulation of miR-7e-5p by c-MYC overexpression is associated with a poor prognosis in patients with FL[44]. Furthermore, a study reported several abnormally altered miRNAs in FL, specifically, 39 significantly downregulated and 27 significantly upregulated miRNAs. MiR-451 was the most downregulated (345-fold decrease), while miR-338-5p was the most upregulated (172-fold increase) compared with those in healthy donors[45].

Recurrent mutations in hsa-miR-142 and editing of the hsa-miR-376 cluster were observed in diffuse large B-cell and FLs. A direct physical interaction between miRNA and mRNA was observed using argonaute-2 photoactivated ribonucleoside-enhanced cross-linking and immunoprecipitation experiments. This integrated analysis revealed several regulatory pathways associated with lymphoma pathogenesis, including the Ras, PI3K-Akt, and MAPK signaling pathways affected by lymphoma mutations. The regulation of mRNAs *via* miRNAs is highly relevant to lymphoma pathogenesis[46]. The onset of malignant tumors, such as malignant lymphomas and other cancers, as well as the proliferation of tumor cells, is regulated quantitatively by miRNAs, not qualitatively. The intricate mechanisms regulating expression are balanced through elaborate and judicious positive and negative regulation.

MiRNAs exhibit duplicity among subgroups of malignant lymphomas. Many miRNAs regulating tumor cell growth overlap between DLBCL and FL. Aberrantly expressed miRNAs have been identified in patients with hematological malignancies, suggesting that they may be novel clinical diagnostic and prognostic biomarkers. Hershkovitz-Rokah *et al* [47] experimentally validated miRNA expression signatures in DLBCL and FL. The authors identified unique miRNA expression patterns for each disease, with some overlap and abnormal expression of miRNAs between them. Their analysis also led to the discovery of several relevant miRNA–mRNA pairs in each disease. Furthermore, potential regulatory pathways were highlighted *via* gene ontology analysis. Specific miRNAs (miR-15a, miR-16, miR-17, miR-106, miR-21, miR-155, and miR-34a-5p) were aberrantly expressed in DLBCL tumor tissues; their specific expression patterns proved useful for DLBCL diagnosis. The overexpression of these miRNAs influences FL pathogenesis. However, given the highly complex functions and interactions of miRNAs, various aspects remain to be elucidated. Understanding how these specific miRNAs are involved in the development and progression of FL is essential. In particular, genes and signaling pathways targeted by these miRNAs should be identified, as their regulation can alter cancer cell behavior[47].

MiRNAs are promising biomarkers for FL diagnosis and prognosis and are relatively stable in the blood and tissues. Hence, they can be measured using relatively simple experimental techniques. This could have potential applications for non-invasive diagnosis and prognosis of FL. Notably, the functions of individual miRNAs are complex and can each affect numerous genes and signaling pathways, requiring comprehensive and cumulative interpretation of research results to enable the development of effective treatments and new biomarkers.

Indeed, miRNAs represent potential new treatment targets for FL as they can be easily produced with simple 20-30 nucleotide sequences. Hence, if they can be administered at high concentrations to tumors *via* intravascular infusion, transarterial injection, or direct injection, miRNAs can elicit therapeutic effects by upregulating or downregulating relevant oncogenes.

GWAS

GWAS examine the association between genetic polymorphisms (SNPs) and specific diseases. In FL, GWAS have identified genetic variants associated with the risk of disease development. For example, Skibola *et al*[48] conducted a large GWAS comprising 4523 cases and 13344 controls of European descent and identified five non-human leukocyte antigen (HLA) FL susceptibility loci and mutations in the *HLA* gene associated with FL risk: 11q23.3 near CXCR5, 11q24.3 near ETS1, 3q28 at LPP, 18q21.33 near BCL2, and 8q24.21 near PVT1. In addition, four HLA-DRβ1 multichain amino acids and independent signals of HLA class II rs17203612 and HLA class I rs3130437 were associated with FL risk. Therefore, mutations at several common loci outside the HLA region significantly contribute to FL risk[48]. Indeed, mutations at specific gene loci other than HLA have been identified for each of the five subtypes of non-Hodgkin lymphoma, namely, DLBCL, FL, chronic lymphocytic leukemia (CLL), MZL, and primary central nervous system lymphoma (PCNSL)[45]. The WEE1 Locus is a "potential therapeutic marker"[49]. Moore *et al*[50] estimated the odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression for the subjects' height and the risk of developing the four non-Hodgkin lymphoma subtypes. A slight significant difference was observed in an increased risk of developing CLL (OR = 1.08, 95%CI: 1.00-1.17, $P = 0.049$), whereas no significant association was observed with DLBCL, FL, or MZL[50].

Epidemiological studies have shown that the risk of non-Hodgkin lymphoma, including FL, is higher in patients with autoimmune disease. This association has been analyzed in a GWAS[51,52]. No association was reported between B-cell-mediated autoimmune diseases and the risk of FL or MZL[51]. Moreover, there was no association between autoimmune diseases and the risk of developing non-Hodgkin lymphoma; however, non-Hodgkin lymphoma shared more genetic etiologies with autoimmune diseases ($P = 0.0041$) than solid tumors[52]. In particular, genes involved in apoptosis and telomere length are associated with autoimmune diseases and non-Hodgkin lymphoma[52]. Additionally, Choi *et al*[53] constructed a site-specific polygenic risk score (PRS) using GWAS and identified risk variants for nine tumor types, including FL. They also estimated the hazard ratio for each tumor type in relation to the PRS in 400807 people of European descent from the United Kingdom Biobank; significant capacity reactivity was observed, with a two-fold higher risk in FL[53].

An association between other tumor types and blood lipid levels was reported by Kleinstern *et al*[54]. The authors analyzed the association between each non-Hodgkin lymphoma subtype and lipid traits using GWAS. High-density lipoprotein (HDL) cholesterol positively correlated with FL, DLBCL, and MZL, whereas triglyceride (TG) negatively correlated with MZL. There was no strong correlation between the OR and *P* value. Meanwhile, Wang *et al*[55] analyzed 2686 cases of FL through GWAS using SNP2HLA; the risk of developing FL increased with an increasing number of homozygous HLA class II loci. This supports the role of HLA zygosity in non-Hodgkin lymphoma cases and indicates that different immune pathways could underlie the etiology of non-Hodgkin lymphoma[55].

The association between prognostic predictors of FL and various loci has also been evaluated using a GWAS[56]. In a multicenter meta-analysis of 586 patients diagnosed with FL in Denmark, Sweden, and the United States, loci strongly associated with lymphoma-specific mortality were observed at SNPs on 17q24 (rs10491178)[56]. In addition, two high-binding SNPs in interleukin-8 (rs4073) were associated with OS[56]. Conde *et al*[57] showed that multiple independent loci on the X chromosome could be involved in the etiology of FL. The Xq21.1 signal was also observed in DLBCL, indicating the sharing of a susceptibility locus with FL[57]. Multiple loci involved in the development of B-cell non-Hodgkin lymphoma, including FL, have been identified. For example, several loci, beyond the five loci reported by Waller *et al*[58], are associated with developing non-Hodgkin lymphoma; a GWAS identified approximately 150 Loci associated with non-Hodgkin lymphoma development[58]. However, these loci have been identified in fragments; therefore, several other important loci could remain unknown. Further GWAS studies and advances in analysis methods will lead to the discovery of more new loci associated with the development of FL, elucidation of the mechanisms underlying the interactions that lead to lymphoma development, development of new therapeutic agents targeting these loci, and the era of FL cure (Figure 2).

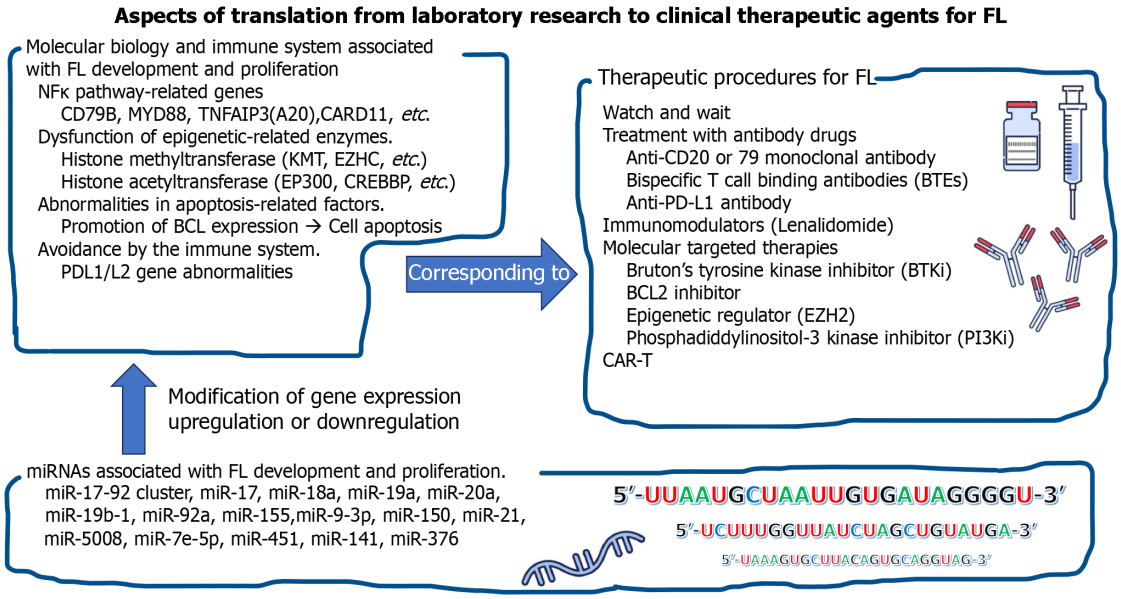

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Figure 2 Genetic mutations that cause follicular lymphoma development and microRNAs that regulate onset and tumor cell growth. Novel therapeutic agents have been developed to target FL-associated gene mutations involved in follicular lymphoma (FL) pathogenesis and proliferation. MicroRNAs are secreted from the FL cytoplasm as exosomes and internalized into recipient cells where they are transduced and regulate gene expression *via* upregulation or downregulation. FL: Follicular lymphoma; BCR: B-cell receptor; BTE(i): Bispecific T-cell binding antibodies (inhibitor); PD-L1(i): Programmed death ligand 1 antibody (inhibitor); miRNA: MicroRNA; mRNA: Messenger RNA; PI3K(i): Phosphatidylinositol-3 kinase (inhibitor); BTK(i): Bruton's tyrosine kinase (inhibitor); AKT: Protein kinase B; mTOR: Mechanistic target of rapamycin; EZH2(i): Enhancer of zeste homolog 2 (inhibitor).

Imaging studies

Imaging studies are used to assess the disease stage. Common imaging modalities include chest radiography, ultrasonography, computed tomography, and magnetic resonance imaging. These assess the lymph node and visceral enlargement, presence of metastases, and location and size of the lesion.

Additional tests

Additional tests could be required in advanced stages or under certain circumstances. Bone marrow biopsies and cerebrospinal fluid tests help confirm the extent of the disease and the sites involved. Several diagnostic specialists (pathologists, hematologists, and radiologists) are involved in the diagnosis process to increase accuracy. Once the diagnosis is confirmed, an appropriate treatment strategy (chemotherapy, radiotherapy, immunotherapy, or surgery) is selected based on the disease stage and general health of the patient.

CHOICE OF TREATMENTS

The following factors influence treatment selection for GI-FL[59,60]: (1) Lesion characteristics and progression: The lesions' location, size, and spread are assessed to determine the characteristics and progression of the disease. This provides a basis for determining disease risk and prognosis; (2) Establishing a pathological diagnosis: The pathological diagnosis of FL should be based on appropriate laboratory and histological evaluations. A pathological diagnosis forms the basis for choosing the most appropriate treatment[61]; and (3) Accurate staging assessment: Staging of the disease is based on accurate diagnosis and evaluation. The stage is important for assessing disease progression and developing a proper treatment plan[59]. Treatment decisions are determined by histological subtype and the extent of the disease[62], that is, stage (Lugano classification) (Table 2)[60]. Although histological grade is a good predictor of prognosis in nodal FL, this alone cannot determine the treatment. Stage, which indicates the extent and spread of the disease, determines the treatment; both stage and histological grade can be used concurrently. In primary GI-FL, personalized treatment choices are determined based on the individual circumstances of each case and the changes over time, in addition to the Lugano classification and histological grade[7].

For advanced stages (III and IV), irradiation is used if the disease is confined to a small number of areas that can be irradiated; this is augmented with chemotherapy with or without immunotherapy depending on the extent of organ involvement, spread, number of sites, and presence of distant metastases. In stages III and IV, if there are one, two, or three fewer lesions and irradiation is feasible in the area, radiation plus the extent of organ-specific invasion, spread, number of sites, and presence of distant metastases may be considered. Additionally, chemotherapy, chemotherapy plus immunotherapy (or in some cases a triple therapy), and, if appropriate, for example, if the patient has symptoms of obstruction of the digestive tract, where resection would improve symptoms and patient quality of life, surgical resection

Table 2 Lugano staging of gastrointestinal tract lymphoma[60]

Stage	
I	Tumor confined to GI tract
	Primary site or multiple, non-contiguous lesions
II	Tumor extends into the abdomen from primary GI site
	Nodal involvement
II_1	Local: Paragastric in cases of gastric lymphoma and para-intestinal for intestinal lymphoma
II_2	Distant: Mesenteric in case of an intestinal primary lymphoma; otherwise paraaortic, paracaval, pelvic, or inguinal
IIE	Penetration of serosa involving adjacent organs or tissues: Enumerate sites of involvement, *e.g.*, IIE (pancreas), IIE (large intestine), IIE (post-intestinal wall)
	Where there is nodal involvement and penetration involving adjacent organs, the stage is denoted using a subscript (1 or 2) and E, *e.g.*, II_1E (pancreas)
IV	Disseminated extra-nodal involvement or a GI tract lesion with supradiaphragmatic nodal involvement

GI: Gastrointestinal.

can be considered. Treatment should be customized to individual cases considering the specific requirements and constraints. In GI-FL, the characteristics of the gastrointestinal tract should be considered, and treatment measures are tailored to the disease status of each patient[7]. GI-FL treatment should be personalized based on the disease stage and patient's general health status. If necessary, the treatment plan should be reviewed regularly and modified based on disease progression, treatment efficacy, and patient response.

TREATMENT

"Watch-and-wait" approach

A watch-and-wait approach may be adopted in some patients to treat GI-FL. In this approach, patients diagnosed in the early stages of the disease are not immediately administered treatment but are regularly observed and monitored. When GI-FL is detected early, patients have a good prognosis. In these cases, a good 10-year prognosis is possible with surgical resection alone or with R-CHOP treatment (a combination of chemotherapy and targeted therapy). However, "watch-and-wait" is often the mainstay of treatment owing to the side effects and reduced patient quality of life. Schmatz *et al*[63] compared 63 patients with stage I GI-FL in treatment and "watch-and-wait" groups; there was no difference in progression-free survival (PFS) or OS. In another study, patients with GI-FL and low tumor volume were divided into a "watch-and-wait" group (15 patients) and a rituximab combined with chemotherapy group (14 patients); no difference in prognosis was observed[64]. Given the broad distribution of GI-FL at the time of diagnosis, localized treatment options are often deemed inappropriate. However, certain pathological features, including lower tumor extension and invasiveness than those in nodal FL, suggest that “watch-and-wait” is a viable option. This approach is promising in some subtypes of GI-FL with a favorable prognosis. Iwamuro *et al*[65] recently reported a long-term retrospective study for localized (stage I or II) GI-FL patients managed with a watch-and-wait strategy, revealing 5-year and 10-year event-free survival rates of 91.1% and 86.9%, respectively. Hence, this strategy is reasonable for GI-FL patients in early disease stages. However, the individual patient’s condition prior to treatment, the overall effect of possible treatments, and their side effects should be considered. The “watch-and-wait” approach should be explained to the patient as an option before choosing the treatment.

Radiotherapy

Radiotherapy can be used to treat localized lesions, particularly when the lesions are localized or when used in combination with other treatment modalities. Radiotherapy uses high-energy radiation to destroy tumor cells. However, several factors have led to radiotherapy not being effectively adopted for treating nodal or gastrointestinal FL[66,67].

Wound avoidance: Radiotherapy can affect the surrounding tissues, particularly in the gastrointestinal tract, an area prone to wounding; therefore, other treatment options could be chosen to avoid wounding. Wound avoidance can reduce treatment-related complications and improve quality of life.

Dose limitation: The gastrointestinal tract is an area where radiation doses must be limited as it is sensitive to radiation damage, which could be associated with an increased risk of complications and side effects. Therefore, elective treatments may be preferred.

Advances in chemotherapy: Following the introduction of drug combinations and target-directed therapies, chemotherapy has proven more effective and selective than radiotherapy.

Risk of side effects and complications: Radiotherapy risks severe side effects and complications in some patients. Particularly in treating the gastrointestinal tract, radiotherapy can significantly impact daily activities, such as food intake and bowel movements. To minimize these risks, other treatment options are preferred.

These factors tend to reduce the use of radiotherapy in favor of more elective treatments and a multifaceted approach centered on chemotherapy. However, the indications of radiotherapy and the balance of benefits and risks must be assessed for each case. Radiotherapy could be beneficial for certain conditions to prevent FL progression.

Treatment agents

Several novel FL agents are used primarily for treating nodal FL. This is the mainstay of treatment for advanced primary GI-FL, which is expected to show an increased incidence in the near future.

Antibodies: Monoclonal antibodies: Immunotherapy, particularly with rituximab and other anti-CD antibodies, is exceedingly effective in managing FL and constitutes a vital and superior component of modern treatment regimens. The success rate of rituximab monotherapy is 67% in untreated FL and 46% in patients with relapsed FL[68]. A German low-grade lymphoma study showed that rituximab plus CHOP was superior to CHOP alone in patients with advanced-stage FL and that OS was significantly improved[69]. In addition to rituximab, several other antibody-based agents have been developed, including tafatasitamab[70], polatuzumab vedotin[9], loncastiximab tecilin[71], maglorimab[72], and obinutumab[73]. Further, the combination of tafacitumab and lenalidomide[70] and polatuzumab vedotin[9] is effective. These therapies are promising for treating FL and other lymphomas; hence, clinical trials are ongoing. Additionally, biosimilars of rituximab have been approved, including CT-P10, which has shown efficacy and safety comparable to those of rituximab[74]. These developments are important for the treatment of FL and could contribute to reducing healthcare costs.

Bispecific T-cell-binding antibodies: Bispecific T-cell-binding antibodies (BTEs) are potent therapeutics that target specific tissues and cells by binding multiple antigens. They are commonly used to treat FL, with CD3 and CD20 being the primary targets. Mosnetuzumab and glofitumab are notable BTEs. Mosnetuzumab, a CD20 × CD3 bispecific antibody, demonstrated a high overall response rate (ORR) of 66% and a complete response rate (CRR) of 49% in a phase I study on r/r FL[75]. When combined with lenalidomide, an ORR of 92% and a CRR of 77% were achieved. Glofitamab, another BTE, showed positive results in a phase I study on r/r B-cell non-Hodgkin lymphoma, including painless lymphoma, with an ORR of 65.7% and CRR of 57.1%. Higher response rates were observed when the drug was combined with obinutuzumab[76]. The anti-CD3 and anti-CD20 BTE, epcoritamab, showed promising efficacy in phase I/II trials of r/r non-Hodgkin lymphoma and FL with a high ORR and CRR[77,78]. Similarly, odronestamab, a CD20 × CD3 bsAb, showed impressive results in phase I trials of r/r FL[79,80]. Overall, BTE is an effective immunotherapeutic strategy for FL. Further progress is expected from ongoing clinical trials.

Anti-PD-L1 antibodies: Anti-PD-L1 antibodies such as atezolizumab and pembrolizumab have shown promising efficacy in FL by enhancing T-cell function and antibody-dependent cellular cytotoxicity (ADCC) in NK cells. In a phase I study that combined atezolizumab and obinutumab, the ORR was found to be 54% (CRR: 23%) in patients with r/r FL and DLBCL, while an ORR of 17% (CRR: 4%) was observed for DLBCL alone. The PFS reached 9 mo for FL and 3 mo for DLBCL[81]. Another trial using pembrolizumab in combination with rituximab exhibited an ORR of 67% and a CRR of 50% in patients with r/r FL. The median PFS and 3-year OS rates were 12.6 mo and 97%, respectively. During the median follow-up duration of 35 mo, 23% of the patients maintained remission[82]. By enhancing T-cell function and NK cell ADCC, PD-1 blockade represents a fundamental mechanism of action that could further improve the therapeutic efficacy of anti-PD-1 antibody therapy in patients with FL.

Immunomodulatory drugs: Lenalidomide is an oral immunomodulatory drug used to treat FL and exhibits tumor-killing and immunomodulatory properties[83]. Clinical trials have shown that combining lenalidomide and rituximab (R2) has a higher ORR and a longer time to progression than lenalidomide alone[84]. The phase III AUGMENT trial showed a significant improvement in PFS with R2 compared with that with rituximab alone[85]. The phase IIIb MAGNIFY trial investigated the extension of R2 treatment and confirmed an ORR of 69% and a PFS of 40 mo[86]. The phase II GALEN trial tested a single arm of lenalidomide and obinutuzumab and reported an ORR of 95%, 2-year PFS of 65%, and OS of 87%[87]. Meanwhile, the phase III RELEVANCE trial showed no superiority of R2 over chemoimmunotherapy for frontline FL[88]. The phase II E2408 trial assessed the efficacy of three different approaches: Bendamustine/rituximab with bortezomib (BR) induction with R2 maintenance, BR induction with rituximab maintenance, and BR induction with maintenance using the proteasome inhibitor bortezomib and rituximab. The trial found that all three groups demonstrated similar and high CRRs[89]. Separately, a clinical trial utilizing lenalidomide and obinutuzumab, as the frontline therapy for patients with advanced untreated FL, reported encouraging results, with an ORR of 98%, CRR of 92%, and 2-year PFS of 96%[90]. Network meta-analyses of randomized controlled trials comparing treatment efficacy[91] were conducted to determine the optimal treatment regimens, sequences, and combination therapies. The evolution and diversification of therapeutics for FL have expanded the combinations of drugs with different mechanisms of action and treatment sequences; however, further clinical trials are needed to determine the most effective treatment regimen and sequence.

Molecular targeted therapies (small-molecule compounds): Bruton's tyrosine kinase inhibitors: Bruton's tyrosine kinase (BTK) plays a crucial role in regulating B-cell differentiation and activation in immune cells. For blood cancers like B-cell non-Hodgkin lymphoma and CLL, including FL, BTK inhibitors (BTKis) are currently under examination for their potential therapeutic benefits. First-generation ibrutinib, second-generation acalabrutinib and zanubrutinib, and third-generation piltobrutinib have demonstrated high effectiveness against B-cell non-Hodgkin lymphoma and CLL[92-96].

However, zanubrutinib monotherapy has limited efficacy in r/r FL[92]. Combination therapy with ibrutinib and rituximab resulted in better response rates in patients with r/r FL[95]. Acarabrutinib showed good efficacy and was well-tolerated by untreated and r/r FL patients[97]. In patients with r/r FL, the combination of zanubrutinib and obinutuzumab showed better PFS than obinutuzumab alone[98]. BTKis exhibited superior activity when used in combination therapies; hence, future studies should focus on evaluating various combinations of novel BTKis and other agents in patients with FL.

Proapoptotic pathway inhibitors (BCL2 inhibitors): Venetoclax, a potent BCL2 inhibitor, exhibits strong binding to BCL2, an anti-apoptotic protein often found in high levels in various blood cancers. By releasing apoptosis-promoting proteins, venetoclax triggers rapid and irreversible apoptosis in blood cancer cells. In a phase I trial of patients with FL, venetoclax monotherapy demonstrated an ORR of 38% and median PFS of 11 mo[99]. The phase II CONTRALTO trial compared different treatment groups, with the combination of venetoclax and rituximab presenting a CRR of 17%, venetoclax plus BR showing a CRR of 75%, and BR combined with venetoclax having a CRR of 69%[100]. Moreover, a phase I/II study reported promising results with the combination therapy of venetoclax and ibrutinib, showing an ORR of 69% and CRR of 25%[101]. BCL2 inhibitors have great potential for improving outcomes in patients with FL caused by BCL2 overexpression.

Epigenetic regulators: Drugs targeting the epigenetic regulation of gene expression, such as DNA methylation, are promising in treating blood cancers. Tazemetostat, an inhibitor of epigenetic regulator (EZH2) and histone methyltransferase, has shown positive outcomes. In a phase II study, tazemetostat treatment resulted in a higher ORR in patients with EZH2 mutations than in wild-type patients[19]. Patients with high-risk FL exhibited particularly favorable responses. The combination therapy of tazemetostat with rituximab and R2 is currently under investigation. Treatment with vorinostat, a histone deacetylase inhibitor, resulted in an ORR of 49% and median PFS of 20 mo in a phase II study of patients with r/r FL[102]. In a phase II study, vorinostat, in combination with rituximab, achieved an ORR of 50% and CRR of 41%[103]. Mocetinostat, another histone deacetylase inhibitor, showed limited efficacy, with an ORR of 12% in a phase II trial in patients with r/r FL[104].

EZH2 and histone deacetylase inhibitors hold promise as epigenetic regulators for the treatment of FL and are expected to yield improved therapeutic outcomes, particularly for tFL.

Phosphatidylinositol-3 kinase inhibitors: The BCR signaling pathway is homeostatically activated in B-cell tumors, leading to the development of inhibitors targeting this pathway. Phosphatidylinositol-3 kinase (PI3K) is a lipid kinase that mediates the phosphorylation of inositol ring 3 of inositol phospholipids—a membrane component[105]. Class I PI3Ks play an important role in signaling and are subdivided into four isoforms, α, β, ϒ, and δ[106]. Idelalisib, which selectively inhibits the δ isoform, is used in the treatment of r/r FL; it has shown the highest efficacy among PI3K inhibitors[107]. Nevertheless, most patients experience adverse events, and life-threatening adverse events limit the use of these agents[108,109]. Duvelisib, a dual inhibitor of PI3K-δ and PI3K-γ, has demonstrated impressive efficacy (70%); however, its use is also associated with a relatively high occurrence of adverse events (grade 3 or higher)[110,111]. It is the only PI3K inhibitor approved for treating r/r FL. Umbralisib is a selective PI3K-δ and casein kinase-1-ε (CK1ε) inhibitor and a fourth-generation PI3Ki with a potential therapeutic role in FL[112,113]. Parsaclisib is a potent PI3K isoform inhibitor that effectively treats r/r FL; however, it is also associated with frequent adverse events[114,115]. Zandelisib, a novel PI3-Kδ inhibitor, has shown good efficacy in the treatment of r/r indolent non-Hodgkin lymphoma with minimal adverse events[116]. However, clinical trials of these inhibitors are ongoing and require careful patient selection and implementation owing to the high frequency of adverse events.

PI3K/Akt/mTOR signaling pathway inhibitors: The PI3K/Akt/mTOR signaling pathway promotes abnormal regulation of tumor cell growth, metabolism, and survival[117]. Dual inhibitors aimed at this pathway have been developed and exhibit encouraging therapeutic effects, including high efficacy at minimal doses and low drug resistance [118]. Temsirolimus, a TOR inhibitor, when used with lenalidomide, showed synergistic effects in untreated advanced lymphomas, particularly in r/r classical Hodgkin lymphoma. However, hematological adverse events were frequent, with three grade 5 adverse events reported[119]. Further development of this novel PI3K/Akt/mTOR dual inhibitor and additional clinical trial data will enhance therapeutic outcomes and promote its use in FL treatment.

Cell-based therapies: CAR-T cell therapy is an innovative treatment for r/r hematological malignancies involving genetically engineered autologous T cells with chimeric antigen receptors (CARs) to target cancer cells. To date, CAR-T cell therapy has shown high efficacy; however, it is limited by hematological toxicity, including post-treatment cytoreduction[120,121]. In addition, several other challenges exist in the development of CAR-T therapies, including complex logistics, manufacturing constraints, toxicity concerns, and economic burdens[122]. Clinical trials have contrasted the effectiveness and safety of three cellular therapies: Autologous, allogeneic, and CAR-T cell therapies[123]. Recent clinical trials have evaluated autologous anti-CD19 CAR-T agents, such as axicabtagene ciloleucel (axi-cell), tisagenlecleucel (tisa-cell), and lisocabtagene maraleucel (liso-cell), all of which have shown remarkable efficacy against r/r DLBCL and FL. Various clinical trials have suggested that axi-cells can achieve an ORR of 94%, CRR of 79%, and sustained remission rate of 40%[124,125]. Tisa-cells have also shown substantial efficacy, achieving an ORR of 86% and CRR of 69% after a median follow-up of 17 mo[126-128]. Liso-cells, which are autologous anti-CD19 CAR-T cells, displayed high efficacy in r/r DLBCL where hematopoietic stem cell transplantation is not planned[129] and may be utilized in r/r FL in the future. The TRANSFORM and PILOT studies have also highlighted the potent efficacy of liso-cells in second-line treatment of r/r large BCL, leading to their approval as a third-line treatment option for aggressive BCL[130]. Despite these challenges, CAR-T-cell therapy could be developed as a fundamental treatment modality for FL.

Response-adapted post-induction strategy: The FOLL12 study compared standard rituximab maintenance therapy with experimental post-induction therapy for patients with FL. Experimental therapy ranged from observation of patients with

the complete metabolic response (CMR) and minimal residual disease (MRD) negativity to administering four doses of rituximab to patients with CMR and MRD positivity until MRD became negative, to one dose of ibritumomab tiuxetan for non-CMR patients, followed by three doses of the standard treatment with rituximab maintenance. With a median follow-up of 53 mo, patients in the standard treatment group had significantly better PFS than those in the experimental group (3-year PFS, 86% *vs* 72%; $P < 0.001$). However, for patients with FL who responded positively to induction therapy, the standard 2-year rituximab maintenance therapy extended PFS following the remission induction[131].

SUMMARY OF GI-FL TREATMENTS

Similar to that for nodal FL, the treatment strategy for GL-FL should be carefully considered based on Lugano stage and the histological grade. Furthermore, personalized treatment choices should be made based on the circumstances of individual patients and their changes over time[7]. The possibility of gastrointestinal perforation due to tumor reduction should always be considered, as this could be a lethal side effect. Moreover, the watch-and-wait strategy has been the mainstay option for GI-FL treatment. However, with the current advances in novel therapeutic agents, many advanced cases are increasingly being treated aggressively with chemotherapy, immunotherapy, or a combination of the two. Surgical resection and postoperative chemotherapy, immunotherapy, or a combination of these, could be an option in some instances, such as gastrointestinal obstruction[7]. Therefore, the therapeutic approach should be more individualized in GI-FL. Depending on the site and stage of the lesion and the patient's general health, a combination of surgery, chemotherapy, radiotherapy, and immunotherapy may be chosen. Conservative surgical or endoscopic treatment of the gastrointestinal tract may be an option depending on the tumor's specific anatomy and histological characteristics.

CONCLUSION

Molecular genetic analysis of FL has revealed overexpression of the *BCL2* gene, rearrangement of the *IGH* gene, and genetic mutations in NF-κB pathway-related factors. To this end, genetic abnormalities related to histone methyltransferases and histone acetylases have been characterized. Approximately 150 miRNAs involved in the development and proliferation of FL, including the miR-17-92 cluster, miR-155, miR-155-5p, miR-9-3p, miR-150, miR-155, and miR-5008, have been identified. Their role as prognostic predictors has been investigated. Recently, GWAS have identified several loci involved in FL development.

There has been significant progress in the development of novel FL therapeutics targeting the genes responsible for FL pathogenesis and tumor growth, including anti-CD20, CD79 monoclonal antibodies, BTEs, anti-PD-L1 antibodies, lenalidomide, and immunomodulators such as lenalidomide, BTKis, BCL2 inhibitors, EZH2 inhibitors, PI3K inhibitors, dual inhibitors of the PI3K/Akt/mTOR signaling pathway, and CAR-T cell therapy. The efficacy of these novel agents is demonstrated in numerous clinical trials, not only as single agents but also in combination. Recent research focuses on identifying the best combination and the order of treatments in multi-drug therapy. The number of advanced cases of primary GI-FL and nodal cases is expected to increase. Gastroenterologists must be trained and offered sufficient practice in treating these patients, especially those in advanced stages.

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Artificial intelligence system for the detection of Barrett's esophagus

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Abstract

BACKGROUND

Barrett's esophagus (BE), which has increased in prevalence worldwide, is a precursor for esophageal adenocarcinoma. Although there is a gap in the

detection rates between endoscopic BE and histological BE in current research, we trained our artificial intelligence (AI) system with images of endoscopic BE and tested the system with images of histological BE.

AIM
To assess whether an AI system can aid in the detection of BE in our setting.

METHODS
Endoscopic narrow-band imaging (NBI) was collected from Chung Shan Medical University Hospital and Changhua Christian Hospital, resulting in 724 cases, with 86 patients having pathological results. Three senior endoscopists, who were instructing physicians of the Digestive Endoscopy Society of Taiwan, independently annotated the images in the development set to determine whether each image was classified as an endoscopic BE. The test set consisted of 160 endoscopic images of 86 cases with histological results.

RESULTS
Six pre-trained models were compared, and EfficientNetV2B2 (accuracy [ACC]: 0.8) was selected as the backbone architecture for further evaluation due to better ACC results. In the final test, the AI system correctly identified 66 of 70 cases of BE and 85 of 90 cases without BE, resulting in an ACC of 94.37%.

CONCLUSION
Our AI system, which was trained by NBI of endoscopic BE, can adequately predict endoscopic images of histological BE. The ACC, sensitivity, and specificity are 94.37%, 94.29%, and 94.44%, respectively.

Key Words: Barrett's esophagus; Artificial intelligence system; Endoscopy; Narrow-band imaging; Gastroesophageal reflux disease

Core Tip: The prevalence of Barrett's esophagus (BE) diagnosed by endoscopy significantly differs from BE diagnosed by histology (7.8% *vs* 1.3%). Current research showed that image-enhanced endoscopy can only increase the detection ability for dysplasia lesions in BE. Our artificial intelligence prediction system, which was trained by endoscopic BE images with the Olympus narrow-band imaging system, still provided good prediction results for images of histological BE. The accuracy, sensitivity, and specificity are 94.37%, 94.29%, and 94.44%, respectively, in the final test, which indicates that endoscopic BE images have characteristics similar to images of histological BE.

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INTRODUCTION

Barrett's esophagus (BE), which is characterized by a columnar lined esophagus with documented intestinal metaplasia[1,2], is a precursor for esophageal adenocarcinoma (EAC)[3,4]. The prevalence of BE has been increasing in western countries[5,6] for decades, and this trend has also recently been observed in Asian countries[7]. The annual incidence of EAC is approximately 0.3% in non-dysplasia BE[8], while the annual incidence of EAC increases to 0.76% in low-grade dysplasia BE[9] and to 6% in high-grade dysplasia BE[10]. Although the importance of the detection of BE in patients is recognized by endoscopists worldwide, the prevalence of BE diagnosed by endoscopy significantly differs from BE diagnosed by histology[7] (7.8% *vs* 1.3%).

The methods of biopsy in Seattle protocol[11] have been under debate, as random four-quadrant biopsies at 2-cm intervals are time-consuming and intolerable under some situations. A recent study showed that targeted biopsy using image-enhanced endoscopy and molecular biomarkers can increase the detection ability for dysplasia lesions in BE[12]. The above issues indicate that there is a large degree of interobserver disagreement in the recognition and biopsy methods of BE. One previous study showed that the prevalence of histological BE is 2.6% in a health examination center data[13] but that the utilization of biopsy during endoscopy is far less in daily practice in Taiwan. We propose that an artificial intelligence (AI) system should be helpful for promoting awareness of BE in daily endoscopic practice.

AI systems are already applied in many fields of modern medicine, which started with diabetic retina observation[14,15], diabetes mellitus care[16], and the detection and classification of Alzheimer's disease[17] and later extended to the fields of gastroenterology and endoscopy. AI systems can help detect and differentiate the classification of colon polyps [18,19], gastric cancer[20], neoplastic and non-neoplastic small bowel lesions[21], pancreatic lesions[22,23], BE[24-26], and EAC[24,26,27]. Despite recent improvements in the AI prediction of BE and EAC, the accuracy (ACC) of such models has

Table 1 Baseline characteristics of development and test sets

NBI image number	Training set, *n* = 771		Label balance, *n* = 1187		Valid set, *n* = 193		Test set, *n* = 160	
	n	%	*n*	%	*n*	%	*n*	%
No Barrett's esophagus	563	73.02	563	47.43	141	73.06	90	56.25
Barrett's esophagus	208	26.98	624	52.57	52	26.94	70	43.75

NBI: Narrow-band imaging.

remained approximately 90%; one of the insurmountable problems is that there are insufficient images of histologically proven BE to train AI models. Our healthcare system faced the same dilemma, and we tried to conquer this problem by establishing a relationship between images of endoscopic BE and images of histological BE.

We trained our AI model with images of endoscopic BE and endoscopic images from patients without BE and then tested our AI prediction system using cases both with and without histologically proven BE. We also compared the outcomes of our prediction system with data from similar recent studies.

MATERIALS AND METHODS

Data acquisition and preparation

The endoscopic images of patients with typical gastroesophageal reflux symptoms were collected from Chung Shan Medical University Hospital (Taichung City, Taiwan) and Changhua Christian Hospital (Changhua County, Taiwan) retrospectively from January 1, 2020 to June 30, 2020, resulting in 724 cases, with 86 patients having complete histological results. The collection of clinical data was de-identification and sent for further image annotation. This study reviewed and approved by the institutional review board (IRB) on May 28, 2020 with IRB number CS1–20075 (the application of artificial intelligence in advanced endocopy) and conducted under IRB regulations to ensure the rights and welfare of the participants.

The images utilized in this study were obtained from the hospitals' endoscopy systems, which were operated by professional physicians. The narrow-band imaging (NBI) mode in the Olympus endoscopy system was selected for capturing images. We collected both histological BE images and other image data from patients with typical symptoms of gastroesophageal reflux disease (GERD) to establish a study dataset.

Data annotation

The development set of this study consisted of 964 images. Three senior endoscopists who were instructing physicians of the Digestive Endoscopy Society of Taiwan independently annotated the images in the development set to determine whether each image was classified as endoscopic BE. After a majority vote among the three senior endoscopists, the final classification was determined. The test set consisted of 160 images of 86 cases, and the categorization was based on the histological results.

Dataset

The development set consisted of 704 images without BE and 260 endoscopic BE images. To avoid overfitting, the image dataset was divided using the holdout method, with 80% of the data employed as the training set and 20% of the data employed as the validation set for model training and development. The test set, which consists of complete histological result images, was used to evaluate the predictive performance of the model. The test set included 90 images without BE and 70 histological BE images. These details are shown in Table 1.

Model design

This section describes the deep learning model architecture utilized in the study to accomplish the binary classification task for BE. First, the image size was resized to 224 pixel × 224 pixel for inputting into the model. To address the issue of class imbalance in the dataset, this study employed a resampling technique to augment the data from the minority class. Data class balancing was performed only on the training set. The original training dataset consisted of 771 images, of which 563 images did not have BE, and 208 images showed endoscopic BE. After data augmentation, the training dataset expanded to 1,187 images, of which 563 did not show BE and 624 images showed endoscopic BE. The effects of data augmentation are listed in the Supplementary Table 1.

In this study, a transfer learning technique was applied for model training. Six pretrained models were compared, including EfficientNetV2B1, EfficientNetV2B2, EfficientNetV2B3, DenseNet201, ResNet50, and VGG16. Ultimately, EfficientNetV2B2 was selected as the backbone architecture for further evaluation due to its better prediction ACC.

The deep neural network in this study employed the sigmoid function as the activation function in the output layer. An early stopping technique was utilized to monitor the maximization of validation ACC, which allowed the model to stop training at a suitable convergence point. After completion of the training, the best model weights were saved. To further enhance the model's generalization ability, the development set was repeatedly sampled to create multiple

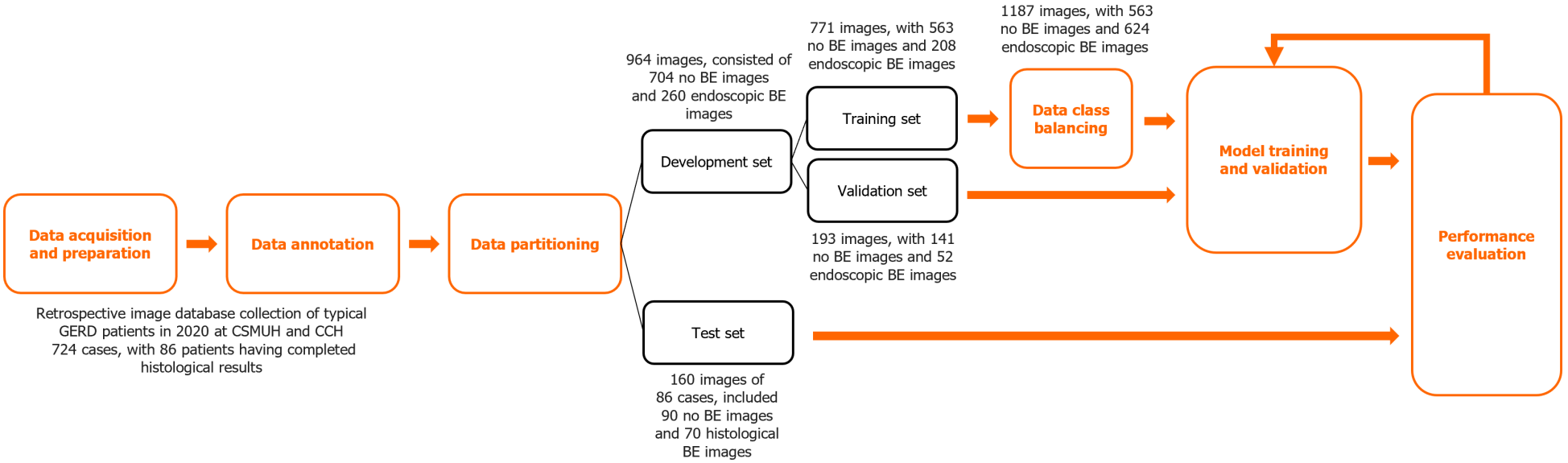

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Figure 1 Flow chart of study design and data acquisition. BE: Barrett's esophagus; GERD: Gastroesophageal reflux disease; CSMUH: Chung Shan Medical University Hospital; CCH: Changhua Christian Hospital.

training sets for model training and validation, with the aim of developing an optimal AI model. A flow chart of the study design and data acquisition is shown in Figure 1.

Classification performance evaluation

The evaluation metrics applied in this study include ACC, sensitivity (SEN), and specificity (SPE), which determine the classification performance of the model. ACC indicates the classification ACC of the model, SEN reveals the proportion of actual positive samples correctly identified as positive, and SPE indicates the proportion of actual negative samples correctly identified as negative.

RESULTS

We collected endoscopic images of the gastroesophageal junction in a total of 724 cases, with 86 patients having complete histological results. There were 771 original images in the training set, including 563 images that did not show BE and 208 images that showed endoscopic BE (26.98% of the total number of images). A total of 193 original images were included in the validation set, including 141 images without BE and 52 images with endoscopic BE (26.94% of the validation images). Due to the imbalanced distribution in these two categories and the limited number of endoscopic BE images, we augmented the BE images in the training set to obtain a total of 624 images of endoscopic BE, which provided 52.57% of

Table 2 Comparison of performance using different pretrained models

Pre-trained model	Training			Validation			Test		
	ACC	SEN	SPE	ACC	SEN	SPE	ACC	SEN	SPE
EfficientNetV2B1	0.9196	0.8702	0.9378	0.7876	0.6346	0.8440	0.7625	0.6857	0.8222
EfficientNetV2B2	0.9702	0.9663	0.9716	0.8497	0.7500	0.8865	0.8500	0.8286	0.8667
EfficientNetV2B3	0.9170	0.8798	0.9307	0.7824	0.6154	0.8440	0.8125	0.7429	0.8667
ResNet50	0.8962	0.7885	0.9361	0.8290	0.5577	0.9291	0.7063	0.5429	0.8333
DenseNet201	0.8755	0.8173	0.8970	0.7772	0.5577	0.8582	0.7312	0.6143	0.8222
VGG16	0.2698	1.0000	0.0000	0.2694	1.0000	0.0000	0.4375	1.0000	0.0000

ACC: Accuracy; SEN: Sensitivity; SPE: Specificity.

Table 3 Confusion matrices

Confusion matrices		Predicted class					
		Training, *n* = 771		Validation, *n* = 193		Test, *n* = 160	
		BE	No BE	BE	No BE	BE	No BE
Actual class	BE	206	2	50	2	66	4
	No BE	2	561	3	138	5	85

BE: Barrett's esophagus.

the images in the training set. These baseline characteristics are shown in Table 1.

Comparisons of different pre-trained models

We compared the prediction outcomes in different pre-trained models, which included EfficientNetV2B1, EfficientNetV2B2, EfficientNetV2B3, ResNet50, DenseNet201, and VGG16. ACC was comparable in the training process of most of the pre-trained models, with the exception of VGG16, which had an ACC of only 26.98%. The same trends were observed in the validation set, with an ACC of 78.76%, 84.97%, 78.24%, 82.90%, 77.72%, and 26.94% for EfficientNetV2B1, EfficientNetV2B2, EfficientNetV2B3, ResNet50, DenseNet201, and VGG16, respectively. For final testing, EfficientNetV2B2 achieved the best ACC of 85%, which was superior to the other pre-trained models; hence, we chose EfficientNetV2B2 as the training model for our BE prediction system. Detailed data on SEN, SPE, and ACC for the pre-trained model comparisons are shown in Table 2.

Results of AI image-based model performance

For the training set, two images were incorrectly classified as having no BE among 208 images of endoscopic BE. For images that showed no BE, two images were incorrectly classified as having BE, while the other 561 images were correctly classified as having BE. Overall, ACC, SEN, and SPE were 99.48%, 99.04%, and 99.64%, respectively.

For the validation set, two images were incorrectly classified as having no BE among 52 images of endoscopic BE, while three images were incorrectly classified as having BE among 141 non-BE images. Overall, ACC, SEN, and SPE were 97.41%, 96.15%, and 97.87%, respectively.

For the test set, four images were incorrectly classified as having no BE among 70 images of histological BE, and five images were incorrectly classified as having BE among 90 non-BE images (as proven by histology) in the final test. The AI BE prediction system had an ACC of 94.37%, a SEN of 94.29%, and a SPE of 94.44%. Detailed information is provided in Table 3 and Figure 2.

Representative images of BE with successful detection (panel A), no BE with successful detection (panel B), BE with false detection (panel C), and no BE with false detection (panel D) are demonstrated in Figure 3.

DISCUSSION

We used only NBI images from the Olympus endoscopy system in our study instead of white light endoscopy images because our previous data showed the superiority of NBI images over white light endoscopy images for the differentiation of GERD classification[28]. However, some studies have shown that NBI images do not display differences in detecting BE esophagus compared to white light endoscopy images[29,30], while another recent convincing study

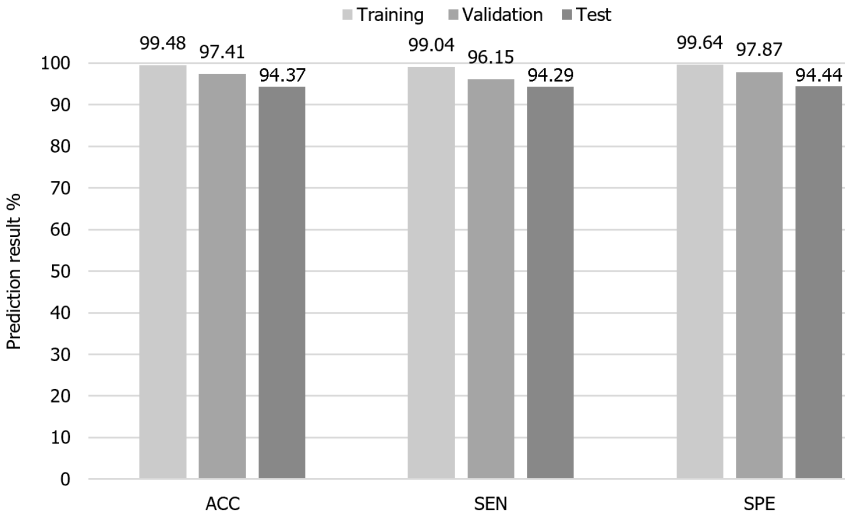

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Figure 2 Artificial intelligence image-based Barrett's esophagus prediction model performance. ACC: Accuracy; SEN: Sensitivity; SPE: Specificity.

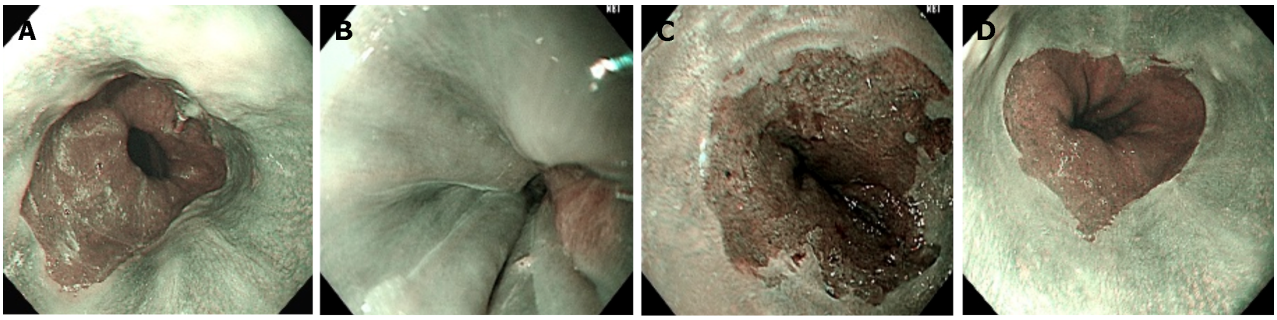

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Figure 3 Narrow-band images of the artificial intelligence prediction system. A: Barrett's esophagus with successful detection; B: Normal esophagus with successful detection; C: Barrett's esophagus with false detection; D: Normal esophagus with false detection.

showed benefits in neoplasia detection with an NBI system[12]. The concept that targeted biopsies using NBI enable the detection of more dysplastic areas and thus reduce the number of biopsies required is widely accepted by the Japan Esophageal Society[12,31]. As a result, we exclusively utilized NBI still images in our AI prediction system training.

Our study is unique in that training of the pretrained model and selected AI prediction model was performed with images of endoscopic BE rather than images of histological BE. The AI prediction system was still able to maintain a high ACC in the final prediction test that used images of histological BE. This finding indicates that the images of endoscopic BE have characteristics similar to those of histological BE in our study. The final prediction results using the EfficientNetV2B2 training model showed that SEN, SPE, and ACC were 94.29%, 94.44%, and 94.37%, respectively. Previous data have highlighted that ACC is currently unsatisfied in AI systems of BE endoscopic interpretation[32], but the AI prediction of early cancer or dysplasia were impressive. Our database employed larger real-case numbers and more original images than previous studies and showed improved SEN, SPE, and ACC. We believe that the data augmentation technique limited the ACC in the final test results due to the large number of training images with similar detailed characteristics.

The most similar recent studies focused on BE neoplasia detection. Ebigbo *et al*[33] demonstrated an ACC of 89.9% using only 14 test patients in 2020, while Hussein *et al*[26] improved the ACC to 93% with 118 patients in 2022. Another study by Abdelrahim *et al*[25] in 2023, which utilized real-time video for the identification of BE dysplasia, showed a similar ACC of 92% with 75 cases.

Our study selected 771 original images from 579 patients as the training set, 193 original images from 145 patients as the validation set, and 160 images from 86 patients as the test set; the final ACC was 94.37%. Detailed information on these results is provided in Table 4. We focused on building an AI reminder system that helps endoscopists decide whether to perform a biopsy when BE is suspected instead of a system that reminds endoscopists which BE lesions may already have dysplastic changes.

Table 4 A comparative summary of the state-of-the-art approaches for the binary classification of Barrett's esophagus

Ref.	Validation (train:valid:test)	Valid	Test
Ebigbo *et al*[33], 2020	Holdout; Image (129:N/A:62); Test patients:14	ACC: N/A	ACC: 89.9%
		AUC: N/A	AUC: N/A
		SEN: N/A	SEN: 83.7%
		SPE: N/A	SPE: 100.0%
		PPV: N/A	PPV: N/A
		NPV: N/A	NPV: N/A
Hussein *et al*[26], 2022	Holdout; Video (64:11:44); Patients: 118	ACC: N/A	ACC: N/A
		AUC: N/A	AUC: 93%
		SEN: N/A	SEN: 91%
		SPE: N/A	SPE: 79%
		PPV: N/A	PPV: N/A
		NPV: N/A	NPV: N/A
Abdelrahim *et al*[25], 2023	Holdout; Image (816:471:N/A); Video (161:N/A:75); Case (161:34:75)	ACC: 94.7%	ACC: 92.0%
		AUC: 0.898	AUC: 0.964
		SEN: 95.3%	SEN: 93.8%
		SPE: 94.5%	SPE: 90.7%
		PPV: 83.6%	PPV: 88.2%
		NPV: 98.6%	NPV: 95.1%
Our approach, 2023	Holdout image; Image (771:193:160); Case (579:145:86)	ACC: 97.41%	ACC: 94.37%
		AUC: 97.01%	AUC: 94.37%
		SEN: 96.15%	SEN: 94.29%
		SPE: 97.87%	SPE: 94.44%
		PPV: 94.00%	PPV: 92.96%
		NPV: 98.57%	NPV: 95.51%

ACC: Accuracy; AUC: Area under the curve; N/A: Not applicable; NPV: Negative predictive value; PPV: Positive predictive value; SEN: Sensitivity; SPE: Specificity.

Limitations

Due to the lack of awareness regarding the diagnosis of BE, cases of histological BE are relatively rare in central Taiwan. First, we initiated this study and collected endoscopic images of the lower esophagus and esophagogastric junction in two medical centers in central Taiwan. The endoscopic BE dataset was developed using the Delphi method, which involved blind voting on endoscopic images by three endoscopists, instead of histological BE images, which can lead to selection bias due to the previously known difference in prevalence between endoscopic BE and histologic BE[7] (7.8% *vs.* 1.3%). Second, the method of biopsy was not recorded in all cases, which meant that some of the cases involved random biopsies, while other cases were NBI-targeted biopsies. Third, the images in our database were obtained from an Olympus endoscopic system with lower esophageal still NBI pictures. It is not known whether our system can adequately perform for white light or other endoscopic systems. The main limitations were attributed to the limited cases and limited original images in our study and the limited number of cases of histological BE. However, our AI prediction system, which was trained by endoscopic BE images, still provided good prediction results for images of histological BE.

CONCLUSION

Our AI prediction system can provide good prediction results for images of histological BE obtained with Olympus NBI after training with images of endoscopic BE.

ARTICLE HIGHLIGHTS

Research background

The prevalence of endoscopic Barrett's esophagus (BE) differs significantly from histological BE. We believe the endoscopic characteristics are similar with endoscopic BE and histological BE.

Research motivation

We want to train an artificial intelligence (AI) system to identified images of BE under endoscopic environments.

Research objectives

To construct an AI system for the detection of endoscopic images of histological BE.

Research methods

Endoscopic narrow-band images of 724 cases, were collected from two medical centers at central Taiwan, with 86 patients having pathological results. Images of endoscopic BE was classified using independent annotation by three senior endoscopists, who were instructing physicians of the Digestive Endoscopy Society of Taiwan. The test set consisted of 160 endoscopic images in 86 histological BE cases.

Research results

EfficientNetV2B2 [accuracy (ACC): 0.85] was selected as the backbone architecture from six training model due to better ACC result. In the final test, the AI system obtained 94.37%, 94.29%, and 94.44%, in ACC, sensitivity, and specificity respectively.

Research conclusions

Our AI prediction system can provide good prediction results after training with images of endoscopic BE.

Research perspectives

Our result implies that images of endoscopic BE share similar characteristics with images of histological BE even in the perspectives of AI system. The gap from endoscopic BE to histological BE maybe comes from biopsy or sampling bias. This opinion needs further prospective studies to confirm. Meanwhile, a better AI prediction system for endoscopic video BE detection is an ongoing task in the near future.

FOOTNOTES

Co-corresponding authors: Chi-Chih Wang and Ming-Hseng Tseng.

Author contributions: Tsai MC and Wang CC were responsible for the conception and design of the study; Yen HH, Tsai HY, Huang YK, Luo YS, Sung WW, and Tseng MH were responsible for the acquisition, analysis, or interpretation of data; Tsai MC and Wang CC were responsible for drafting the manuscript; Edy Kornelius and Lin CC were responsible for critically revising the manuscript for important intellectual content; Tsai HY and Tseng MH were responsible for the statistical analyses; Tseng MH and Sung WW were responsible for obtaining the funding; Tseng MH and Wang CC were responsible for supervising the study; Wang CC and Tseng MH contributed equally to this work as co-corresponding authors. The reasons for designating Wang CC and Tseng MH as co-corresponding authors are as follows. The research was performed as a collaborative effort, and the designation of co-corresponding authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. This also ensures effective communication and management of post-submission matters, ultimately enhancing the paper's quality and reliability. The overall research team encompassed authors with a variety of expertise and this also promotes the most comprehensive and in-depth examination of the research topic, ultimately enriching readers' understanding by offering various expert perspectives. Wang CC contributed to the study design, endoscopic image collection, and endoscopic image interpretation while Tseng MH constructed the AI model. The choice of these researchers as co-corresponding authors acknowledges and respects this equal contribution, while recognizing the spirit of teamwork and collaboration of this study. In summary, we believe that designating Wang CC and Tseng MH as co-corresponding authors of is fitting for our manuscript as it accurately reflects our team's collaborative spirit, equal contributions, and diversity.

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ORIGINAL ARTICLE

Observational Study

Clinical value of the Toronto inflammatory bowel disease global endoscopic reporting score in ulcerative colitis

Xin-Yue Liu, Zi-Bin Tian, Li-Jun Zhang, Ai-Ling Liu, Xiao-Fei Zhang, Jun Wu, Xue-Li Ding

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Abstract

BACKGROUND

Endoscopic evaluation in diagnosing and managing ulcerative colitis (UC) is becoming increasingly important. Several endoscopic scoring systems have been established, including the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score and Mayo Endoscopic Subscore (MES). Furthermore, the Toronto Inflammatory Bowel Disease Global Endoscopic Reporting (TIGER) score for UC has recently been proposed; however, its clinical value remains unclear.

AIM

To investigate the clinical value of the TIGER score in UC by comparing it with the UCEIS score and MES.

METHODS

This retrospective study included 166 patients with UC who underwent total colonoscopy between January 2017 and March 2023 at the Affiliated Hospital of Qingdao University (Qingdao, China). We retrospectively analysed endoscopic scores, laboratory and clinical data, treatment, and readmissions within 1 year. Spearman's rank correlation coefficient, receiver operating characteristic curve, and univariate and multivariable logistic regression analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, United States) and GraphPad Prism version 9.0.0 for Windows (GraphPad Software, Boston, Massachusetts, United States).

RESULTS

The TIGER score significantly correlated with the UCEIS score and MES (r = 0.721, 0.626, both P < 0.001), showed good differentiating values for clinical severity among mild, moderate, and severe UC [8 (4–112.75) *vs* 210 (109–219) *vs* 328 (219–426), all P < 0.001], and exhibited predictive value in diagnosing patients with severe UC [area under the curve (AUC) = 0.897, P < 0.001]. Additionally, the TIGER (r = 0.639, 0,551, 0.488, 0.376, all P < 0.001) and UCEIS scores (r = 0.622, 0,540, 0.494, and 0.375, all P < 0.001) showed stronger correlations with laboratory and clinical parameters, including C-reactive protein, erythrocyte sedimentation rate, length of hospitalisation, and hospitalisation costs, than MES (r = 0.509, 0,351, 0.339, and 0.270, all P < 0.001). The TIGER score showed the best predictability for patients' recent advanced treatment, including systemic corticosteroids, biologics, or immunomodulators (AUC = 0.848, P < 0.001) and 1-year readmission (AUC = 0.700, P < 0.001) compared with the UCEIS score (AUC = 0.762, P < 0.001; 0.627, P < 0.05) and MES (AUC = 0.684, P < 0.001; 0.578, P = 0.132). Furthermore, a TIGER score of ≥ 317 was identified as an independent risk factor for advanced UC treatment (P = 0.011).

CONCLUSION

The TIGER score may be superior to the UCIES score and MES in improving the accuracy of clinical disease severity assessment, guiding therapeutic decision-making, and predicting short-term prognosis.

Key Words: Ulcerative colitis; Toronto Inflammatory Bowel Disease Global Endoscopic Reporting score; Ulcerative Colitis Endoscopic Index of Severity; Mayo Endoscopic Subscore; Endoscopy; Severity

Core Tip: The manuscript introduces the clinical value of the Toronto Inflammatory Bowel Disease Global Endoscopic Reporting (TIGER) score for ulcerative colitis (UC). Our study, for the first time, validated that the TIGER score accurately reflects disease activity and is significantly correlated with laboratory parameters in patients with UC. We also defined TIGER score thresholds for upgraded treatment and 1-year readmission, providing treatment strategies and personalised disease management for patients with UC.

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INTRODUCTION

Ulcerative colitis (UC) is a multifactorial disease that is characterised by continuous mucosal inflammation of the colon and rectum with an increasing incidence, resulting in a high-cost burden worldwide[1,2]. Endoscopy is the principal technique for visualising lesions in the intestinal mucosa and is regarded as the gold standard for evaluating mucosal inflammation[3]. It can reflect endoscopic disease activity and plays a vital role in assessing therapeutic effects, colorectal cancer surveillance, and UC management[4-6]. These data indicate that the endoscopic score, as a concretisation of endoscopic evaluation, may be a prognostic indicator in UC[7,8]. Several endoscopic scoring systems have been developed, including the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score and Mayo Endoscopic Subscore (MES), which are commonly used in clinics and trials[9,10]. The UCEIS score, proposed by Travis *et al*[9] in 2012, ranges between 0 and 8, with higher scores indicating increased endoscopic severity, whereas the MES, created by Schroeder *et al* [10] in 1987, categorises severity into a 4-point scoring grade where patients with normal or inactive, mild, moderate, or severe disease are given scores of 0, 1, 2, or 3, respectively. Recently, the extent of mucosal inflammation has been emphasised, and the controversy on the accuracy of the UCEIS score and MES has arisen since both tools provide final scores aimed only at the most severely inflamed segment without highlighting the number of segments exhibiting moderate-to-severe inflammation[11,12]. Endoscopic scores focusing only on the severity during the medical treatment may be flawed because of the presence of segmental remission[13,14]. Therefore, attempts have been made in the past 10 years to assess disease extent and score the entire colonic mucosa[15,16]. In 2022, Zittan *et al*[17] proposed a reliable and useful endoscopic score, the Toronto Inflammatory Bowel Disease Global Endoscopic Reporting (TIGER) score. The TIGER score, constructed for both UC and Crohn's disease, can accurately and comprehensively evaluate the disease severity in all colonic segments and optimise treatment strategies in patients with UC[17].

However, whether the TIGER score has better clinical value than the UCEIS score and MES remains unclear. Therefore, this study aimed to analyse the clinical value of the TIGER score in UC by comparing its relationship with disease severity, predictive potential of treatment options, and prognosis with those of the UCEIS score and MES.

MATERIALS AND METHODS

Study design and patients

This retrospective study included 166 patients aged 18–75 years with a confirmed diagnosis of UC who were initially admitted to the Affiliated Hospital of Qingdao University (Qingdao, China) between January 2017 and March 2023. The following were the exclusion criteria: (1) Patients who did not undergo a colonoscopy, those with inadequate bowel preparation, or those with difficulty in undergoing a full colonoscopy, including the terminal ileum; and (2) presence of comorbidities, such as gastrointestinal neoplasia, infectious bowel disease, previous colorectal surgery, severe cardiac or pulmonary disease, and haemopathy. Figure 1 presents the selection process of the patients.

This study was conducted in accordance with the principle of the Declaration of Helsinki (World Medical Association, 2013) and was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (Approval number: QYFY WZLL 28085). Additionally, the Ethics Committee of the Affiliated Hospital of Qingdao University waived the requirement for written informed consent due to this study's retrospective design.

Clinical and laboratory parameters

Demographic and clinical data, including age, sex, body mass index (BMI), symptoms (abdominal pain, diarrhoea, bloody stool, and fever), disease duration, medical history, assessment of disease severity, treatment programs, length of hospitalisation, hospitalisation costs, and baseline endoscopy results, were collected. Laboratory data, including C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, neutrophil (NE) count, lymphocyte (LYM) count, platelet (PLT) count, haemoglobin (Hb) level, albumin (Alb) level, urea nitrogen (BUN) level, uric acid level, and creatinine (Cr) level, were also collected. Based on the Montreal classification system, the extent of UC was classified into three types as follows: Proctitis, left-sided colitis, and extensive colitis defined as E1, E2, and E3, respectively[18]. Furthermore, disease severity in patients with UC was assessed using the Truelove and Witts Severity Index[19].

Endoscopic scores

Colonoscopy images were read independently by two experienced gastroenterologists (Ding XL and Liu AL) who were blinded to the clinical information of the patients, and the TIGER score, UCEIS score, and MES were calculated. When the results were inconsistent, a third senior physician (Tian ZB) confirmed the diagnosis and made the final decision. The TIGER score of each segment was evaluated by summing the following five items: General mucosal appearance, ulcer/erosion size, percentage of ulcer/erosion surface area, percentage of affected surface area per segment, and degree of narrowing[17]. Segments with a TIGER score of ≥ 5, which represent moderate-to-severe endoscopic disease activity, received an additional 100 points[17,20]. The total TIGER score was the sum of the five segmental scores (terminal ileum, ascending, transverse, descending colon, and rectum), and the first digit implied the number of moderate-to-severe endoscopic active segments[17]. The UCEIS score was determined using the following three descriptors with a total score ranging from 0 to 8: Erosion and ulcers, bleeding, and vascular pattern[9]. Furthermore, the MES was scored between 0 and 3 according to the following parameters: Erythema, vascular pattern, bleeding, friability, and ulcerations[10].

Statistical analysis

Statistical analyses and the construction of charts were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, United States) and GraphPad Prism version 9.0.0 for Windows (GraphPad Software, Boston, Massachusetts, United States), respectively. Quantitative variables with a normal distribution were presented as mean (± SD), while those with a non-normal distribution were presented as median [interquartile range (IQR)]. Qualitative variables were presented as numbers (percentages). The correlations between the TIGER score and the UCEIS score, MES, and laboratory and clinical parameters were tested using Spearman's rank correlation coefficient (r). For continuous variables, the two-sample t-tests or Mann–Whitney U test was performed to compare two groups, and the ANOVA or Kruskal–Wallis H test was used to compare multiple groups, as appropriate. For categorical variables, the chi-square test was used for the univariate analysis. Multivariable logistic regression analysis was used to identify independent risk factors. Furthermore, we used the area under the curve (AUC) of the receiver operating characteristic (ROC) curve to compare the discrimination abilities across different scoring systems. Two-sided hypothesis tests were considered, and statistical significance was set at $P < 0.05$.

RESULTS

Baseline characteristics of patients with UC

Overall, 166 patients were selected in this study. Notably, 98 (59%) males and 68 (41%) females were included, with a mean age of 42.29 ± 12.05 years. The patients with UC were categorised into mildly, moderately, and severely active groups comprising 44 (26.5%), 51 (30.7%), and 71 (42.8%) patients, respectively, according to Truelove and Witts Severity Index[19]. The median TIGER score, UCEIS score, and MES were 214, 5, and 3, respectively. Furthermore, 161 (97.0%), 40 (24.1%),18 (10.8%), 3 (1.8%), 2 (1.2%), 2 (1.2%), and 1 (0.6%) patients received 5-aminosalicylates (5-ASAs) orally or rectally, used systemic corticosteroids, were treated with biologics (including infliximab or vedolizumab), used immunomodulators, received tofacitinib, used thalidomide, and underwent colorectal surgery, respectively (Table 1).

Table 1 Patient demographic and clinical characteristics

Characteristics	Values (*n* = 166)
Gender, *n* (%)	
Male	98 (59.0)
Female	68 (41.0)
Age (yr)	42.29 ± 12.05
BMI (kg/m^2)	22.49 ± 3.47
Duration (mo)	36.0 (12.0, 96.0)
Montreal classification, *n* (%)	
E1 = proctitis	21 (12.7)
E2 = left-sided colitis	47 (28.3)
E3 = extensive colitis	98 (59.0)
Truelove and Witts Severity Index, *n* (%)	
Mild	44 (26.5)
Moderate	51 (30.7)
Severe	71 (42.8)
Total TIGER score	214 (109, 324)
UCEIS score	5 (3, 6)
MES	3 (2, 3)
Treatment, *n* (%)	
5-ASAs	161 (97.0)
Systemic corticosteroids	40 (24.1)
IFX	8 (4.8)
VDZ	10 (6)
Immunomodulators	3 (1.8)
Tofacitinib	2 (1.2)
Thalidomide	2 (1.2)
Colectomy	1 (0.6)
Length of hospitalization (d)	7.00 (4.75-10.00)
Hospitalisation costs (CNY)	9088.11 (6788.40-12500.72)
Readmission within 1 yr, *n* (%)	48 (33.8)
CRP (mg/L)	3.58 (1.44-9.96)
ESR (mm/60 min)	10 (6-18)
WBC (× 10^9/L)	6.95 (5.53-8.62)
NE (× 10^9/L)	4.29 (3.03-5.59)
LYM (× 10^9/L)	1.81 (1.45-2.27)
PLT (× 10^9/L)	273.50 (216.75-365.50)
Hb (g/L)	127.00 (104.75-143.00)
Alb (g/L)	42.65 (37.50-54.10)
BUN (mmol/L)	3.96 (3.01-5.02)
UA (μmol/L)	296.39 ± 103.49
Cr (μmol/L)	57.50 (49.00-69.00)

BMI: Body mass index; TIGER: Toronto Inflammatory Bowel Disease Global Endoscopic Reporting; UCEIS: Ulcerative Colitis Endoscopic Index of Severity; MES: Mayo Endoscopic Subscore; 5-ASAs: 5-Aminosalicylates; IFX: Infliximab; VDZ: Vedolizumab; CRP: C-reactive protein; CNY: Chinese Yuan; ESR: Erythrocyte sedimentation rate; WBC: White blood cell; NE: Neutrophil count; LYM: Lymphocyte count; PLT: Platelet count; Hb: Haemoglobin; Alb: Albumin; BUN: Urea nitrogen; UA: Uric acid; Cr: Creatinine.

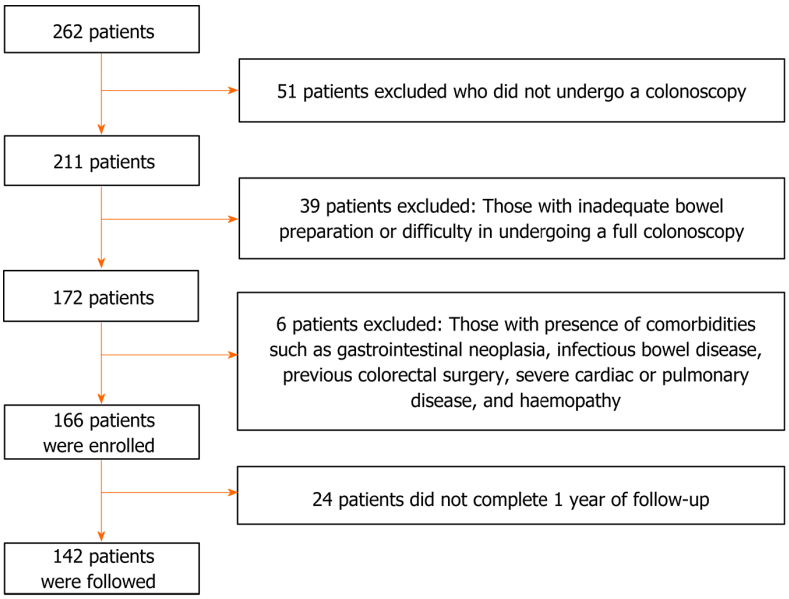

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Figure 1 Selection process of the patients.

Correlations among the TIGER score, UCEIS score, and MES

The TIGER score was strongly correlated with the UCEIS score (r = 0.721, P < 0.001) and moderately correlated with MES (r = 0.626, P < 0.001). Additionally, a moderate correlation was observed between the UCEIS score and MES (r = 0.681, P < 0.001).

Comparison of the endoscopic scores in different clinical severities

Using the Truelove and Witts Severity Index[19], the performance of the three endoscopic scoring systems in patients with different clinical severities was evaluated. Significant differences were observed in the three endoscopic scoring systems among patients with different disease severities (all P < 0.001). Comparison within each group revealed that both the TIGER and UCEIS scores showed significant distinctions from each other [median (IQR): 8 (4–112.75) *vs* 210 (109–219) *vs* 328 (219–426), all P < 0.001; 3 (2–4) *vs* 4 (4–5) *vs* 6 (5–6), all P < 0.001], although no significant difference was found in the MES between moderate and severe disease severities (P > 0.05) (Figure 2).

Comparison of the diagnostic value of the endoscopic scores for patients with severe UC

To evaluate the diagnostic performance of the three endoscopic scoring systems for severe UC, assessed using the Truelove and Witts Severity Index[19], the patients were further categorised into mild-to-moderate (n = 95) and severe (n = 71) groups, and the diagnostic value was analysed using the ROC curve. The results revealed that the TIGER score exhibited superior diagnostic performance, with an AUC, sensitivity, specificity, and cut-off value of 0.897, 80.3%, 83.2%, and 217.5, respectively (P < 0.001), demonstrating a good diagnostic capability for severe UC. The UCEIS score ranked second, with an AUC, sensitivity, specificity, and cut-off value of 0.839, 81.7%, 72.6%, and 4.5, respectively (P < 0.001), whereas the MES exhibited relatively poor accuracy for diagnosing severe patients with UC, showing an AUC, sensitivity, specificity, and cut-off value of 0.727, 74.6%, 67.4%, and 2.5, respectively (P < 0.001) (Figure 3).

Correlations between the three endoscopic scores and laboratory/clinical parameters

The three scoring systems were positively correlated with CRP level, ESR, WBC count, NE count, PLT count, length of hospitalisation, and hospitalisation costs (all P < 0.001) but were negatively correlated with Hb, Alb, and BUN levels (all P ≤ 0.001). Furthermore, the correlations between the TIGER score and CRP level, ESR, WBC count, NE count, PLT count, Alb level, and BUN level (r = 0.639, 0.551, 0.387, 0.458, 0.429, -0.422, and -0.320, all P < 0.001) were higher than those between MES and the respective parameters (r = 0.509, 0.351, 0.268, 0.310, 0.248, -0.278, and -0.251, all P ≤ 0.001). However, the correlation between the TIGER score and Hb level was lower than that of the UCEIS score but higher than the MES (all P ≤ 0.001). The correlations between the TIGER score and CRP level, ESR, length of hospitalisation, and hospitalisation costs were similar to those of the UCEIS score (all P < 0.001) and higher than those of the MES (all P <

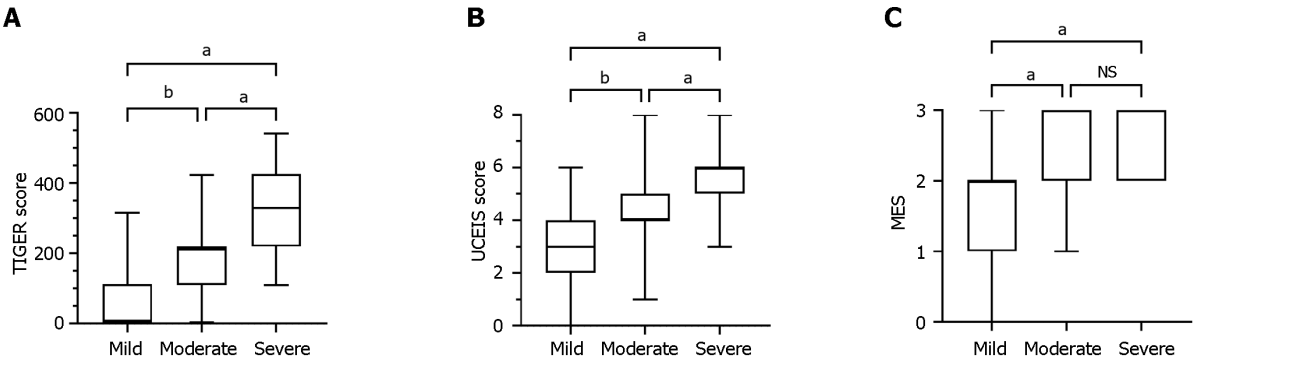

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Figure 2 Comparisons of endoscopic scores among the mild (*n* = 44), moderate (*n* = 51), and severe (*n* = 71) patients. A: Comparison of Toronto Inflammatory Bowel Disease Global Endoscopic Reporting score among different severities of ulcerative colitis (UC); B: Comparison of Ulcerative Colitis Endoscopic Index of Severity score among different severities of UC; C: Comparison of Mayo Endoscopic Subscore among different severities of UC. $^{a}P < 0.0001$; $^{b}P < 0.001$; NS: Not significance; TIGER: Toronto Inflammatory Bowel Disease Global Endoscopic Reporting; UCEIS: Ulcerative Colitis Endoscopic Index of Severity; MES: Mayo Endoscopic Subscore.

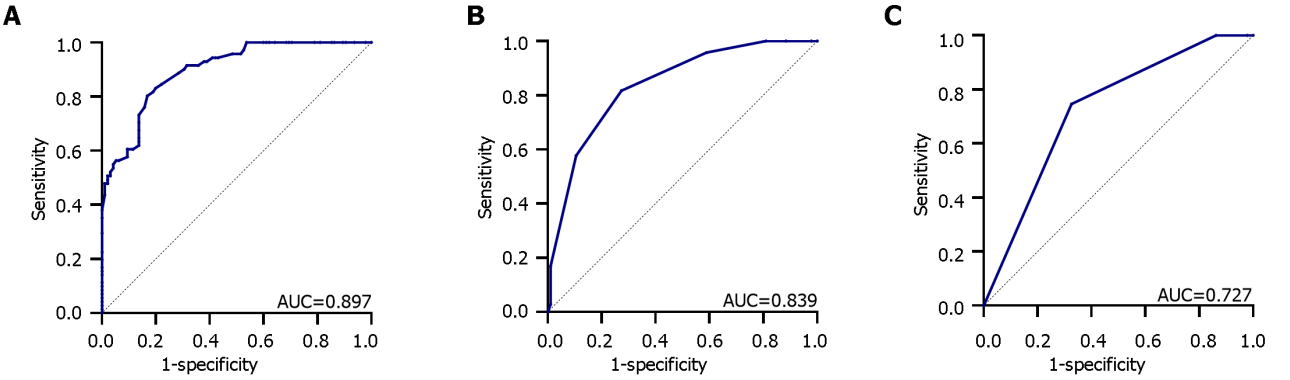

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Figure 3 Comparison of the diagnostic value of endoscopic scores for patients with severe ulcerative colitis. A: The diagnostic value of Toronto Inflammatory Bowel Disease Global Endoscopic Reporting score for severe patients; B: The diagnostic value of Ulcerative Colitis Endoscopic Index of Severity score for severe patients; C: The diagnostic value of Mayo Endoscopic Subscore for severe patients. AUC: Area under the curve.

0.001). No correlations were observed among BMI, disease duration, LYM count, or Cr level in any of the three scoring systems (all $P > 0.05$) (Table 2).

The relationship between the endoscopic scores and advanced treatment

Overall, 113 (68.1%) and 53 (31.9%) patients received 5-ASAs alone and advanced treatment during this admission or within 1 month of discharge, respectively, classifying them into the 5-ASAs-alone and advanced treatment groups, respectively. Advanced treatments included systemic corticosteroids, biologics, immunomodulators, thalidomide, and surgery. Notably, patients in the advanced treatment group demonstrated significantly higher TIGER score [median (IQR): 328 (220.5–426) *vs* 116 (8.5–218); $Z = -7.210$, $P < 0.0001$], UCEIS score [median (IQR): 6 (4–6) *vs* 4 (3–5); $Z = -5.543$, $P < 0.0001$], and MES [median (IQR): 3 (2–3) *vs* 2 (2–3); $Z = -4.272$, $P < 0.0001$], as shown in Figure 4A-C.

ROC analysis was performed in patients with UC to compare the predictive capability for advanced treatment. The TIGER score had an AUC of 0.848 with a sensitivity, specificity, and cut-off value of 69.8%, 86.7%, and 317, respectively ($P < 0.001$), showing the best predictive potential for treatment escalation. The UCEIS score had an AUC of 0.762, with a sensitivity, specificity, and cut-off value of 60.4%, 83.2%, and 5.5, respectively ($P < 0.001$), indicating a moderate predictive capability. Furthermore, the AUC of the MES was 0.684 with a sensitivity, specificity, and cut-off value of 73.6%, 60.2%, and 2.5, respectively ($P < 0.001$), indicating lower predictive potential than the TIGER and UCEIS scores for advanced therapies (Figure 4D-F).

Based on the ROC curves (Figure 4D-F), we selected the TIGER score, UCEIS score, and MES of 317, 5.5, and 2.5, respectively, as the cut-off values and patients were classified into the low- and high-score groups. The TIGER score, UCEIS score, MES, extent of UC, CRP level, Hb level, and Truelove and Witts Index exhibited significant differences between the 5-ASAs-alone and advanced treatment groups (all $P < 0.001$; Table 3). Furthermore, the TIGER score of ≥ 317 [odds ratio (OR): 3.891; 95% confidence interval (95%CI): 1.360–11.136; $P = 0.011$) and extent of E3 (OR: 6.488; 95%CI: 1.617–26.027; $P = 0.008$) were the significant risk factors for treatment escalation (Table 4).

Table 2 Correlations between three endoscopic scores and laboratory/clinical parameters

Parameters	TIGER score		UCEIS score		MES	
	Spearman coefficient (*r*)	*P* value	Spearman coefficient (*r*)	*P* value	Spearman coefficient (*r*)	*P* value
BMI	-	0.146	-	0.035	-	0.191
Duration	-	0.660	-	0.604	-	0.814
CRP	0.639	< 0.001	0.622	< 0.001	0.509	< 0.001
ESR	0.551	< 0.001	0.540	< 0.001	0.351	< 0.001
WBC	0.387	< 0.001	0.319	< 0.001	0.268	< 0.001
NE	0.458	< 0.001	0.369	< 0.001	0.310	< 0.001
LYM	-	0.349	-	0.823	-	0.790
PLT	0.429	< 0.001	0.395	< 0.001	0.248	< 0.001
Hb	-0.353	< 0.001	-0.388	< 0.001	-0.245	0.001
Alb	-0.422	< 0.001	-0.306	< 0.001	-0.278	< 0.001
BUN	-0.320	< 0.001	-0.295	< 0.001	-0.251	0.001
UA	-	0.233	-0.164	0.035	-	0.510
Cr	-	0.305	-	0.399	-	0.144
Stay	0.488	< 0.001	0.494	< 0.001	0.339	< 0.001
Costs	0.376	< 0.001	0.375	< 0.001	0.270	< 0.001

BMI: Body mass index; TIGER: Toronto Inflammatory Bowel Disease Global Endoscopic Reporting; UCEIS: Ulcerative Colitis Endoscopic Index of Severity; MES: Mayo Endoscopic Subscore; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; WBC: White blood cell; NE: Neutrophil count; LYM: Lymphocyte count; PLT: Platelet count; Hb: Haemoglobin; Alb: Albumin; BUN: Urea nitrogen; UA: Uric acid; Cr: Creatinine.

Relationship between the endoscopic scores and 1-year readmission

A 1-year follow-up was conducted on 142 patients to investigate the relationship between the endoscopic scores and 1-year readmission (Figure 1). Notably, 142 patients were classified into the non-readmission (n = 94) and readmission (n = 48) groups to compare the predictive value of the assessed endoscopic scoring systems for readmission within 1 year. Mann–Whitney test results revealed that the readmission group had a higher TIGER [median (IQR): 319.5 (210–425.75) *vs* 210 (106–222.5); Z = -3.889, P < 0.001] and UCEIS [median (IQR): 5 (4–6) *vs* 4 (3–6); Z = -2.529, P = 0.011] scores than the non-readmission group, whereas no significant difference was observed in the MES [median (IQR): 3 (2–3) *vs* 2(2–3); Z = -1.701, P = 0.089] between the two groups (Figure 5A-C).

ROC curves were drawn to compare the predictive value of the scoring systems for readmission within 1 year (Figure 5D-F). The AUC of the TIGER score was 0.700, indicating a sensitivity, specificity, and cut-off value of 60.4%, 73.4%, and 220.5, respectively (P < 0.001), demonstrating a predictive capability for readmission. The AUC of the UCEIS score was 0.627, with a sensitivity, specificity, and cut-off value of 43.8%, 74.5%, and 5.5, respectively (P < 0.05), exhibiting inferior predictive ability for readmission. Conversely, MES showed no significant predictive value compared with the two endoscopic scores mentioned above, with an AUC of 0.578 (P = 0.132).

DISCUSSION

In this study, we compared the clinical utility of the TIGER score, UCEIS score, and MES and identified their roles in predicting disease burden and short-term clinical outcomes.

The three indices consistently reflected endoscopic findings, and the TIGER score strongly and moderately correlated with the UCEIS score and MES, respectively. Xu *et al*[20] indicated that the TIGER score correlated with the UCEIS score and MES, with correlation coefficients of 0.6193 and 0.4527, respectively, similar to our results. These findings demonstrate a better correlation between the TIGER and UCEIS scores, which we attribute to the better definition and grading of the descriptors in the two scoring systems, such as more detailed scoring criteria for erosions and ulcers[21].

Furthermore, using the Truelove and Witts Severity Index[19], we discovered that the TIGER and UCEIS scores could distinguish between the different UC severities. Notably, the TIGER score demonstrated optimal diagnostic performance for severe UC. This superior performance may be because the TIGER score assesses total bowel segments and considers both inflammation and the extent of UC, whereas the UCEIS score and MES exclude the extent of UC. Interestingly, the

Table 3 Univariable analysis of risk factors for the advanced treatment, *n* (%)

Variables	5-ASAs-alone group (*n* = 113)	Advanced treatment group (*n* = 53)	*P* value
Age			0.373
< 40 yr	45 (39.8)	25 (47.2)	
≥ 40 yr	68 (60.2)	28 (52.8)	
Sex			0.054
Male	61 (54.0)	37 (69.8)	
Female	52 (46.0)	16 (30.2)	
Extent			< 0.001
E1 or E2	65 (57.5)	3 (5.7)	
E3	48 (42.5)	50 (94.3)	
CRP			< 0.001
< 5 mg/L	85 (75.2)	14 (26.4)	
≥ 5 mg/L	28 (24.8)	39 (73.6)	
Hb			< 0.001
≥ 110 g/L	94 (83.2)	26 (49.1)	
< 110 g/L	19 (16.8)	27 (50.9)	
Truelove and Witts index			< 0.001
Mild or moderate	84 (74.3)	11 (20.8)	
Severe	29 (25.7)	42 (79.2)	
TIGER score			< 0.001
< 317	98 (86.7)	16 (30.2)	
≥ 317	15 (13.3)	37 (69.8)	
UCEIS score			< 0.001
< 6	94 (83.2)	21 (39.6)	
≥ 6	19 (16.8)	32 (60.4)	
MES			< 0.001
≤ 2	68 (60.2)	14 (26.4)	
> 2	45 (39.8)	39 (73.6)	

TIGER: Toronto Inflammatory Bowel Disease Global Endoscopic Reporting; UCEIS: Ulcerative Colitis Endoscopic Index of Severity; MES: Mayo Endoscopic Subscore; 5-ASAs: 5-Aminosalicylates; CRP: C-reactive protein; Hb: Haemoglobin.

extent is one of the dimensions used to evaluate endoscopic severity and can influence the overall severity of UC[22]. Osada *et al*[23] demonstrated that total colonoscopy could provide complete information on patients with UC and improve the accuracy of clinical disease assessment, which is consistent with our conclusion. Nevertheless, because of factors including discomfort and complications, total colonoscopic studies included fewer acute severe cases, which might have resulted in different results. Gomes *et al*[24] revealed a poor correlation between total colonoscopic findings and clinical manifestations. Moreover, a finer categorization and larger scale of the scoring system may be more advantageous and accurate in reflecting inflammatory burden and treatment response[22,25]. The UCEIS score, ranging from 0 to 8, provides a larger scale and finer gradings of ulcers and bleeding than the MES. Song *et al*[26] also demonstrated that the UCEIS score was superior to MES in diagnosing UC severity. Therefore, we infer that the TIGER score can provide a detailed description of the ulcers (size and percentage of surface) and localised inflammation in relation to the bowel segment and a wide range of scores between 0 and 560, resulting in optimal performance when reflecting the overall severity[17].

In this study, we observed that the TIGER score was significantly correlated with the clinical parameters of active inflammation, particularly CRP levels, and the burden parameters including length of stay and cost, whereas the MES exhibited disadvantages in evaluating the clinical activity of UC compared to the TIGER and UCEIS scores. Previous studies have demonstrated that objective blood markers, including CRP, ESR, WBC, PLT, Alb, and Hb, are relevant to UC endoscopic severity and disease activity[27,28]. Additionally, the association between endoscopic scores and inflam-

Table 4 Multi-variable logistic regression analysis for the risk factors of the advanced treatment

Variables	Odds ratio	95%CI	*P* value
Extent, E3	6.488	1.617-26.027	0.008
CRP ≥ 5 mg/L	2.554	0.956-6.819	0.061
Hb < 110 g/L	1.736	0.615-4.899	0.297
Truelove and Witts Index, Severe	1.390	0.452-4.278	0.566
TIGER score ≥ 317	3.891	1.360-11.136	0.011
UCEIS score ≥ 6	2.171	0.736-6.403	0.160
MES > 2	0.945	0.335-2.667	0.914

95%CI: 95% confidence interval; CRP: C-reactive protein; Hb: Haemoglobin; TIGER: Toronto Inflammatory Bowel Disease Global Endoscopic Reporting; UCEIS: Ulcerative Colitis Endoscopic Index of Severity; MES: Mayo Endoscopic Subscore.

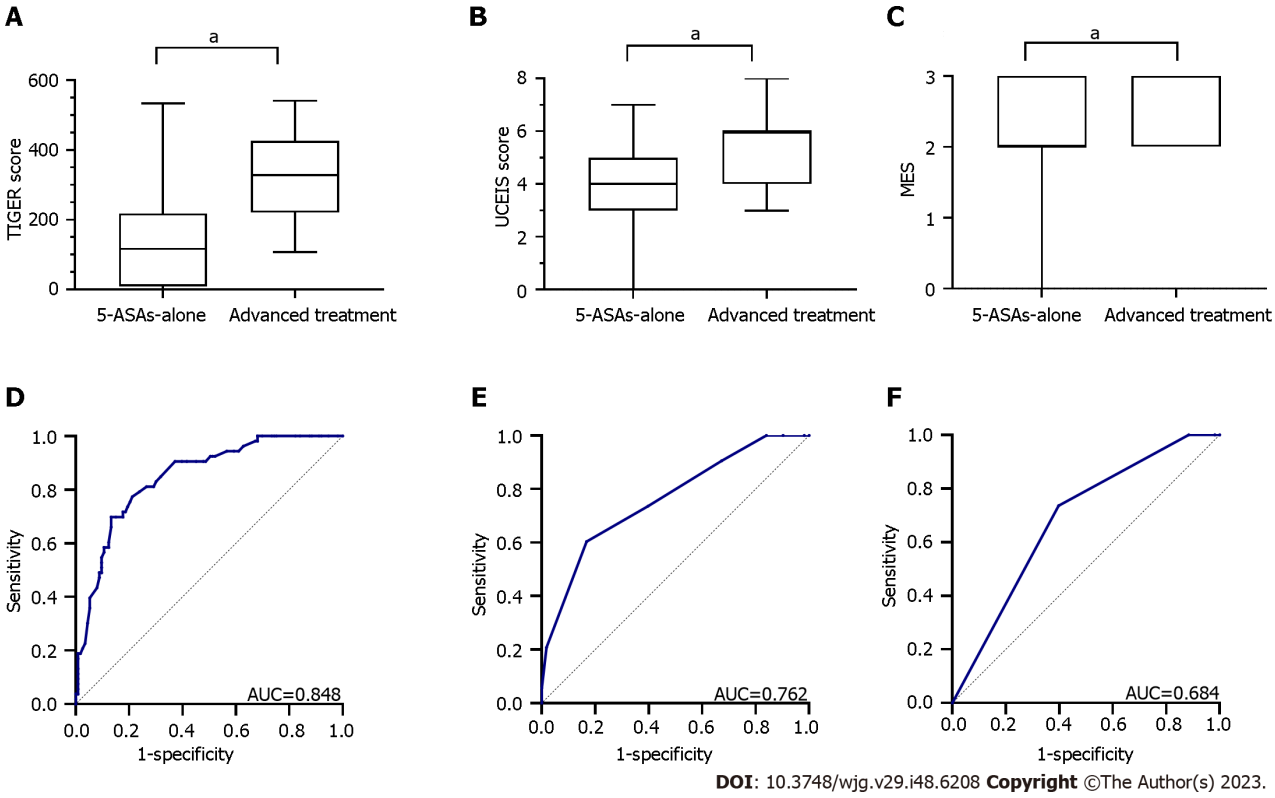

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Figure 4 Comparisons of endoscopic scores between the 5-aminosalicylates-alone group (*n* = 113) and the advanced treatment group (*n* = 53). A: Comparisons of Toronto Inflammatory Bowel Disease Global Endoscopic Reporting (TIGER) score between 2 groups; B: Comparisons of Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score between 2 groups; C: Comparisons of Mayo Endoscopic Subscore (MES) between 2 groups; D: The predictive value of TIGER score for advanced treatment recently; E: The predictive value of UCEIS score for advanced treatment recently; F: The predictive value of MES for advanced treatment recently. [a]*P* < 0.0001. AUC: Area under the curve.

matory burden has been confirmed in other studies[29,30]. Recent studies have shown that CRP, a typical acute-phase protein, reflects the inflammatory state of the entire colon, which aligns with our findings that demonstrate the existence of a correlation between the TIGER score and CRP levels[31]. Additionally, Zittan *et al*[17] reported that the TIGER score was positively correlated with faecal calprotectin levels and the inflammatory bowel disease (IBD) Disk score, indicating that the disease condition and burden of UC may be observed using the TIGER score. Inflammatory biomarkers can exacerbate damage to the epithelial barrier and imbalance of the intestinal mucosal immune system and influence the synthesis of related protein synthesis in UC[32,33], which may be manifested in endoscopic mucosal inflammation and reflected by the TIGER score since it contains a clear description of mucosal appearance and ulcer conditions and could precisely describe and assess the entire intestine.

Five-ASAs are still recommended as the standard treatment and maintenance strategies for patients with mild-to-moderate UC, whereas systemic corticosteroids, thiopurines, biologics, immunomodulators, and tofacitinib are

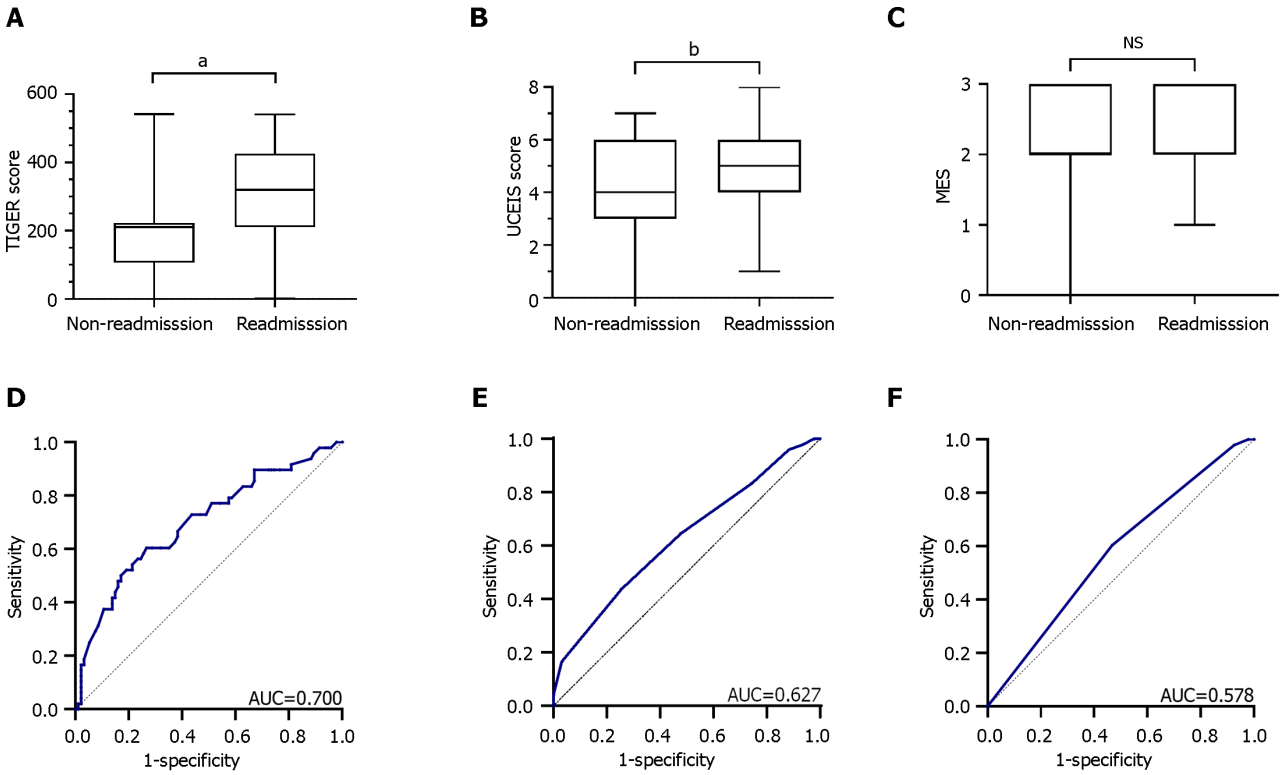

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Figure 5 Comparisons of the endoscopic scores between the non-readmission group (*n* = 94) and the readmission group (*n* = 48). A: Comparisons of Toronto Inflammatory Bowel Disease Global Endoscopic Reporting (TIGER) score between 2 groups; B: Comparisons of Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score between 2 groups; C: Comparisons of Mayo Endoscopic Subscore (MES) between 2 groups; D: The predictive value of TIGER score for 1-year readmission; E: The predictive value of UCEIS score for 1-year readmission; F: The predictive value of MES for 1-year readmission. [a]*P* < 0.001; [b]*P* < 0.05; ns: No significance; AUC: Area under the curve.

considered upgraded treatment options for those with moderate-to-severe disease activity or those with 5-ASAs failure or intolerance[34,35]. In this study, we observed that the TIGER score had a superior predictive potential for advanced treatment in patients with UC and demonstrated that a TIGER score of ≥ 317 was an independent risk factor for indicating that patients with three or more segments involved in moderate-to-severe endoscopic activity were more likely to upgrade their treatment programs. This could be a useful indication of escalating treatment. Severe endoscopic activity and extensive colitis may represent a severe degree of disease, leading to therapy escalation and poor prognosis[36-38], which could explain why patients with higher TIGER scores were at a higher risk of advanced treatment in this study. Bálint *et al*[39] suggested that a scoring system should provide additional information on the localization and extent of the disease and argued that this could guide treatment choices, which is consistent with the above mentioned results.

Meanwhile, a high readmission rate indicates increased disease severity and poor prognosis in UC[40]. Therefore, the prediction of readmissions could help clinicians develop healthcare plans and manage patients. We observed that the TIGER score in the readmission group was higher than that in the non-readmission group and it displayed the best predictive capability for readmission within 1 year. These results indicate that the TIGER score could help predict short-term prognosis in patients with UC. Higher TIGER scores indicate more severe UC, which may also be associated with a higher readmission rate[40]. Notably, the UCEIS score was observed to be an independent risk factor for the 1-year readmission, demonstrating that endoscopic scores might be associated with early readmission[41].

The TIGER score requires further validation in clinical practice before its broad adoption. Five descriptors in each of the five segments were evaluated during the endoscopic examination to assess the mucosal inflammation of the entire intestine, resulting in 25 items that required scoring. For segments with a TIGER score of ≥ 5, 100 bonus points were counted, and the segmental scores were summed to determine the final total TIGER score. With the recent development of high-performance computers, advanced optical technologies, molecular imaging, and artificial intelligence algorithms, a computer-aided diagnostic system for patients with IBD to improve endoscopic assessment has become possible[42-45]. Although the TIGER score requires calculation and total colonoscopy, it can be intelligently calculated currently and may be automatically scored in the future by analysing the entire colonic mucosa.

Furthermore, we validated, for the first time, that the TIGER score could accurately reflect disease activity and significantly correlate with laboratory parameters in patients with UC. Moreover, we also defined TIGER score thresholds for upgraded treatment and 1-year readmission, providing treatment strategies and personalised disease management for patients with UC. Additionally, this study included a larger cohort of patients with UC than a previous study[17].

However, this study had some limitations. First, this was a single-centre retrospective study. Second, some patients with acute severe UC could not undergo total colonoscopy and were excluded from the study. Therefore, multicenter prospective studies are warranted.

CONCLUSION

The TIGER score is a useful scoring method that provides an overall intestinal evaluation of endoscopic activity and demonstrates a significant correlation with the UCEIS score, MES, and laboratory indices, particularly CRP levels. Furthermore, the TIGER score may be superior to the UCEIS and MES scoring systems in improving the accuracy of clinical disease severity assessment, guiding therapeutic decision-making to some extent, and predicting short-term clinical outcomes.

ARTICLE HIGHLIGHTS

Research background

Endoscopy is crucial in the diagnosis, assessment, and management of ulcerative colitis (UC). Several endoscopic scoring systems have been established to make endoscopic evaluation quantified and objective, including the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score and Mayo Endoscopic Subscore (MES). The Toronto Inflammatory Bowel Disease Global Endoscopic Reporting (TIGER) score for UC, which considers the extent of UC involvement and reflects the number of segments with moderate-to-severe inflammation, was proposed in 2022.

Research motivation

Although the TIGER score is a novel and reliable tool for reflecting complete endoscopic inflammation, its clinical value remains unclear.

Research objectives

To assess the clinical value of the TIGER score by comparing it with the UCEIS score and MES.

Research methods

We performed a retrospective study that included 166 patients with UC who underwent total colonoscopy. Spearman's rank correlation coefficient was used to estimate the linear associations of three scores and laboratory/clinical parameters. The receiver-operating characteristic curve was performed to compare the predictive potentials of the three scores for predicting severe UC, patients' recent advanced treatment, and 1-year readmission. Univariate and multivariable logistic regression analyses were performed to investigate the independent risk factors for treatment escalation.

Research results

The TIGER score showed a significant correlation with the UCEIS score, MES, and laboratory indices, particularly C-reactive protein levels. Additionally, the TIGER score exhibited the best predictive capability for diagnosing patients with severe UC, upgrading treatment options, and 1-year readmission and a TIGER score of ≥ 317 was found to be an independent risk factor for treatment escalation in UC.

Research conclusions

The TIGER score exhibits an advantage in assessing the disease severity of UC, guiding treatment decisions, and predicting short-term prognosis compared to the UCEIS score and MES.

Research perspectives

The TIGER score may have significant clinical utility in evaluating, treating, and managing patients with UC, although multicenter prospective studies are required to promote its use.

FOOTNOTES

Author contributions: Ding XL, Liu XY, and Tian ZB designed this study; Zhang LJ evaluated and reviewed the statistical methods; Ding XL, Liu XY, Liu AL, Zhang XF, and Wu J collected data; Liu XY analysed the data; Liu XY edited the manuscript; Ding XL, Liu AL, Tian ZB revised the manuscript; and all authors have read and approved the final manuscript.

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Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (Approval No. QYFY WZLL 28085).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: All authors declare no potential conflicts of interest related to this study.

Data sharing statement: The data used in this study can be obtained from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Basic Study

Single-cell analysis identifies phospholysine phosphohistidine inorganic pyrophosphate phosphatase as a target in ulcerative colitis

Yan-Fei Wang, Ruo-Yu He, Chan Xu, Xiao-Ling Li, Yan-Fei Cao

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Abstract

BACKGROUND

Ulcerative colitis (UC) is a chronic gastrointestinal disorder characterized by inflammation and ulceration, representing a significant predisposition to colorectal cancer. Recent advances in single-cell RNA sequencing (scRNA-seq) technology offer a promising avenue for dissecting the complex cellular interactions and molecular signatures driving UC pathology.

AIM

To utilize scRNA-seq technology to dissect the complex cellular interactions and molecular signatures that underlie UC pathology.

METHODS

In this research, we integrated and analyzed the scRNA-seq data from UC patients. Moreover, we conducted mRNA and protein level assays as well as pathology-related staining tests on clinical patient samples.

RESULTS

In this study, we identified the sustained upregulation of inflammatory response pathways during UC progression, characterized the features of damaged endo-

thelial cells in colitis. Furthermore, we uncovered the downregulation of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) has a negative correlation with signal transducer and activator of transcription 3. Significant downregulation of LHPP in UC patient tissues and plasma suggests that LHPP may serve as a potential therapeutic target for UC. This paper highlights the importance of LHPP as a potential key target in UC and unveils its potential role in inflammation regulation.

CONCLUSION

The findings suggest that LHPP may serve as a potential therapeutic target for UC, emphasizing its importance as a potential key target in UC and unveiling its role in inflammation regulation.

Key Words: Ulcerative colitis; Single-cell RNA sequencing; Phospholysine phosphohistidine inorganic pyrophosphate phosphatase

Core Tip: Ulcerative colitis (UC), a chronic inflammatory bowel disease linked to colorectal cancer, was investigated using single-cell RNA sequencing technology. The study unveiled sustained upregulation of inflammatory response pathways and characterized damaged endothelial cells during UC progression. Notably, the downregulation of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) exhibited a negative correlation with signal transducer and activator of transcription 3. LHPP's significant downregulation in UC patient tissues and plasma suggests its potential as a therapeutic target. The findings highlight LHPP as a key target in UC and emphasize its role in inflammation regulation, offering insights for potential therapeutic interventions.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) characterized by mucosal inflammation and ulceration primarily affecting the colon and rectum[1]. It is a multifactorial[2,3]. Individuals with UC often experience debilitating symptoms such as abdominal pain, bloody diarrhea, diarrhea, rectal bleeding, and an increased risk of colorectal cancer (CRC), severely impacting their quality of life. Extensive research over the years has yielded valuable insights into the pathogenesis of UC, highlighting the importance of aberrant immune responses and the gut microbiome in disease development and progression[4-6]. However, a comprehensive understanding of the cellular and molecular mechanisms underlying UC development and progression remains elusive, and there is a growing need for more precise and targeted therapeutic interventions[7].

Recent advances in single-cell RNA sequencing (scRNA-seq) technology have revolutionized our ability to dissect the heterogeneity of cell populations within complex tissues[8-10]. This revolutionary technique empowers researchers to discern distinct cell subsets, unveil novel cellular pathways, and delve into the dynamics of immune responses with unparalleled resolution[11,12]. In the context of UC, scRNA-seq offers a unique opportunity to decipher the intricate cellular interactions and molecular signatures driving disease pathology[13-17].

Phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP), a histidine phosphatase protein, has been implicated in diverse biological processes, including tumor suppression in hepatocellular carcinoma[18], increasing the expression of cleaved-poly (ADP-ribose) polymerase and cleaved-Casp3 protein to promote apoptosis[19], and inflammation regulation and immune response modulation. Recent studies have suggested its potential correlation with survival of CRC patients[20]. Another study reports that the loss of LHPP in intestinal epithelial cells correlate with colitis in mice, suggests the involvement of LHPP in IBD[21,22]. However, the precise involvement and impact of LHPP in the context of UC remain an unexplored territory, offering a promising avenue for further investigation.

In this study, we integrated the current single-cell sequencing data of UC[15,16], shedding light on the specific cell types, transcriptional profiles, and immune signaling pathways that play pivotal roles in UC pathogenesis. By interrogating the single-cell landscape of UC-affected colonic tissues, we identified sustained upregulation of inflammatory response pathways during the progression of UC and characterized the features of damaged endothelial cells (EC) in colitis. Through integrated analysis of UC disease databases utilizing single-cell data, we uncovered that LHPP exhibits sustained downregulation in UC, displaying a negative correlation with signal transducer and activator of transcription 3 (*STAT3*). Notably, our experimental results in UC patients corroborated that when *STAT3* expression is upregulated, LHPP expression is downregulated, suggesting a potential role for *STAT3* in transcriptionally inhibiting LHPP expression. Furthermore, LHPP not only exhibited decreased expression in the intestinal tissue of UC patients but also displayed reduced expression in their plasma samples. This observation suggests that LHPP may serve as a critical

factor in the pathogenesis of UC.

MATERIALS AND METHODS

Construction of cell atlas of healthy and UC human clonal tissues

The raw data of healthy and UC human clonal tissues were from GSE125527 in GEO database. The R package Seurat (version 4.0.2) was used for construction of cell atlas of human clonal tissues. In brief, the function "CreateSeuratObject" was used to load gene expression matrix of each sample. The function 'SCTransfrom' was used for finding high variable genes, normalization and scaling of the gene expression matrix for each sample, the 'PrepSCTIntegration' and 'FindIntegrationAnchors' functions were used for selecting the anchors for integration all samples. The function "IntegrateData" was used for the following integration. After integration, the function "ScaleData" was used to scale the integrated expression matrix, then the principle component analysis and Dimensionality reduction of dataset were performed by the functions "RunPCA" and "RunUMAP", the functions "FindNeighbors" and "FindClusters" were used to cell clustering and identification. The function 'FindAllMarkers' (|avg_log2FC| ≥ 0.5 and p_val_adj ≤ 0.05) was used to calculate marker genes for each cell type.

Identification of differential expression genes (DEGs) between healthy and UC group across tissues and cell compartments

The 'FindMarkers' function in Seurat was used to identify differentially expressed genes in diseased *vs* healthy group (diseased/healthy) of each cell type, which were based on normalized data and the Wilcoxon test. The screening criteria for significantly differentially expressed genes were selected by BH-adjusted *P* value < 0.05 and $|\log_2 FC| > 0.25$.

Pseudo-time trajectory inference

To characterize the mobilization and of EC during UC, the R package Monocle2 was used to perform pseudotime trajectory inference for EC of healthy and UC tissues. The top 3000 high variable genes were used to calculate the pseudotime. The functions "plot_pseudotime_heatmap" and "plot_genes_in_pseudotime" were used to perform time-related gene analysis.

Gene set score analysis

The gene sets were downloaded from MSigDB (https://www.gsea-msigdb.org/gsea) and the 'AddModuleScore' function of Seurat was used to calculate gene set scores.

Patients and tissues

The biopsy samples were collected from 12 heathy control subjects and 15 UC patients from the Department of Gastroenterology, The Third Affiliated Hospital of Zhejiang Chinese Medical University (Hangzhou, China, ZSLL-KY-2023-031-01). The protocol was approved by the Institutional Ethics Committee of The Third Affiliated Hospital of Zhejiang Chinese Medical University and was in concordance with the Helsinki Declaration. Biopsy specimens were collected and washed twice with phosphate-buffered saline, then frozen by liquid nitrogen.

Quantitative real-time polymerase chain reaction (PCR)

Colonic samples were subjected to physical homogenization and total RNA extracted by TRIzol Reagent (Invitrogen). Then cDNA was synthesized using the high-capacity reverse transcription kit (Thermo Fisher) following the manufacturer's instructions. The qPCR reactions were performed using TaqMan gene expression and assays on ABI QuantStudio 5 (Applied Biosystems, Thermo-Fisher).

Western blotting

Tissues were lysed using SDS lysis buffer (containing 100 mmol/L Tris-HCl (pH = 7.0), 1% SDS and 2% 2-mercaptoethanol, supplemented with 1 x protease inhibitors from Roche) and incubated at 105 ℃ for 10 min. Total protein was extracted and quantified using the BCA Kit (Abcam). Equal amounts proteins were subjected to SDS-PAGE electrophoresis and subsequently transferred onto PVDF membranes (Millipore). After blocking with 5% skim milk, the membranes were treated overnight at 4 ℃ with the following antibodies: Mouse monoclonal antibody against GAPDH (1: 2000 dilution; 0411; sc47724); Rabbit monoclonal antibody against *STAT3* (1: 1000 dilution; abcam; ab68153). Rabbit monoclonal antibody against *STAT3* (1: 500 dilution; abcam; ab254788). Following incubation with HRP-conjugated secondary antibodies, the PVDF membrane was visualized using an enhanced chemiluminescence (ECL) kit (Thermo).

Enzyme-linked immunosorbent assay (ELISA)

The protein levels of IL6 (Biolegend) and LHPP (Abbexa) in the blood were determined using ELISA according to the manufacturer's instructions. Following incubation with the detection kit, the plate was analyzed at 450 nm using a chemiluminescence immunoassay system (Dxl800Access, Beckman, United States).

Statistical analysis

All data were statistical analyzed using GraphPad Prism software version 8.0. The data were presented as the mean ±

SEM. Comparisons were conducted using the two-tailed student's *t* test or one-way ANOVA. *P* values < 0.05 were considered statistically significant.

RESULTS

Single-cell transcriptomics identified major cell types in human colon microenvironment

To systematically elucidate the microenvironmental changes occurring during the development of UC, we conducted an analysis utilizing publicly available single-cell datasets from healthy individuals and UC patients. Following quality control (Supplementary Figures 1 and 2), a total of 64643 high quality cells were filtered from healthy (n = 8) and UC (n = 8) individuals, we identified eight major cell types, which were $CD4^+$ T cells (37.44%), $CD8^+$ T cells (4.70%), natural killer T cells (NKT) (15.00%), natural killer cells (7.98%), B cells (13.81%), myeloid immune cells (8.80%), and EC (2.45%) (Figure 1A, Supplementary Figure 1). The accuracy of cell identification was confirmed by the expression of canonical marker genes and top marker gene annotation of each cell type (Figure 1B-D). The distribution between healthy and inflamed tissues indicated that there is no specific cell types generated during the inflammatory process (Figure 1E, Supplementary Figure 2).

Regarding alterations in cellular composition, we observed that during UC development, the proportions of $CD8^+$ T cells and NKT cells were increased (Figure 1F and G). This suggests a pronounced activation response by the host immune system in the face of inflammation, mobilizing these immune cell populations to target and eliminate diseased cells. The heightened presence of $CD8^+$ T cells, renowned for their cytotoxic capabilities, signifies a concerted effort by the immune system to target and eliminate inflamed UC tissues. NKT cells, with their unique properties bridging the innate and adaptive immune responses, may also play a crucial role in anti-inflammatory. This bolstering of cytotoxic immune cells within the colon microenvironment is an encouraging sign, suggesting that the immune system is actively engaged in combating the UC. However, the observed decline in the proportions of $CD4^+$ T cells and B cells is a matter of concern (Figure 1F and G). $CD4^+$ T cells play a vital role in mediating diverse immune responses, including antigen presentation to cytotoxic CD8+ T cells and regulation of immune tolerance. A reduction in their presence could suggest a potential weakening of the immune system's ability to recognize inflammatory cells. Similarly, B cells, which are instrumental in antibody production and antigen presentation, also exhibited diminishing proportions. This decline might impede the cytotoxic ability against UC of $CD8^+$ T and NKT cells. The reduction in $CD4^+$ T cells and B cells might be indicative of an evolving immune evasion strategy employed by UC cells, where the colon microenvironment becomes less conducive to an effective immune response and gains a tendency of the chronic, long-term inflammation.

Concurrently, we observed a progressive increase in the proportion of ECs within the colon microenvironment (Figure 1F and G). This phenomenon underscored the significant role of angiogenesis in the UC's progression. The increasing angiogenesis has been proved a strong link with inflammation in various inflammatory diseases. Angiogenesis and inflammation cooperative with each other, and hypoxia acts as a common stimulus for both[23]. Furthermore, a robust vascular network can facilitate the intravasation and extravasation of inflammation factors, enabling metastasis to distant sites within the body.

These findings offer valuable insights into the alterations taking place within the microenvironment during the progression of UC. The body responds to inflammation through a series of immune cell activities. The augmented angiogenesis underscores the significance of vascularization in the inflammation microenvironment.

Characterization of cell-type-specific transcriptomic changes in UC progression

We systematically investigated the differential gene expression profiles across all cell types during the process of UC development. Overall, we identified 706 upregulated and 1471 downregulated differential genes. Notably, ECs exhibited the highest number of both upregulated and downregulated DEGs. These trends in differential gene expression to some extent reflect the inflammatory response of these cells during the progression of UC (Figure 2A-D).

Upon examining the upregulated differential genes within each cell type, we identified a substantial association with the regulation of the innate immune response, such as "regulation of innate immune response", "cytokine Signaling in the Immune system", and "neutrophil degranulation". These findings suggested an elevated immune response and immune cell infiltration within the inflamed tissue. During the UC, the body recruited immune cells to the inflamed tissue for the purpose of clearing infections, necrotic cells, or other pathological cells. Notably, the upregulation of the "epithelial to mesenchymal transition" pathway was also observed, hinting at the possibility that sustained inflammation may induce tissue fibrosis. Additionally, the upregulation of the "Angiogenesis" pathway signifies the presence of neovascularization within the inflamed tissue, which aligns with our previous observation of an increased proportion of ECs. These findings underscored the dynamic response of the body to inflammation, involving the recruitment of immune cells to the site of inflammation, concurrent local tissue fibrosis, and angiogenesis.

In the realm of downregulated genes, a notable decrease in the expression of genes associated with various metabolic processes, including "xenobiotic metabolic process", "oxidative phosphorylation", and "fatty acid metabolic process". These findings indicated that inflammation may lead to a functional decline in affected tissues. Moreover, the downregulation of genes related to "chromatin organization", "cell activation" and "intracellular protein transmembrane transport" indicated alterations in cellular states within the inflamed tissue. These observations collectively imply that inflammation induced substantial changes in cellular function and identity.

These findings of differential gene expression profiles across diverse cell types during UC process has unveiled intriguing insights into the dynamic molecular changes occurring within the inflammation microenvironment, corresponding with the high frequency DEGs across all cell types (Figure 2E and F). The activation of inflammation-related

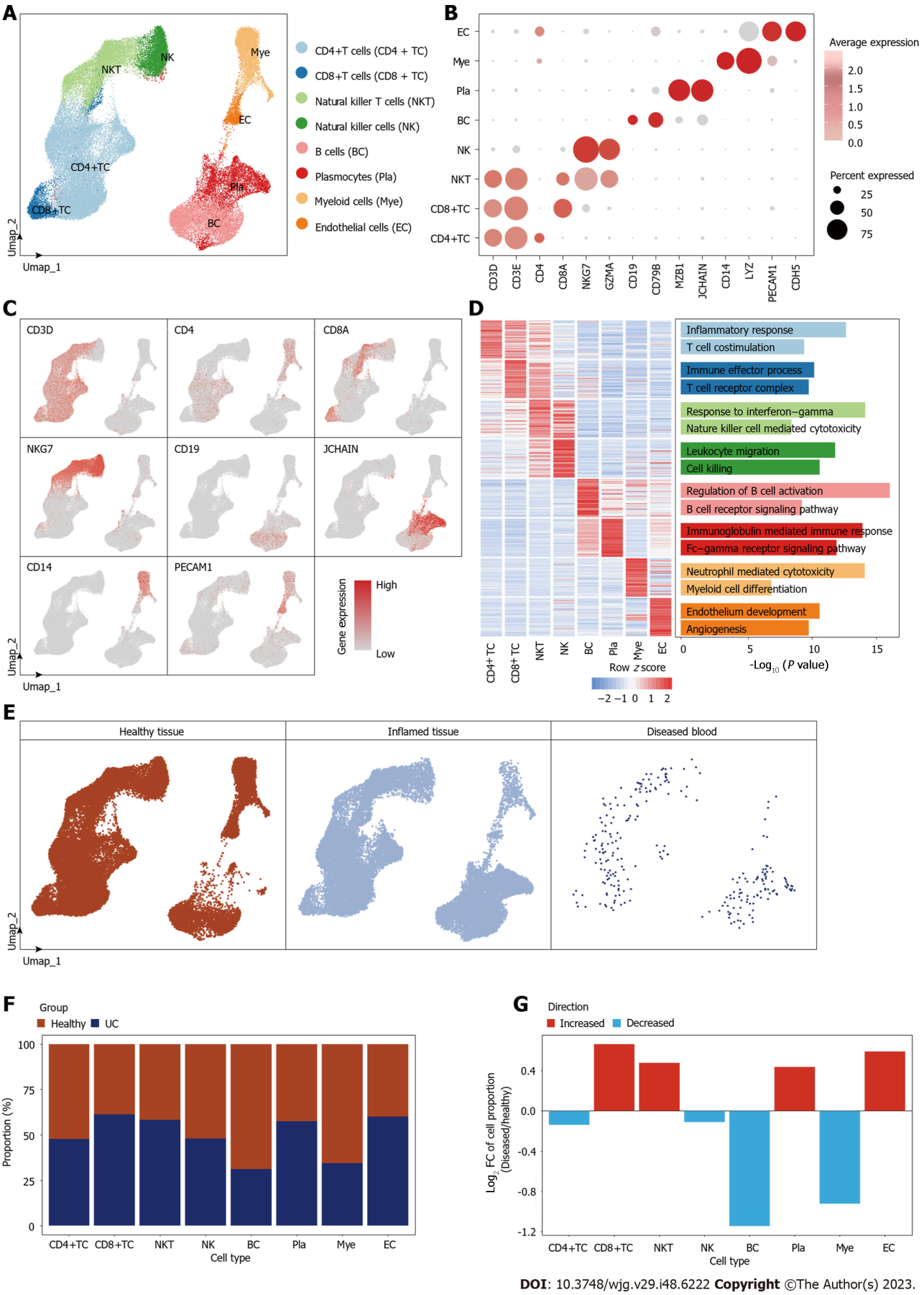

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Figure 1 Cell type identification and cell proportion diversity during ulcerative colitis. A: Umap plot showing different cell types distribution in human clonal tissues; B: Dot plot showing the gene expression levels of the classic marker genes of each cell type; C: Umap plots showing the expression profiles of

indicated cell-type-specific marker genes. The color key from gray to red indicates low to high gene expression levels; D: Heatmap showing the gene expression levels of the top 50 cell-type-specific marker genes for each cell type, with corresponding functional annotations on the right. The color key from blue to red represents low to high gene expression levels; E: Umap plots showing distribution of each group of human clonal tissues; F: Bar plot showing the cell proportion distribution of each cell type in healthy and ulcerative colitis tissues; G: Bar plot showing the fold change level of cell proportion during lesion. CD4+ TC: $CD4^+$ T cells; CD8+ TC: $CD8^+$ T cells; NKT: Natural killer T cells; NK: Natural killer cells; BC: B cells; Pla: Plasmocytes; Mye: Myeloid cells; EC: Endothelial cells.

pathways, immune cell recruitment, tissue fibrosis, and angiogenesis collectively reflect the body's response to inflammation. Concurrently, the downregulation of metabolic pathways and changes in cellular state suggest a functional decline within the affected tissues. These differential gene transcription profiles provide a valuable landscape for assisting in the identification of potential therapeutic targets for UC.

Compromised endothelial mobility during UC progression

Prior research has emphasized the regulatory role of ECs in immune responses, encompassing functions like filtration, endocytosis, antigen presentation, and leukocyte recruitment[24,25]. Aberrant crosstalk between ECs and immune cells were established in injury tissues[26,27]. Furthermore, ECs undergo significant alterations themselves, contributing to inflammation[28]. These findings not only underscore the intricate interplay between ECs and the inflammatory microenvironment but also accentuate the potential of targeting EC-specific mechanisms as a promising avenue for UC therapy.

To gain a deeper understanding of the specific cellular changes that ECs undergo during UC, we conducted pseudotime analysis to elucidate the progressive changes in EC states. Notably, we identified a distinct differentiation trajectory from healthy to UC-associated ECs. Healthy ECs were predominantly situated at the front end of the differentiation trajectory, while UC-associated subpopulations were concentrated toward the middle and end, providing compelling evidence that ECs undergo a series of alterations in their cellular states during UC (Figure 3A and B).

We then focused on the transcriptomic profiles alterations during UC by conducting gene expression patterns that gradually evolved along the pseudotime trajectory. These genes were then categorized into two distinct groups based on their expression trends: the cluster 1 displayed increasing expression as pseudotime advanced, while the cluster 2 exhibited decreasing expression (Figure 3C).

The upregulated genes offer insights into the transcriptional alterations occurring in ECs during the inflammatory process. Notably, they were primarily associated with critical signaling pathways, including the phosphatidylinositol-3-kinase-protein kinase B signaling pathway, known as a key regulator in multiple inflammations. the TGF-beta pathway, known for its role in cellular proliferation and differentiation, as well as tumor genesis. The blood vessel development pathway, indicated ECs' active engagement in inflammation-related angiogenesis processes. Additionally, the anaplastic lymphoma kinase pathway in cancer pathway, suggested that ECs possibly implicating them in a potential predisposition towards carcinogenesis (Figure 3C and D). Intriguingly, these observations underscored the active involvement of ECs in angiogenesis and their responsiveness to various signals driving inflammation. Moreover, it hints at a proclivity of ECs themselves towards malignant transformation. Conversely, the downregulated genes were predominantly linked to pathways such as the Hippo signaling pathway, which plays a pivotal role in regulating cell proliferation and organ size. The apoptosis pathway, responsible for programmed cell death and damaged cell clearance. Furthermore, cellular homeostasis pathways, indicated the disorder of cell state (Figure 3C and E). Collectively, the suppression of these pathways suggests that ECs might undergo functional changes, contributing to their altered roles during inflammation progression.

Among genes whose expression exhibited the most significant changes along the pseudotime trajectory, *TGFB3*, a member of the transforming growth factor-beta family, is well-known for its role in regulating cell growth, differentiation, and immune responses. Its upregulation in ECs implies its potential involvement in the activation of signaling pathways critical for inflammation-related angiogenesis and microenvironment remodeling. Similarly, the upregulation of *EGFR* and *FGFR2* suggests the heightened responsiveness of ECs to growth factor signaling, possibly fueling their angiogenic activities and interaction with inflammation microenvironment. The increased expression of *EPHB4*, a receptor involved in cell-cell communication and tissue patterning, hints at its role in EC differentiation and response to inflammation. Moreover, the upregulation of *ILA* and *COL1A2* highlights the active participation of ECs in immune responses and extracellular matrix remodeling within the UC microenvironment (Figure 3F). Conversely, we observed a downregulation of LHPP, a phosphatase implicated in cell cycle regulation, may suggest a reduced control over EC proliferation and differentiation during UC progression. *HMOX1*, a stress-responsive enzyme, exhibited decreased expression, possibly indicating altered oxidative stress responses in ECs within the UC microenvironment. The decreased expression of *ATP5PD* may implicate alterations in energy metabolism within ECs during inflammation. The downregulation of *ANXA2*, a protein involved in cellular processes such as membrane trafficking and cell adhesion, suggests potential changes in cell-cell interactions. The downregulation of *BCL2* may indicate alterations in apoptosis regulation in ECs within the UC pathological process (Figure 3G).

LHPP was identified as a core regulator for UC

To deepen our insight of the correlation among dysregulated genes in the UC transcriptome profile and uncover key regulator into the molecular mechanisms underlying UC pathogenesis, we conducted an integrated analysis of UC by combining data from disease databases and scRNA-seq DEGs. We identified 13 commonly upregulated genes, including *CFB*, *SPINK4*, *FOXP1*, *TIMP1*, *CSF3R*, *STAT3*, *DUOXA2* and *MMP1*, and 7 downregulated genes (*LHPPP*, *CK1*, *CLDN8*, *ABCG2*, *FMO5*, *CKB*, *GUCA2A*) across these datasets (Figure 4A). Notably, apart from pathways associated with inflam-

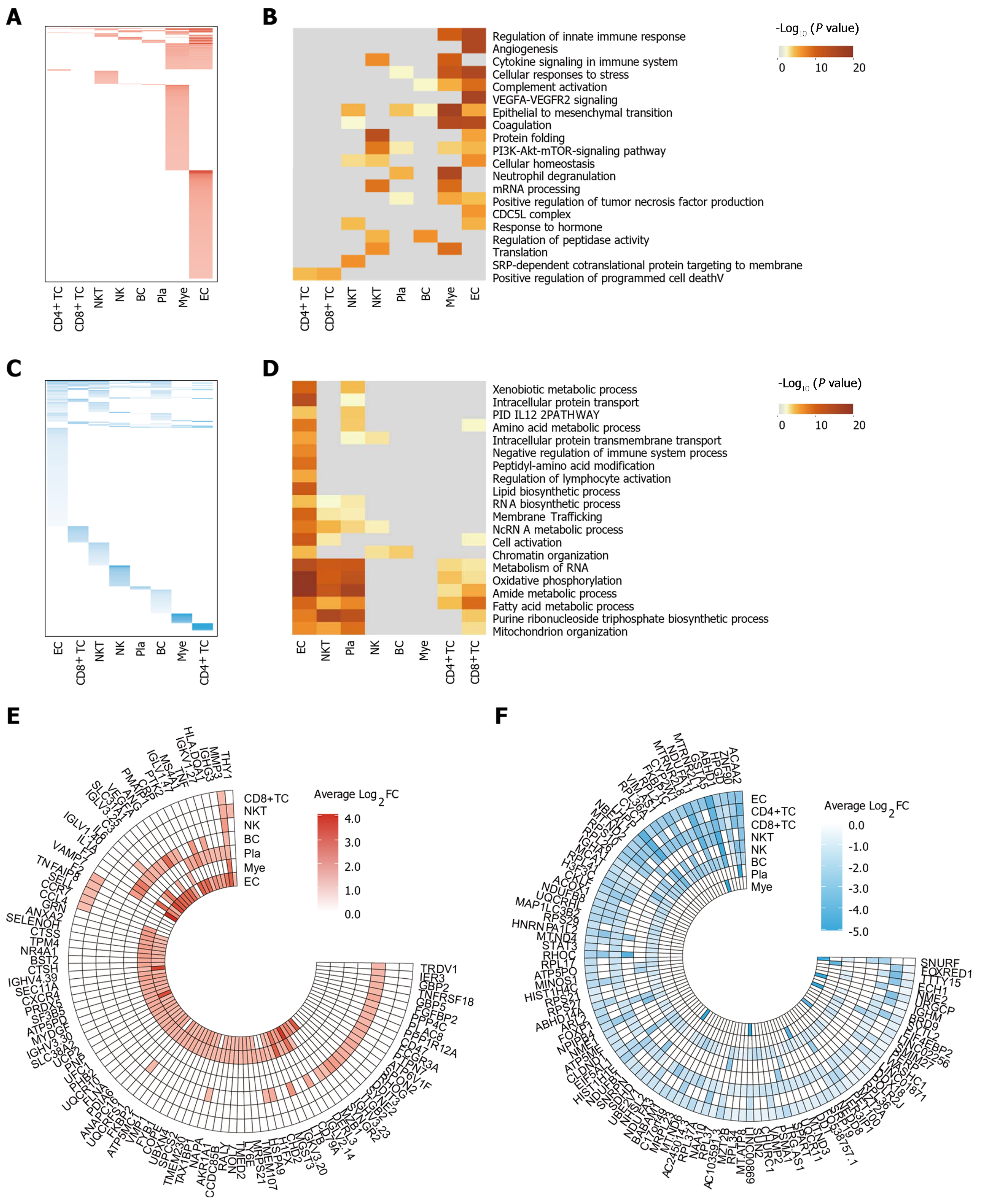

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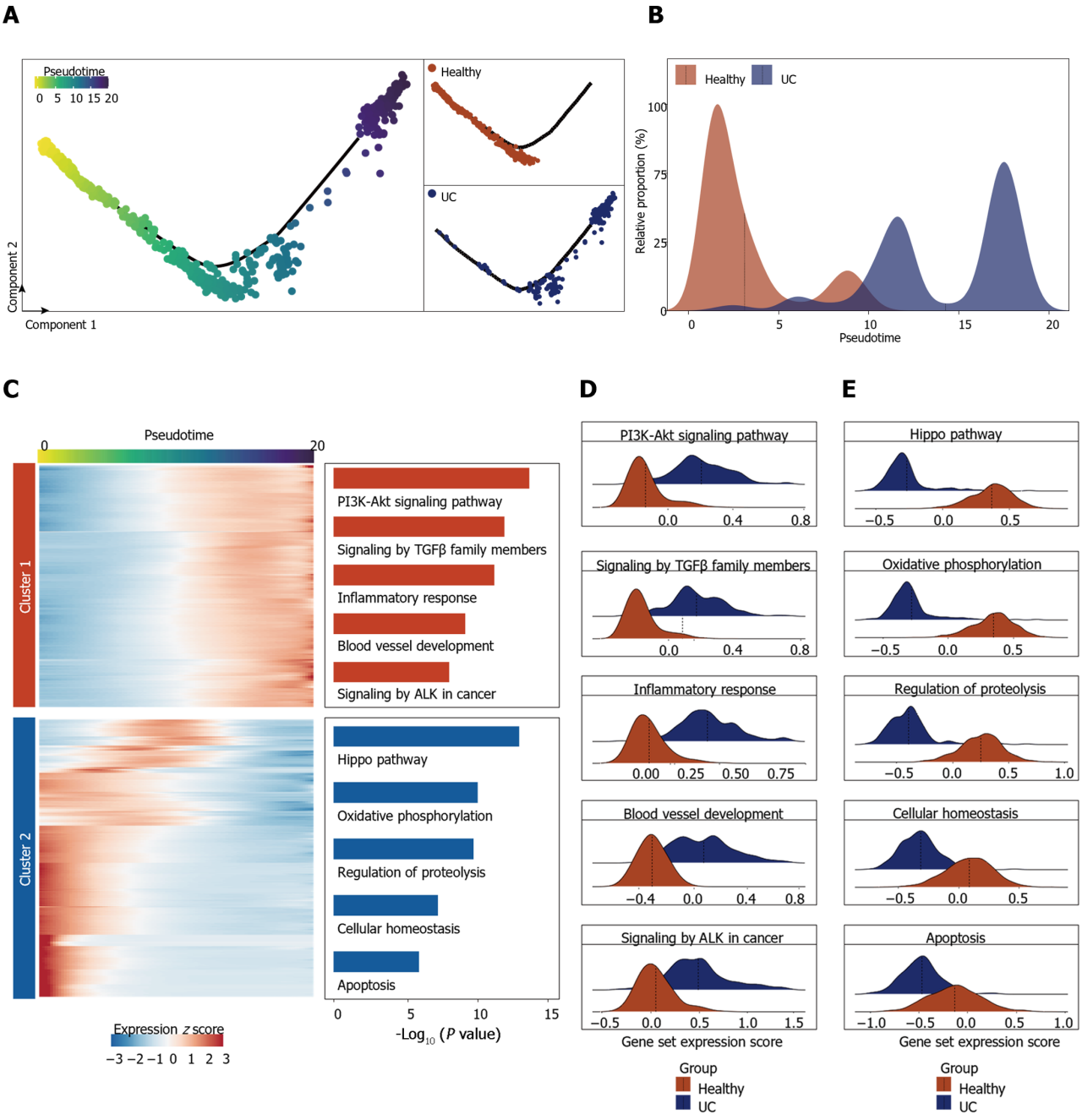
Figure 2 Alterations in transcriptional profiles across different cell types during ulcerative colitis. A: Heatmap showing the distribution of upregulated differential expression genes (DEGs) (adjusted *P* value ≤ 0.05, Log2FC ≥ 0.25) for each cell type between healthy and ulcerative colitis groups (diseased/healthy); B: Heatmap showing the gene function annotations of upregulated DEGs; C: Heatmap showing the distribution of downregulated DEGs (adjusted *P* value ≤ 0.05, Log2FC ≤ 0.25) for each cell type between healthy and ulcerative colitis groups (diseased/healthy); D: Heatmap showing the gene function annotations of downregulated DEGs; E: Ring heatmap showing the top 100 upregulated DEGs during ulcerative colitis; F: Ring heatmap showing the top 100 downregulated DEGs during ulcerative colitis.


A
Pseudotime
0 5 10 15 20
Healthy
UC
Component 2
Component 1
B
Healthy
UC
Relative proportion (%)
100
75
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0 5 10 15 20
Pseudotime
C
Pseudotime
0
20
Cluster 1
Cluster 2
PI3K-Akt signaling pathway
Signaling by TGFβ family members
Inflammatory response
Blood vessel development
Signaling by ALK in cancer
Hippo pathway
Oxidative phosphorylation
Regulation of proteolysis
Cellular homeostasis
Apoptosis
0 5 10 15
-Log10 (P value)
Expression z score
−3 −2 −1 0 1 2 3
D
PI3K-Akt signaling pathway
0.0 0.4 0.8
Signaling by TGFβ family members
0.0 0.4 0.8
Inflammatory response
0.00 0.25 0.50 0.75
Blood vessel development
−0.4 0.0 0.4 0.8
Signaling by ALK in cancer
−0.5 0.0 0.5 1.0 1.5
Gene set expression score
Group
Healthy
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Hippo pathway
−0.5 0.0 0.5
Oxidative phosphorylation
−0.5 0.0 0.5
Regulation of proteolysis
−0.5 0.0 0.5 1.0
Cellular homeostasis
−0.5 0.0 0.5
Apoptosis
−1.0 −0.5 0.0 0.5 1.0
Gene set expression score
Group
Healthy
UC
F
TGFB3
EGFR
FGFR2
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10.0
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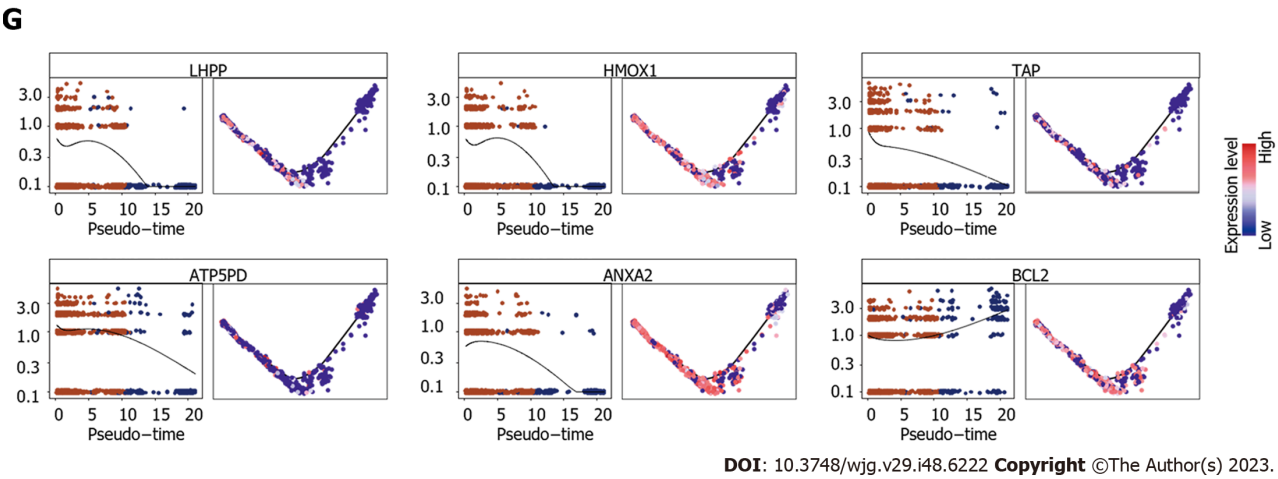

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Figure 3 Cellular and molecular dynamics of endothelial cells during ulcerative colitis progression. A: Pseudotime trajectory analysis of endothelial cell (EC). Left, pseudotime scores of EC. Top right, the distribution of ECs in healthy group. Bottom right, the distribution of ECs in ulcerative colitis group; B: Ridge plot showing the cell number distribution of EC in healthy and ulcerative colitis groups along pseudotime trajectory of Figure 3A; C: Heatmap showing the time-related gene expression profiles during ulcerative colitis, with gene function annotation on the right; D: Ridge plots showing the expression score of gene set from cluster 1 in Figure 3C of healthy and ulcerative colitis groups; E: Ridge plots showing the expression score of gene set from cluster 2 in Figure 3C of healthy and ulcerative colitis groups; F: Scatter plots and trajectory plots showing the expression level of top genes in cluster 1 of Figure 3C; G: Scatter plots and trajectory plots showing the expression level of top genes in cluster 2 of Figure 3C.

mation, we observed the upregulation of pivotal transcription factors such as *STAT3* and *FOXP1*. Correlation analysis of common regulatory factors revealed a negative association between LHPP and the expression of *STAT3* and *FOXP1*. LHPP, known for its role in cell proliferation and tumor suppression, displayed a significant negative correlation with *STAT3* and a less significant correlation with *FOXP1* (Figure 4B and C).

To further substantiate our findings, we recruited a cohort of 15 UC patients and 12 healthy controls, from whom we obtained intestinal tissue samples. Histological assessment (HE staining) of these tissue specimens indicated a significantly elevated presence of chronic inflammation in UC patients ($P < 0.05$; Figure 4D). Subsequent qPCR analysis of these tissue specimens demonstrated elevated mRNA expression levels of inflammation-related genes, including *IL6*, *IL1A*, *CRP*, consistent with our preceding analyses (Figures 2A and C). Notably, in comparison to the healthy control group, we observed a significant reduction in LHPP mRNA expression and an increase in *STAT3* expression within the UC patient group ($P < 0.05$), although the increase in *FOXP1* expression did not reach statistical significances (Figure 4E). Furthermore, western blot analysis of the enrolled individuals provided further confirmation, showing a substantial decrease in LHPP expression and an increase in STAT3 expression among UC patients ($P < 0.05$; Figure 4F and G). These findings suggest a potential transcriptional inhibitory role of STAT3 on LHPP expression.

Finally, we conducted ELISA testing on blood samples collected from 23 matched pairs of healthy individuals and UC patients. Our analysis revealed a noteworthy elevation in IL6 levels among UC patients, underscoring the systemic inflammatory effects associated with UC ($P < 0.01$; Figure 4H). Furthermore, the diminished levels of plasma LHPP in UC patients hint at its potential as a predictive biomarker for UC ($P < 0.05$; Figure 4H). These findings emphasize the systemic impact of UC-related inflammation and point towards the promising prospect of utilizing LHPP as a potential predictive marker for this condition.

DISCUSSION

UC is a complex and chronic IBD that predominantly affects the colon and rectum. Despite significant progress in understanding the pathogenesis of UC, there remains a need for more precise and targeted therapeutic interventions. Our study leveraged scRNA-seq technology to delve into the intricate cellular and molecular landscape of UC. We observed a notable upregulation of inflammatory response pathways during UC progression, indicating an active immune response against inflamed tissues. The increased presence of cytotoxic $CD8^+$ T cells and NKT cells suggests an encouraging antitumor response, but the declining proportions of $CD4^+$ T cells and B cells raise concerns about potential immune evasion strategies employed by UC cells.

A particularly intriguing finding in our study was the sustained downregulation of LHPP in UC, coupled with its negative correlation with *STAT3*. Our experimental data supported the notion that *STAT3* may transcriptionally inhibit LHPP expression, both in intestinal tissue and plasma samples of UC patients. LHPP's multifaceted roles in tumor suppression, apoptosis regulation, and immune response modulation make it a promising candidate for further investigation in the context of UC pathogenesis.

Moreover, the heightened angiogenesis observed in UC underscores the importance of vascularization in disease progression. Enhanced vascularization provides UC cells with access to vital nutrients and oxygen, facilitating their survival and proliferation. This finding may open avenues for targeted therapies aimed at disrupting angiogenic

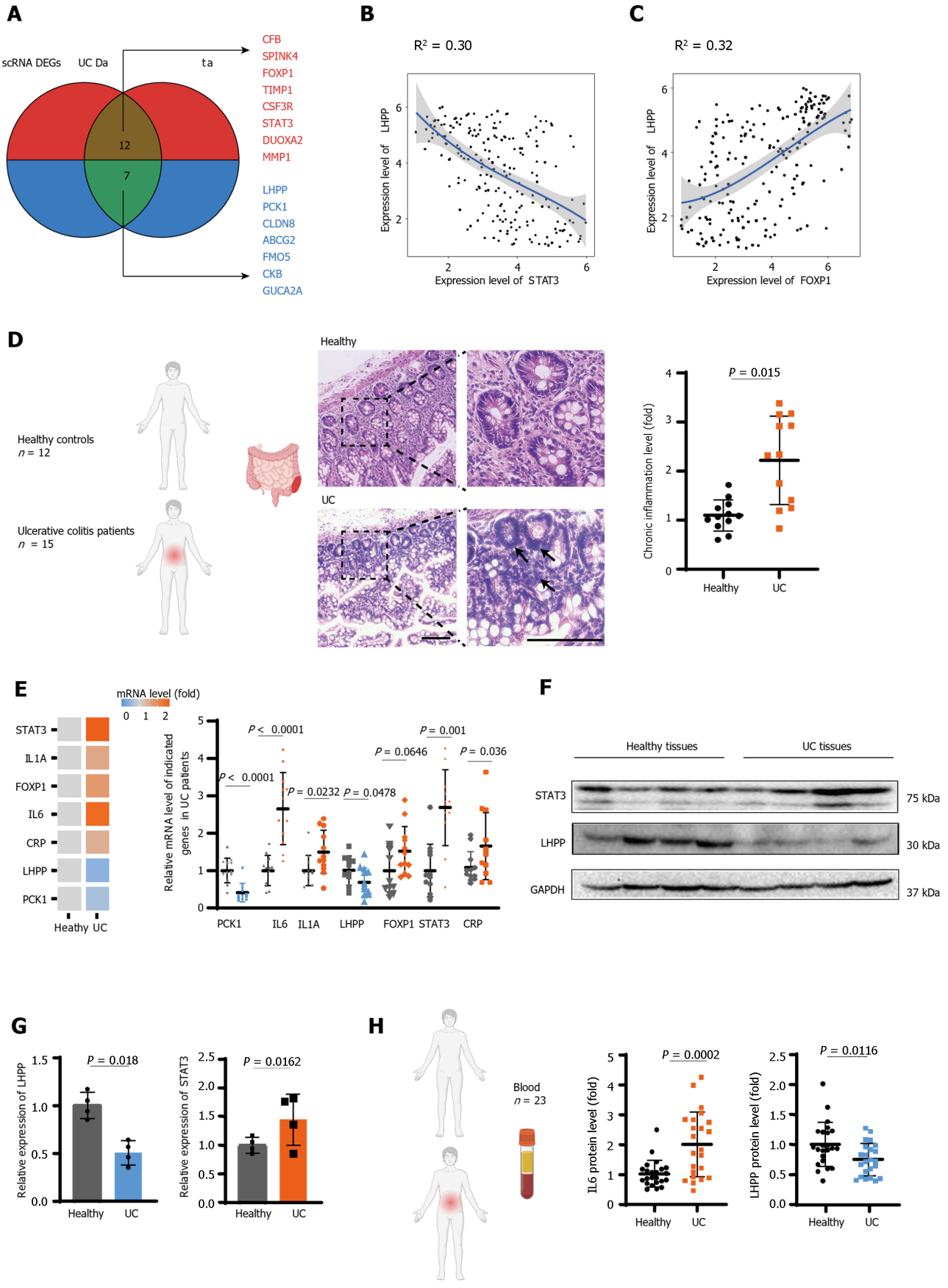

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Figure 4 Phospholysine phosphohistidine inorganic pyrophosphate phosphatase was identified as a core regulator for ulcerative colitis.

A: Venn plot showing the overlap between differential expression genes and public ulcerative colitis data; B: Scatter plot showing the correlation of expression levels between phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) and signal transducer and activator of transcription 3 (*STAT3*); C: Scatter

plot showing the correlation of expression levels between LHPP and FOXP1; D: HE stained colon biopsy samples from healthy individuals and patients diagnosed with ulcerative colitis (UC). Representative images were captured at × 20 magnification. Black arrows: Increased chronic inflammatory infiltrate; E: The relative mRNA level of *STAT3*, *FOXP1*, *IL6*, *IL1A*, *CRP*, LHPP, and *PCK1* was detected in 12 paired heathy and UC patient tissues; F: Western blot verifying differential STAT3 and LHHP expression in heathy and UC patient tissues; G: The relative expression analysis of STAT3 and LHHP in heathy and UC patient tissues; H: Enzyme-linked immunosorbent assay analysis of IL6 and LHPP levels in serum of heathy and UC patient.

processes in UC.

In summary, our study sheds light on the cellular and molecular mechanisms driving UC pathogenesis. These insights provide a foundation for future research into potential therapeutic interventions, with LHPP emerging as a key player in UC regulation and the immune response. Ultimately, unraveling the complexities of UC at the single-cell level holds promise for more effective treatments and improved outcomes for patients with this challenging condition.

CONCLUSION

This study suggests that LHPP may serve as a potential therapeutic target for UC, emphasizing its importance as a potential key target in UC and unveiling its role in inflammation regulation.

ARTICLE HIGHLIGHTS

Research background

Ulcerative colitis (UC) is a chronic intestinal condition characterized by inflammation and ulceration, and it is a significant risk factor for colorectal cancer. Recent advances in single-cell RNA sequencing (scRNA-seq) technology offer a promising avenue for dissecting the complex cellular inter-actions and molecular signatures driving UC pathology.

Research motivation

A comprehensive understanding of the cellular and molecular mechanisms underlying UC development and progression remains elusive, and there is a growing need for more precise and targeted therapeutic interventions.

Research objectives

The object of this study is to utilize scRNA-seq technology to dissect the complex cellular interactions and molecular signatures that underlie UC pathology.

Research methods

We integrated and analyzed the scRNA-seq data from UC patients. Moreover, we conducted mRNA and protein level assays as well as pathology-related staining tests on clinical patient samples.

Research results

We identified the sustained upregulation of inflammatory response pathways during UC progression, characterized the features of damaged endothelial cells in colitis. Furthermore, we uncovered the downregulation of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) has a negative correlation with signal transducer and activator of transcription 3. Significant downregulation of LHPP in UC patient tissues and plasma suggests that LHPP may serve as a potential therapeutic target for UC. This paper highlights the importance of LHPP as a potential key target in UC and unveils its potential role in inflammation regulation.

Research conclusions

This study suggests that LHPP may serve as a potential therapeutic target for UC, emphasizing its importance as a potential key target in UC and unveiling its role in inflammation regulation.

Research perspectives

This study sheds light on the cellular and molecular mechanisms driving UC pathogenesis. These insights provide a foundation for future research into potential therapeutic interventions, with LHPP emerging as a key player in UC regulation and the immune response. Ultimately, unraveling the complexities of UC at the single-cell level holds promise for more effective treatments and improved outcomes for patients with this challenging condition.

FOOTNOTES

Co-first authors: Yan-Fei Wang and Ruo-Yu He.

Author contributions: Cao YF designed and supervised the study, and drafted the manuscript; Wang YF and He RY took the responsibility for statistical analyses and the manuscript; Xu C and Li XL performed manuscript reviewing and editing; all authors have read and approved the article. The reasons for designating Wang YF and He RY as co-first authors are twofold. Wang YF is responsible for all bioinformatics computations and analyses, including handling data. He RY collects samples and conducts experiments. They contributed efforts of equal substance throughout the research process. Second, this study was a team effort, and the co-first authorship accurately reflects how responsibilities and work were shared during the study and paper completion. This helps with effective communication and managing post-submission tasks, ultimately improving the paper's quality and reliability. Summary, we believe that designating Wang YF and He RY as co-first authors of is fitting for our manuscript as it accurately reflects our team's collaborative spirit, equal contributions, and diversity.

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LETTER TO THE EDITOR

Potential therapeutic targets for nonalcoholic fatty liver disease: Glucagon-like peptide 1

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most rapidly growing contributor to liver mortality and morbidity. Hepatocellular injury in nonalcoholic steatohepatitis (NASH) is caused by an increase in metabolic substrates (glucose, fructose, and fatty acids), leading fatty acids to participate in pathways that cause cellular injury and a poor response to injury. The pathogenesis of this disease is largely associated with obesity, type 2 diabetes, and increasing age. To date, there are no Food and Drug Administration-approved treatments for NAFLD/NASH or its associated fibrosis. Since one of the pathogenic drivers of NASH is insulin resistance, therapies approved for the treatment of type 2 diabetes are being evaluated in patients with NASH. Currently, the glucagon-like peptide-1 receptor agonist (GLP-1RA) semaglutide is a safe, well-studied therapeutic for NAFLD/NASH patients. Existing research demonstrates that semaglutide can increase the resolution of NASH but not improve fibrosis. However, improving the fibrosis of NAFLD is the only way to improve the long-term prognosis of NAFLD. Given the complex pathophysiology of NASH, combining therapies with complementary mechanisms may be beneficial. Researchers have conducted trials of semaglutide in combination with antifibrotic drugs. However, the results have not fully met expectations, and it cannot be ruled out that the reason is the short trial time. We should continue to pay increasing attention to GLP-1RAs.

Key Words: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Antidiabetic drugs; Glucagon-like peptide 1; Semaglutide

Core Tip: Semaglutide is effective and safe for nonalcoholic fatty liver disease (NAFLD) but does not improve fibrosis. The treatment of NAFLD requires further combinations of drugs with different and complementary mechanisms of action.

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TO THE EDITOR

We read with great interest the work by Zhu *et al*[1], who further validated that semaglutide can improve the resolution of nonalcoholic steatohepatitis (NASH) but not fibrosis by summarizing the histological results of semaglutide in the treatment of nonalcoholic fatty liver disease[1,2].

To date, there are no Food and Drug Administration-approved treatments for nonalcoholic fatty liver disease (NAFLD)/NASH or its associated fibrosis. Fibrosis or cirrhosis has been recognized in recent guidelines as the main diagnostic and therapeutic target to halt the progression of NASH to end-stage liver disease, change the natural history of the disease, and improve the long-term prognosis of patients with NASH[3,4].

Because insulin resistance is a shared characteristic of type 2 diabetes and obesity and is a key pathogenic driver of NASH[5], pharmacologically, antidiabetic drugs with weight loss effects should be effective against NASH. Therefore, most of the antidiabetic drugs used to treat NASH focus on peroxisome proliferator-activated receptor (PPAR) agonists, glucagon-like peptide-1 receptor agonists (GLP-1RAs), or sodium-glucose transporter 2 (SGLT2) inhibitors[6,7]. Pioglitazone can improve fibrosis in NASH[7]. However, its harmful adverse effects limit its use, and its long-term benefits are not obvious, thus reducing enthusiasm for its use[8,9]. Currently, SGLT-2 is and GLP-1 RAs are gaining more attention in the treatment of NAFLD/NASH metabolic dysfunction-associated fatty liver disease.

GLP-1 RAs may represent the most promising treatment option for improving hepatic steatosis and liver enzyme levels (serum aspartate transaminase, alanine transaminase and gamma-glutamyl transferase) in patients with NAFLD[10]. As a GLP-1 RA, semaglutide appears to be more prominent in the treatment of NASH[11]. Semaglutide activates the hepatic PPAR-α, thereby reducing apolipoprotein C production and breaking down fats and triglycerides in plasma, delaying gastric emptying, prolonging satiety, and reducing waist circumference[3]. Moreover, semaglutide increases insulin production and secretion and decreases glucagon secretion[3]. Current studies have demonstrated that semaglutide is effective in reducing hepatic steatosis and inflammation but not fibrosis[2,5]. The complex pathophysiology of the disease and the multiple often redundant "escape" treatment pathways strongly suggest that combinations of therapies with different but complementary mechanisms of action are considered the best way to improve efficiency, slow disease progression, and even reverse NASH[7].

Alkhouri *et al*[12] conducted a phase 2 clinical trial to validate the potential value of combination therapy[7]. The results of this experiment showed that GLP-1 RA in combination with antifibrotic drug therapy [cilofexor (nonsteroidal FXR agonist)/firsocostat (carboxylase inhibitor)] demonstrates greater improvement in hepatic steatosis, liver biochemistry, and noninvasive fibrosis measured by the magnetic resonance imaging-estimated proton density fat fraction, despite similar weight loss (7%-10%) compared to semaglutide monotherapy[12-15]. The role of GLP-1 RAs is undeniable, and combinations of GLP-1 RAs and other incretin receptor agonists have been developed with good results [16]. Cotadutide, a GLP-1R/GcgR agonist, significantly improves glucose tolerance and decreases C3M plasma levels and P4NP7S circulating levels compared to liraglutide and obeticholic acid in biological experiments[17]. Tirzepatide, a dual agonist of GLP-1 and glucose-dependent insulinotropic polypeptide receptors, has associated therapeutic effects and can induce significant weight loss, improve glycaemic control, and improve plasma lipids in trials related to the treatment of obesity and diabetes[16,18]. In addition, the new drugs lanifibranor (a pan-PPAR agonist) and Aramchol (a partial inhibitor of hepatic stearoyl-CoA desaturase) have performed well as an antifibrotic therapy for NASH, but it is unclear whether there is an unintended effect when combined with GLP-1 RAs[7,19].

Although GLP-RA monotherapy does not perform well as an antifibrotic therapy, it is undeniable that its associated combinations have a role in NASH. In addition, GLP-1RAs have outstanding performance in fat loss, weight loss, and improvement of insulin resistance and have a potential protective effect against the complications of NASH. Thus, we should pay closer attention to GLP-1RAs.

FOOTNOTES

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