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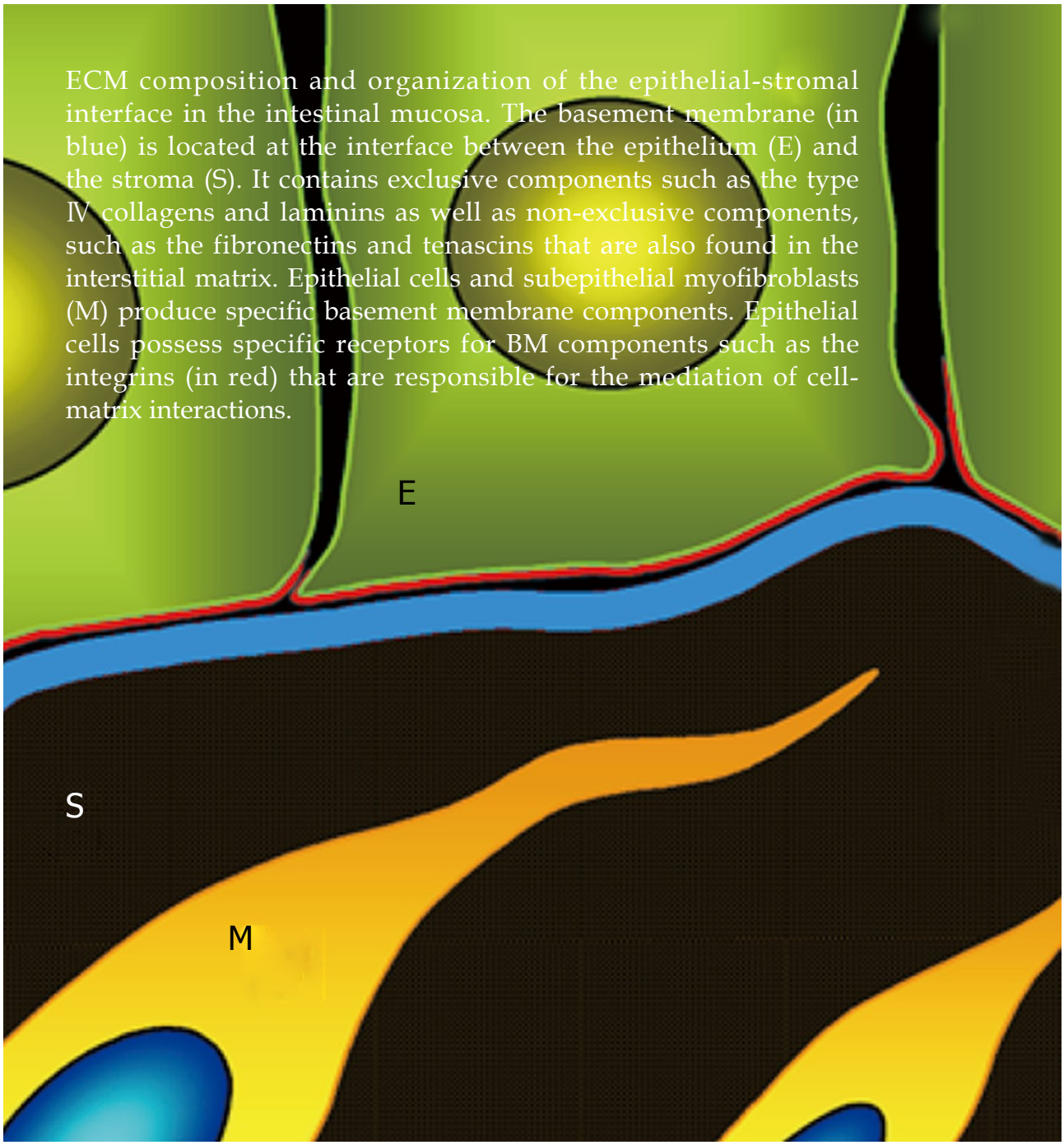

ECM composition and organization of the epithelial-stromal interface in the intestinal mucosa. The basement membrane (in blue) is located at the interface between the epithelium (E) and the stroma (S). It contains exclusive components such as the type Ⅳ collagens and laminins as well as non-exclusive components, such as the fibronectins and tenascins that are also found in the interstitial matrix. Epithelial cells and subepithelial myofibroblasts (M) produce specific basement membrane components. Epithelial cells possess specific receptors for BM components such as the integrins (in red) that are responsible for the mediation of cell-matrix interactions.

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2010-2015

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EDITORIAL

What is the purpose of launching *World Journal of Gastrointestinal Pathophysiology*?

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Abstract

The first issue of *World Journal of Gastrointestinal Pathophysiology* (*WJGP*), whose preparatory work was initiated in June, 2009, is published on April 15, 2010. The *WJGP* Editorial Board has now been established and consists of 154 distinguished experts from 27 countries. Our purpose of launching *WJGP* is to publish peer-reviewed, high-quality articles *via* an open-access online publishing model, thereby acting as a platform for communication between peers and the wider public, and maximizing the benefits to editorial board members, authors and readers.

Key words: Maximization of personal benefits; Editorial board members; Authors; Readers; Employees; *World Journal of Gastrointestinal Pathophysiology*

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INTRODUCTION

I am very pleased to announce that the first issue of *World Journal of Gastrointestinal Pathophysiology* (*World J Gastrointest Pathophysiol*, *WJGP*, online ISSN 2150-5330, DOI: 10.4291) is published on April 15, 2010. The *WJGP* Editorial Board has now been established and consists of 154 distinguished experts from 27 countries.

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. To realize these desired attributes of a journal and create a well-recognized journal, the following four types of personal benefits should be maximized.

MAXIMIZATION OF PERSONAL BENEFITS

The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others.

Maximization of the benefits of editorial board members

The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution.

Maximization of the benefits of authors

Since *WJGP* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJGP* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading.

Maximization of the benefits of readers

Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion[1].

Maximization of the benefits of employees

It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal[2,3]. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, *WJGP* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the experiment is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

SCOPE

The major task of *WJGP* is to report rapidly the most recent results in basic and clinical research on gastrointestinal pathophysiology, including all aspects of normal or abnormal function of the gastrointestinal tract, hepatobiliary system, and pancreas. *WJGP* specifically covers growth and development, digestion, secretion, absorption, metabolism and motility relative to the gastrointestinal organs, as well as immune and inflammatory processes, and neural, endocrine and circulatory control mechanisms that affect these organs. This journal will also report new methods and techniques in gastrointestinal pathophysiological research.

COLUMNS

The columns in the issues of *WJGP* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide Guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Articles: To report innovative and original findings in gastrointestinal pathophysiology; (9) Brief Articles: To briefly report the novel and innovative findings in gastrointestinal pathophysiology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJGP*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of gastrointestinal pathophysiology; and (13) Guidelines: To introduce Consensuses and Guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in gastrointestinal pathophysiology.

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REVIEW

Integrin α6β4 in colorectal cancer

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Abstract

The ability of cells to interact with extracellular matrix macromolecules is at the forefront of the regulation of cell phenotype and organization. Indeed most if not all cells bear specific cell surface receptors for these molecules, namely the integrins, which are specific for the ligation of various macromolecules such as the laminins, fibronectins and tenascins. It is now well established that integrins can regulate a variety of biological activities, most notably cell cycle and tissue-specific gene expression. In the intestine, several observations suggest functional roles for cell-matrix interactions in the regulation of epithelial cell functions. This article focuses on integrin α6β4 as a paradigm to illustrate the importance as well as the complexity of integrins in the mediation of cell-matrix interactions. Indeed, α6β4 has been well-characterized for its involvement as a link between the cytoskeleton and extracellular matrix molecules as well as in the activation of a variety of intracellular signalization processes in cooperation with growth factor receptors. Furthermore, recent studies show that distinct forms of α6 and β4 subunits are expressed in the human intestine and, more importantly, recent work provides experimental evidence that various forms of α6β4 can differentially regulate intestinal epithelial cell functions under both normal and pathological conditions. For instance, it has been discovered that colorectal cancer cells express a hybrid form of α6β4 that is never seen in normal cells. Although further work is needed, integrin α6β4 is emerging as a key regulator of intestinal functions in both intestinal health and disease.

Key words: Human; Integrin; Colon; Intestine; Epithelium; Cell proliferation; Cell differentiation; Colorectal cancer; Adenocarcinoma

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Beaulieu JF. Integrin α6β4 in colorectal cancer. *World J Gastrointest Pathophysiol* 2010; 1(1): 3-11 Available from: URL: http://www.wjgnet.com/2150-5330/full/v1/i1/3.htm
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INTRODUCTION

The digestive epithelium is a highly organized tissue that requires only 3 to 5 d to be completely replaced. The regulation of its renewal and expression of digestive and absorptive functions depends on a number of factors[1-3]. Among these, the extracellular matrix macromolecules such as the fibronectins, tenascins and laminins play an important role[1-5]. As for other epithelia, the intestinal epithelium lies on a specialized thin extracellular matrix, the basement membrane (BM) (Figure 1). BM composition defines the microenvironment required for the expression of cell functions such as proliferation,

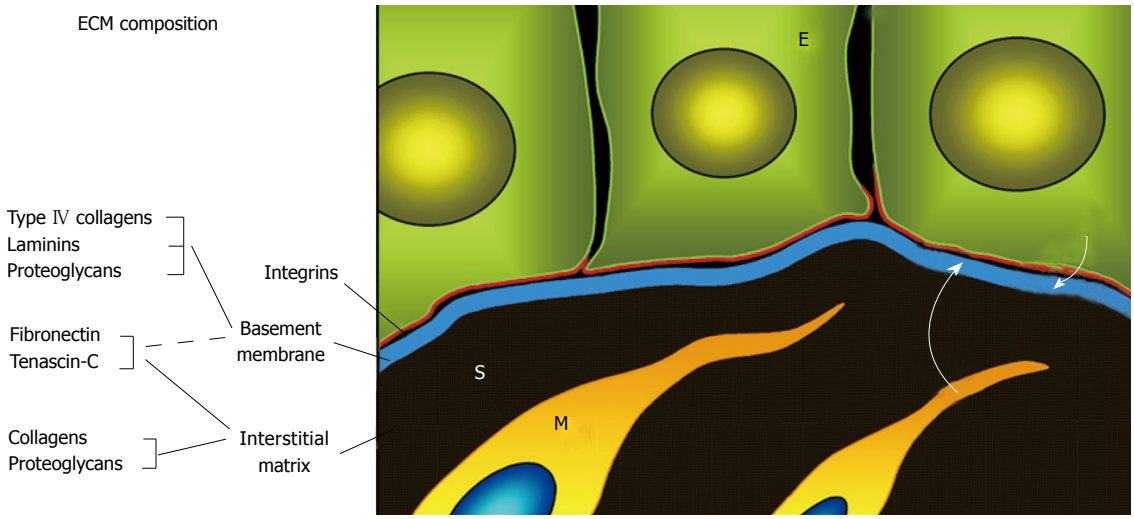

Figure 1 ECM composition and organization of the epithelial-stromal interface in the intestinal mucosa. The basement membrane (in blue) is located at the interface between the epithelium (E) and the stroma (S). It contains exclusive components such as the type Ⅳ collagens and laminins as well as non-exclusive components, such as the fibronectins and tenascins that are also found in the interstitial matrix. Epithelial cells and subepithelial myofibroblasts (M) produce specific basement membrane components. Epithelial cells possess specific receptors for BM components such as the integrins (in red) that are responsible for the mediation of cell-matrix interactions. Adapted from references [4,5].

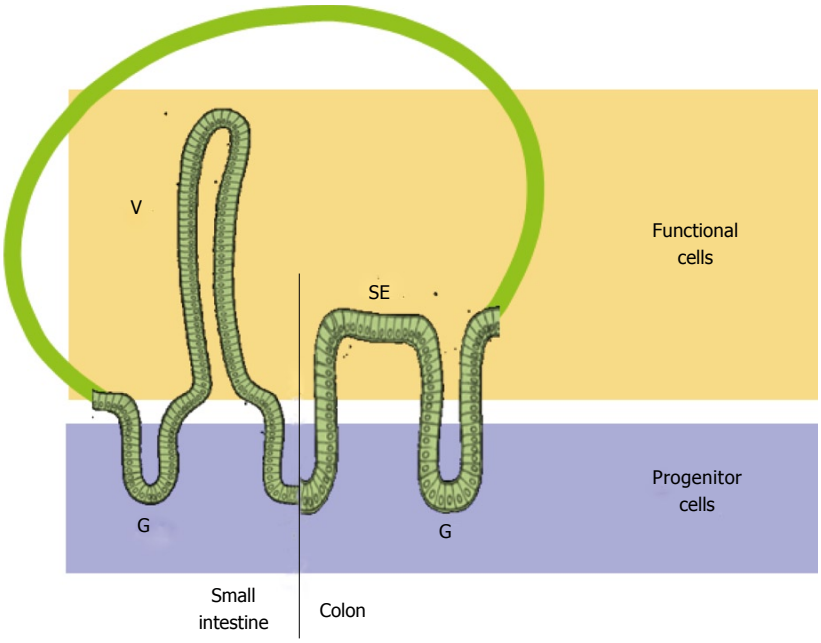

Figure 2 Organization of the renewal units of the human adult small intestine and colon. In the small intestine, the crypt-villus axis consists of stem and progenitor cells, located in the lower and middle third of the gland (G), respectively, with terminal differentiation occurring in the upper third so that all villus (V) cells are mature and functional. In the colon, the stem cells are located at the bottom of the glands (G) while the proliferative cell compartment extends to the middle. Cells in the upper half of the glands and the surface epithelium (SE) are non-proliferative and functional. Adapted from reference [5].

migration, tissue-specific gene expression and apoptosis[6]. Indeed, cell-matrix interactions are mediated by a variety of membrane receptors, many of which are members of the integrin superfamily[7-9].

INTEGRINS AND THEIR LIGANDS IN THE INTESTINE

The intestinal epithelium is a particularly attractive system for deciphering the role of cell-matrix interactions in the regulation of cell functions. In both the normal small and large intestine, the epithelial renewal unit consists of spatially well-separated progenitor and functional cell populations[2,5,10,11]: the crypt-villus axis in the small intestine and the glandular-surface epithelium axis in the colon (Figure 2). Analysis of the expression patterns of integrins and their ligands along the epithelial renewal units of the healthy and pathologic human small intestine and colon has provided crucial basic information on the potential implication of each of these molecules relative to cell state and has identified fundamental differences between the human and rodents[3-5,12-15]. Used in concert with the well-characterized experimental human cell models that replicate the intestinal cell life cycle throughout the epithelial renewal unit[16], these data have led to significant progress in our understanding of the implications of cell-matrix interactions on the regulation of intestinal cell functions.

The human intestinal BM contains all major macromolecules typical of basal lamina such as the type IV collagens and laminins as well as other non-exclusive components such as the fibronectins[4,13]. An important feature of the intestinal BM is that its composition varies considerably along the renewal unit[5,17-31]. For instance, two functionally important laminins[32], LM-211 and LM-511, are subject to a reciprocal pattern of expression along the small intestinal crypt-villus axis, being restricted to the proliferative and differentiated compartments, respectively[25]. Other laminins are also subject to unique spatial and temporal patterns of expression[28,30,31]. Interestingly, patterns of laminin expression are altered in various intestinal pathologies including the chronic inflammatory bowel diseases[33] and cancer[34,35] (see[5] for a review). Taken together, these observations suggest functional roles for these interactions in the regulation of intestinal cell functions.

The biological activities of BM macromolecules depend on the repertoire of specific receptors expressed by the cells involved. Numerous receptors for extracellular matrix molecules have been identified but the integrins are considered to be the main mediators of cell-matrix interactions[8,9]. Indeed, the integrins act as fully functional membrane receptors that can trigger cytoskeletal rearrangements and a variety of intracellular signaling events leading to changes in gene expression[36-38]. Integrins are a superfamily of transmembrane αβ heterodimer glycoproteins that represent a major and ubiquitous class of receptors. There are at least a dozen of them that can interact with extracellular matrix molecules, of which many are expressed by human intestinal epithelial cells[4,5,12-14]. As for their ligands, integrin expression is highly regulated along the intestinal epithelial renewal unit. For instance, the α2β1 and α7β1 integrins are predominantly expressed in the proliferative cells of the glands[18] and newly differentiated cells[39], respectively. Further examples involve other β1 integrins such as α5β1, α8β1 and α9β1[18,40-43].

Surprisingly, α6β4, one of the best characterized integrins involved in the regulation of many cell functions such as proliferation, migration and survival in both health and disease[36,44-47], was initially found to be uniformly distributed at the base of epithelial cells from the bottom of the glands to the tip of the small intestinal villus and the surface epithelium of the colon[25,48,49]. The difficulty in interpreting the ubiquitous expression of α6β4 arose from the existence of splicing variants for both α6 (α6A-B) and β4 (β4A-E)[50] and proteolytically processed forms[51,52]. As reviewed herein, recent studies have shown that distinct forms of the α6 and β4 subunits are expressed in the intestine and, more importantly, recent work provides experimental evidence that various forms of α6β4 can differentially regulate intestinal epithelial cell functions under both normal and pathological conditions.

INTEGRIN α6β4

The α6β4 integrin is expressed at the base of most epithelial cells where it serves as a laminin receptor[53-55]. This integrin is considered to be an exception among the integrins in both structural and functional aspects. One of the most peculiar features of α6β4 is the atypically long cytoplasmic domain of its β subunit, which is involved in the formation of hemidesmosomes[56] as well as in complex signaling functions[57,58].

The cytoplasmic domain of β4 can interact with the keratin network *via* plectin to initiate the formation of hemidesmosomes, which are specialized structures that mediate the attachment of epithelial cells to laminins in the BM[47,59-61]. Although structurally complex, hemidesmosomes are dynamic structures that can rapidly disassemble under specific circumstances such as cell division or migration[62,63]. Cellular α6β4 redistribution[64,65] appears to be regulated by phosphorylation in response to growth factor stimulation[44,59] and involves interaction of α6β4 with the actin cytoskeletal network[45,66,67].

The signaling functions of α6β4 have received much attention. Indeed, its β subunit behaves as a binary tyrosine kinase receptor. Signal transduction is mediated by the activation of a member of the Src kinase family which combines with the juxtamembrane segment of the β4 cytoplasmic domain[64]. Then, Src kinase phosphorylates 5 major tyrosine phosphorylation sites located in the signalling domain of β4[68,69]. Phosphorylation of tyrosine 1526 mediates the recruitment of the adaptor protein Shc and activation of the Ras-MEK-Erk pathway[68] as well as the PI3-K pathway and its targets including Akt, Rac and mTOR[70-72] while phosphorylation of tyrosine 1440 induces the recruitment of Shp2 phosphatase which favours the activation of the Src kinase Shp2[73]. Furthermore, phosphorylation of serines 1356, 1360 and 1364 by PKC is involved in the disassembly of hemidesmosomes[74], the recruitment of the 14-3-3 proteins and the association with Ron[75]. Cooperation with growth factor receptors appears to play an important role in β4 signalization[37]. Indeed, α6β4 can associate with several tyrosine kinase receptors such as EGF, ErbB2, Met and Ron[64,75-77], which once activated, lead to the phosphorylation of the cytoplasmic domain of β4. Conversely, α6β4 can promote the phosphorylation of associated tyrosine kinase receptors *via* Src activation[73,76]. Interestingly, both series of signals appear to be necessary to generate a sustained intracellular response suggesting that in normal cells, ligation of α6β4 to laminin is required for amplifying the signal generated by the tyrosine receptor kinases. In tumor cells, receptor tyrosine kinases are often mutated or amplified and α6β4 is frequently over-expressed. Cooperation between deregulated β4 and receptor tyrosine kinases could contribute to tumoral growth and invasion[64,77-79].

Taken together, these data suggest that the α6β4 integrin plays an important role in normal cells where it is involved in the formation of hemidesmosomes as well in the regulation of a variety of intracellular signalization processes. In tumor cells, cooperation of over-expressed α6β4 with various growth factor receptors enhances sig-

nals leading to the promotion of cellular events linked to tumor progression. However, fundamental questions such as the precise mechanisms involved remain open[44,45,47].

α6β4 IN THE GUT

A better understanding of how α6β4 functions under both normal and pathological conditions would have significant impact on the diagnosis and/or treatment of epithelium-related diseases. Among these, cancers in general and more particularly colorectal cancers, which is one of the major causes of death by cancer[80,81], appear the most prominent pathologies. However, until recently, α6β4 was considered to be expressed ubiquitously in the human intestinal epithelium and its up- or down-regulation in colorectal cancer was controversial[34,35,82,83]. The data obtained in our laboratory over the last few years have led to a different concept. Indeed, the discovery of distinct forms of the α6β4 integrin, which are functionally distinct and differentially expressed in relation to the cell state, suggests an additional level of complexity for this integrin.

α6Aβ4 AND α6Bβ4 INTEGRINS

Although important, the intrinsic signalling potential of β4 appears to be less than the complete α6β4 heterodimer, suggesting that the α6 subunit has a more important role than thought initially[84-89]. The α6 subunit is involved in signalling by different ways including *via* the association with proteins such as CD151[87,90], other proteins that interact with its GFFKR motif[89] or with its PDZ domain at the C-terminal end[84,88].

The α6 integrin mRNA undergoes alternative splicing to yield two distinct isoforms[91] termed α6A and α6B that exhibit distinct cytoplasmic domains and dissimilar spatial and temporal patterns of tissue expression[92-96]. For instance, α6A is found in the mammary gland and in basal keratinocytes while α6B is the predominant variant in the kidney[93]. These distinct patterns of expression for α6A and α6B have been conserved in many species[50]. Their importance is also suggested from work showing the distinct capacity of the two variants to initiate intracellular signalling events[97,98] and the ability to migrate onto laminin[99] when associated with the β1 integrin subunit; the α6Aβ1 integrin being considered to be the "active" variant relative to α6Bβ1[97-101].

Until recently, nothing was known of the importance of α6Aβ4 and α6Bβ4. Based on the fact that α6 only dimerizes with β4 in intestinal cells[102,103] and that both α6 variants are expressed in this tissue[93], the intestinal epithelium was used to characterize any functional differences between the α6Aβ4 and α6Bβ4 integrins. First, distinct patterns of expression of the α6A and α6B variants were found in the normal intestine. In both the small intestine and colon, proliferative cells of the crypt were found to predominantly express α6A while the differentiated cells of the villus, the Paneth cells, as well as the upper gland and surface epithelial cells of the colon were found to express α6B[103,104]. A similar relationship was observed in intestinal experimental cell models. Second, in addition to an upregulation of the total amounts of α6 subunit, a predominant expression of α6A in relation to α6B was found in both primary colon tumors and adenocarcinoma cell lines suggesting that the enhanced α6A/α6B ratios may be linked to the proliferative status of colon cancer cells[103]. Further studies have shown that manipulating the cellular balance of the two α6 variants can alter cell proliferation and influence transcriptional activities related to cell proliferation but not differentiation[103,104]. More specifically, the data suggest that a predominant expression of α6A could favour cancer cell proliferation by a) directly activating the TCF4/β-catenin pathway[104], a well-documented pathway for colorectal cancer progression[105-108] and b) competing with the inhibitory effect of α6B on cell proliferation and c-Myc activity[103].

The specific abilities of α6A to activate pathways linked to cell growth and of α6B to inhibit proliferation are in accordance with their predominant expression in the proliferative and quiescent compartments, respectively, of both the normal small intestine and colon. Up-regulation of α6A expression in primary colon cancers and adenocarcinoma cell lines strongly suggests that the expression and ratio of the α6A and α6B splice variants are inherent to normal intestinal homeostasis and exploited by colon cancer cells.

α6β4ctd+ AND α6β4ctd- INTEGRINS

As mentioned above, the β4 subunit, which is ubiquitously expressed by epithelial cells[12,46,55,109], possesses an unusual β integrin cytoplasmic domain. As for its partner α6, cytoplasmic splice variants have been described but these minor forms of β4 remain poorly characterized compared to the ubiquitous β4A form[50]. On the other hand, a cytoplasmic variant of the β4A subunit that results from the proteolytic cleavage of the C-terminal domain (ctd) has been identified in the human intestinal epithelium[102]. Interestingly, this β4ctd- variant was found to be associated with the proliferative/undifferentiated cells of the crypts in both the small intestine and colon while the non-cleaved β4ctd+ form was only detected in the differentiated cells of the villus and upper gland/surface epithelium in the small intestine and colon, respectively[102,110]. Furthermore, the α6β4ctd- form was not functional for adhesion on purified laminin-332, one of the preferred ligands for α6β4[102].

In colorectal cancer, an overall up-regulation of the expression of the β4 subunit has been found in relation to c-Myc in the primary tumors but the β4ctd- form was lost in both tumors and adenocarcinoma cell lines[110]. Based on the fact that a mutation in β4 generating a deletion of this ctd domain is lethal in man[111], its function appears to be crucial. However, at present, the only potential role for the ctd domain is to self-attach to the connecting

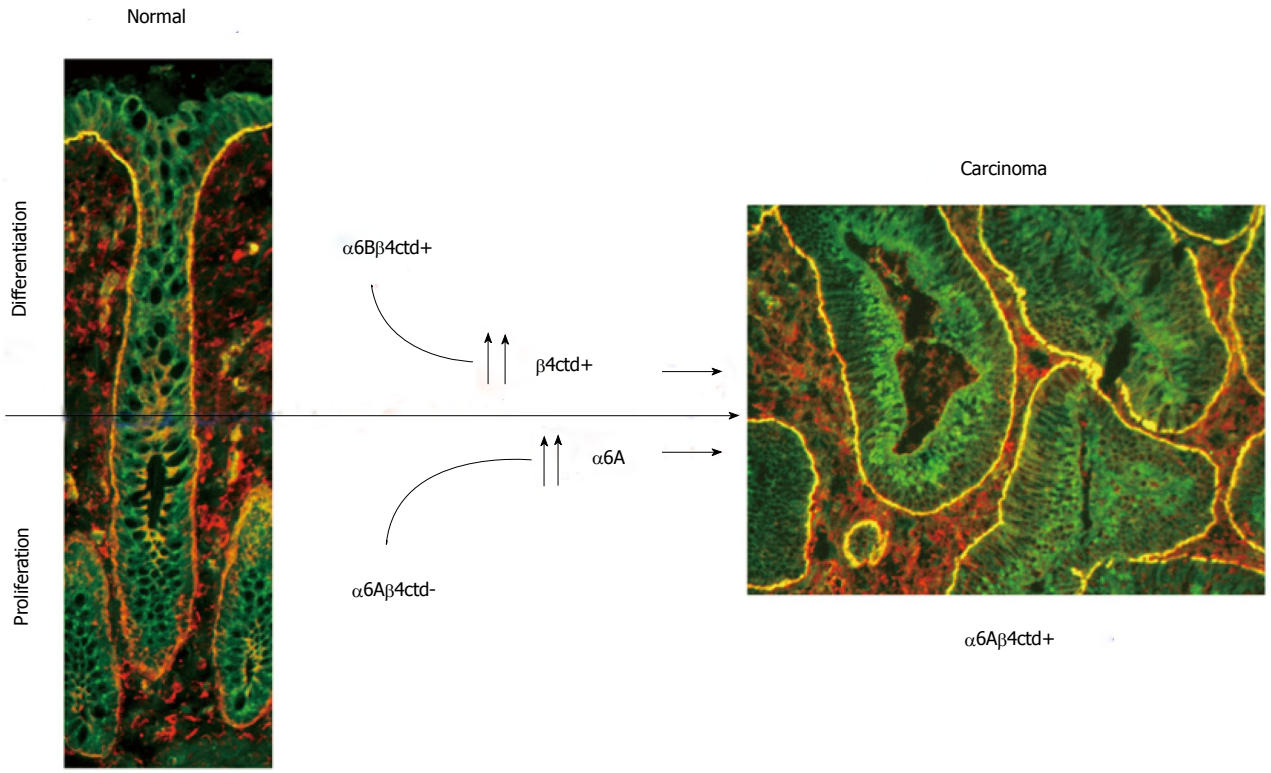

Figure 3 Colorectal cancer cells express a form of α6β4 that is not found in normal colonic cells. In the normal colon, progenitor cells express the α6Aβ4ctd- form which is pro-proliferative but not functional for adhesion while differentiated cells express the α6Bβ4ctd+ form which is anti-proliferative and functional for adhesion. In colorectal carcinoma, the predominant form of α6β4 is α6Aβ4ctd+, which is pro-proliferative and functional for adhesion.

segment, a region located between the two pairs of type Ⅲ fibronectin-like domains of the cytoplasmic β4 forming a loop[112,113] and/or to serve as a binding site for plectin[111,114]. The β4ctd- form, which generates an inactive α6β4 integrin for adhesion, appears to be an exclusive feature of normal proliferative intestinal cells. Its expression as a β4ctd+ form in differentiated normal intestinal cells as well as in adenocarcinoma cells thus raises fundamental questions. For instance, can the fact that the α6β4ctd- integrin is inactive for adhesion be linked to the possibility that the ctd domain is required to maintain a functional conformation of the integrin? While this hypothesis appears to be compatible with the various proposed models of α6β4[47,115-117], further work is needed to verify it. Another interesting challenge would be the identification of the precise mechanism involved in the intracellular cleavage of ctd. Indeed, the characterization of a hypothetical "β4ctd-ase" that could impair tumor growth and promotion may provide an interesting clue in the development of an anti-colorectal cancer therapy.

CONCLUSION

The complexity of the integrin α6β4 has only begun to be unravelled. The α6 and β4 subunits are both up-regulated in various tumor types including colorectal cancer and play key roles in the major intracellular signaling networks. Furthermore, the characterization of cytoplasmic variants for both subunits has revealed new elements to be considered in the equation. Indeed, as illustrated in Figure 3, these findings show on one hand that the α6β4 integrin is present under the α6Aβ4ctd- form (pro-proliferative but not functional for adhesion) in normal proliferative intestinal cells and under the α6Bβ4ctd+ form (anti-proliferative but functional for adhesion) in quiescent and differentiated intestinal cells. In the other hand, in human colorectal adenocarcinoma cells, the predominant form is α6Aβ4ctd+ (pro-proliferative and functional for adhesion), a hybrid form that is never seen in normal cells.

A better understanding of how α6β4 and its variants function under normal and pathological conditions, such as during the tumor progression process, would have significant impact on the diagnosis and treatment of many epithelial cancers including colorectal cancers.

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REVIEW

Molecular mechanisms of cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CC), the malignant tumor of the epithelial cells lining the biliary ducts, has undergone a worldwide increase in incidence and mortality. The malignant transformation of the biliary cells originates from a multistep process evolving through chronic inflammation of the biliary tract to CC. In the last few years several advances have been towards understanding and clarifying the molecular mechanisms implicated in the cholangiocarcinogenesis process. However, many pathophysiologic aspects governing the growth of CC are still undefined. The poor prognosis of this tumor underlines the urgent need to codify the underlying molecular mechanisms involved in the growth and progression of CC in order to design effective preventive measures and valid treatment regimens. This review reports on progresses made in the last few years in clarifying the molecular pathways involved in the process of cholangiocarcinogenesis.

Key words: Cholangiocarcinoma; Molecular mechanism; Cholangiocarcinogenesis; Genetic; Invasion; Apoptosis.

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INTRODUCTION

The malignant transformation of cholangiocytes, the epithelial cells lining the biliary tree, gives rise to cholangiocarcinoma (CC). These tumors may arise from any part of the biliary tree and are anatomically classified as intrahepatic or extrahepatic. 50% of CCs, also called Klatskin tumors, develops in the liver hilum, 42% within extrahepatic biliary ducts and only 8% originate in the intrahepatic biliary ducts. In more of 90% of cases this tumor is histologically classified as an adenocarcinoma[1,2].

Epidemiological data show that the incidence, prevalence and mortality of intrahepatic CC are increasing worldwide[3]. The high mortality and poor outcome of this disease is certainly due to the lack of tools for early diagnosis and treatment[3]. Surgery represents the only curative treatment for CC[1]. However, surgery is only feasible at an early stage of this neoplasia and is characterized by a high rate of recurrence[1]. Unfortunately, because of the lack of specific symptoms coupled with the high level of invasiveness and easy involvement of critical anatomical structures[1,2], in the majority of cases the tumor is usually very advanced at the time of diagnosis. For this reason surgery assumes a palliative more than curative role[1]. Generally, the survival rate is very poor, with less than 5% of the patients surviving up to 5 years[2,3]. Chemotherapy and radiation therapies have been used in an attempt to control this disease and improve the survival and the quality of life of patients with advanced CC. However, these therapeutic strategies are not effective in prolonging long-term survival[4] and their role is only palliative. Recent therapeutic options include brachytherapy and photodynamic therapy, although their effect is not yet established[1,2].

Some risk factors for CC development have been

identified[1]. However, it is a common experience for clinicians in western countries that none of the known specific risk factors are detectable in patients affected by this malignancy.

Recent studies in this field of research have focused on the identification of the molecular mechanisms that regulate CC development. These studies have described many aspects of the intracellular mechanisms associated with the malignant transformation of cholangiocytes that had remained obscure for many years. In particular, several studies have helped to clarify the link between chronic bile duct inflammation and acquisition of a malignant phenotype by cholangiocytes[5]. In this review, we have summarized what is currently known about the mechanisms implicated in the multistep process of cholangiocarcinogenesis.

PROCESS OF CHOLANGIOCARCINOGENESIS

Analogously to other malignant tumors, CC develops as a multistep process of cellular transformation. Biliary epithelium cells undergo genetic and epigenetic alterations in regulatory genes, which accumulate and lead to the activation of oncogenes and the dysregulation of tumor suppressor genes (TSGs)[6-9]. A multitude of mutated genes and pathways have been described in malignant cholangiocytes.

Even if a small number of CCs arise in normal liver, CCs generally develop in a background of chronic inflammation of bile ducts and the consequent cholangiocyte injury associated with cholestasis[1,6].

Some risk factors related to the development of CC are well known[10]. Among them age greater than 65 years, liver fluke infestation by *Opistorchis viverrini* and *Clonorchis sinensis*, primary sclerosing cholangitis (PSC), hepatolithiasis, Caroli's disease, congenital choledochal cysts, bile duct adenoma, anomalous pancreaticobiliary-junction malformations, thorotrast[10]. A number of other factors predisposing to CC development have also been described: smoking, papillomatosis, liver cirrhosis, diabetes mellitus, dioxin and vinyl chloride intoxication, HIV, HBV and HCV infections[10]. However, none of these specific conditions is often detectable in patients affected by this cancer. Independently of the existence of risk factors, malignant transformation of cholangiocytes arises against a background of chronic inflammation[4,6,9,11]. The network of cytokines and molecules present in high concentration during this chronic inflammatory process triggers and maintains the multistep process of cholangiocarcinogenesis[1,6,9,11] (Figure 1). This process is characterized by a progressive accumulation of chromosomal, genetic and epigenetic alterations[7,12,13] (Figure 1). The final result is a sustained overproduction of cytokines, stimulatory or inhibitory growth factors and hormones that drive cholangiocytes to irreversible changes in cell physiology, i.e. (1) dysregulated growth, (2) high capacity of invasiveness; and (3) capacity to metastasize[6,9,11] (Figure 1). Two other particular characteristics of cholangiocarcinoma cells are the epigenetic changes[14] and the process of epithelial-to-mesenchymal transition (EMT)[15] associated with the malignant transformation.

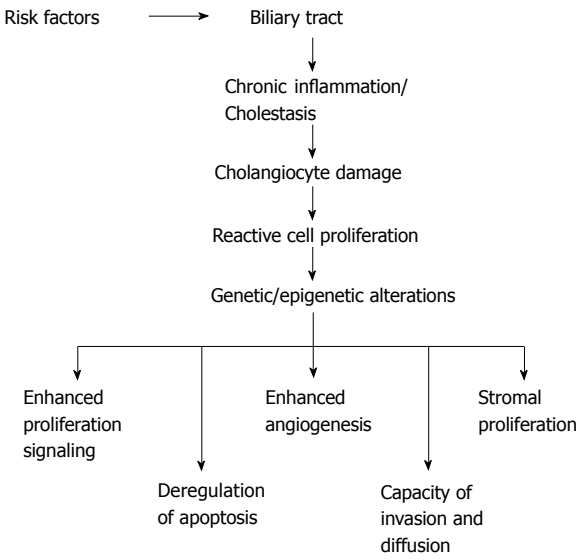

Figure 1 Cholangiocarcinogenesis model. Proposed mechanisms and cellular events linked to malignant transformation of biliary epithelia.

GENETIC ALTERATIONS

Genetic alterations are followed by specific changes in cell physiology such as: (1) stimulation of growth induced by autocrine signals; (2) dysregulated of the mechanisms of replication; (3) insensitivity to growth inhibitory mechanisms; (4) escape from cell apoptosis; and (5) neo-angiogenesis, tissue invasiveness and metastasis[16]. The final result of these altered processes is an uncontrolled cell growth. The principal genes altered during the development of CC, such as *K-ras*, *p53*, *p14ARF*, *p16INK4a* and *β-catenin*, are also altered in other cancer types[7].

K-ras and p53

Mutations of *K-ras* and *p53* oncogenes are described in several epithelial carcinomas. Moreover, *K-ras* and *p53* oncogenes are also often mutated in CC cells[7,17-19]. Such point mutations are located in codon 12 and consist of changes from glycine (GGT) to aspartic acid (GAT) or, less frequently, to valine. Furthermore, mutations have been located at codon 13, involving GGT to GAT, and codon 61, involving CAA to CAC changes[20]. In addition, some Authors described that the expression of *K-ras* correlates with the gross morphology and location of the tumor in the liver[7,20]. In summary, these differences in *K-ras* mutations could be due to the existence of different subtypes of cancers, racial and geographical differences of the patients or use of different assay techniques[7].

p53 is a fundamental tumor suppressor gene with

two important functions: the induction of cell cycle arrest and suppression of Bcl-2 protein expression with consequent blockage of apoptosis. The incidence of *p53* gene mutation is high and varies from 20 to 80% of cases[7]. The evidence that this mutation is more frequent in the mass-forming type tumors means that *p53* mutation could be related to the development of intrahepatic CCs of the peripheral small bile ducts[7,21,22].

In many cases, the p53 protein forms complexes with other molecules such as WAF-1 and mdm-2, which favor its inactivation[23].

p14ARF and p16INK4a are cell cycle regulator genes implicated in the genesis of CCs. However, their mutation or deletion is not frequent in CCs[24,25].

NKG2D

Natural killer (NK) cells are implicated in tumor surveillance by cell-mediated cytotoxicity[26]. The natural killer group 2, member D cell receptor, also known as NKG2D, is expressed by NK cells and T-lymphocytes and is involved in their cytotoxic activity[27]. The role of these cells is highly relevant since some studies have suggested that high levels of cytotoxicity protect PSC patients from the development of CCs in a background of chronic inflammation of the biliary tract[28]. Melum *et al*[28] recently evaluated the *NKG2D* gene in PSC-affected patients and showed that two single nucleotide polymorphisms (SNPs) of the gene were associated with an increased risk of CCs. In addition, a homozygous condition for the non-risk alleles is linked to an extremely low risk of CCs[28]. This finding could be helpful in identifying low risk CC-patients. The development of cancer is a complex biological process, and other yet unknown polymorphisms are likely to be associated with CC risk. Combining *NKG2D* SNPs with other polymorphisms in a panel of markers may be useful in creating a test to assess CC risk[29].

Activation-induced cytidine deaminase

Activation-induced cytidine deaminase (AID) represents a member of the DNA/RNA-editing cytidine deaminase, apolipoprotein B mRNA-editing enzyme catalytic-polypeptide family. Recently, it has been shown that proinflammatory cytokines, abundant in clinical conditions such as PSC and CCs, significantly stimulated AID production in cholangiocytes[30]. Specifically, the DNA mutator *AID* gene is targeted from the IKK-β-dependent NF-κB activation pathway[30] and it is aberrantly expressed during chronic inflammation[30]. Consequently, aberrant expression of *AID* in biliary cells results in the generation of somatic mutations in tumor-related genes, including *p53*, *c-myc*, and the promoter region of the *INK4A/p16* sequences[30]. Komori *et al*[30] speculated that proinflammatory cytokine stimulation is responsible for the aberrant *AID* gene expression in human cholangiocytes, thus providing a possible link between chronic biliary inflammation and the development of CC. However, further studies are necessary to clarify the significance and the role of AID production in stimulating precancerous cells to develop a critical number of genetic changes.

MOLECULAR STEPS OF CHOLANGIOCARCINOGENESIS

Enhanced proliferation signaling

Deregulation of several molecular mechanisms such as ErbB-2, MUC-1, Met, β-catenin, interleukin-6 (IL-6), transforming growth factor-β (TGF-β), signal transducer and activator of transcription-3 (STAT-3), Bcl-2, DCP4/Smad4, hepatocyte growth factor (HGF), reduced glutathione (GSH), Notch-1, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), $p16^{INK4a}$, Ras/Raf and WISP1v[6,9] have been described in CCs as a consequence of the activation of cellular oncogenes or the inactivation of TSGs.

The network of cytokines released into the biliary microenvironment in the course of chronic inflammatory processes is responsible for the induction of malignant transformation of cholangiocytes. The trigger of the cholangiocarcinogenesis process is the activation of an autonomous proliferative signal in the biliary epithelium[6,9]. Cytokines are abundant in the course of chronic inflammation and they are secreted into the liver by a multitude of cell types such as hepatocytes, hepatic stellate cells, sinusoidal endothelial, and Kupffer cells. In addition, recent studies have demonstrated that cholangiocytes themselves produce and release cytokines[31] such as IL-6, TGF-β, IL-8, TGF-α and platelet-derived growth factor B chain[4], which all interact with biliary epithelium in an autocrine/paracrine manner. All these factors are able to regulate biliary cell pathophysiology and several studies have demonstrated that they also play a fundamental role in the development and growth of biliary tract cancers[12]. This suggestion is favored by the fact that cholangiocyte intracellular signaling in response to specific cytokine stimuli is altered during malignant processes[5]. The constitutive activation of cellular receptors and the mitogenic factors abundant in proximity to the biliary epithelium, stimulates the uncontrolled growth of malignant cholangiocytes[1,7].

The development of autonomous proliferative signaling is the principal step in the cholangiocarcinogenesis process (Figure 1). Mitogenic factors are locally secreted by a network of liver cells such as tumoral cells and by the constitutive activation of cellular receptors or various components of the intracellular signaling pathway. They stimulate and enhance the growth of malignant cholangiocytes[1,5].

IL-6 and myeloid cell leukemia-1 (Mcl-1): IL-6 and TGF-β are the two major cytokines involved in the regulation of CC growth[32]. IL-6 is a cytokine secreted in the liver in the course of inflammatory processes by several cell types, including cholangiocytes[21]. Such a cytokine both mediates the immune responses and

stimulates the growth of normal and tumoral cells. Several pieces of data demonstrate a fundamental role of IL-6 in the pathogenesis and growth of CC[21].

IL-6 content is increased in the course of chronic inflammation and the neoplastic processes in biliary ducts. It is also found at increased levels in bile and serum of CC affected patients[33]. This cytokine stimulates cell proliferation by an autocrine/paracrine mechanism[34] and specifically promotes the activation of p44/p42 and p38 mitogen-activated protein kinase (MAPK) pathways[35] which, in turn, decrease the expression of p21 (WAF1/CIP1), a cell cycle controller protein[36]. Several studies have shown that this mitogenic property of IL-6 is mediated by the up-regulation of Mcl-1, a key antiapoptotic Bcl-2 family member protein[37]. More specifically, this effect is dependent upon IL-6 induced increased activation of STAT-3 (constitutively activated in malignant cholangiocytes), which regulates Mcl-1 transcription[22]. These data directly link the inflammatory mediator IL-6 to a potent antiapoptotic signaling mechanism in CC and suggest a critical role of STAT-3 in malignant transformation.

Furthermore, Mcl-1 increases cancer cell resistance to TRAIL[38,39] and inhibition of IL-6 induced expression of Mcl-1 restores sensitivity to TRAIL[40].

JAK/STAT: The activation of the Janus kinase (JAK)/STAT-3 pathway[22] by IL-6 enhances SOCS-3 which, through recruiting Tyr759 of gp130, turns off IL-6 signaling by a negative feed-back to JAK-1. The importance of this pathway in maintaining tumor growth is demonstrated by the fact that this inhibitory loop is inactivated in several human CC cell lines, and re-expression of SOCS-3 reduces STAT-3 activation with consequent inhibition of its target gene Mcl-1. This reduction in Mcl-1 sensitizes CC cells to TRAIL-induced cytotoxicity[39]. In summary, SOCS-3 re-expression can predispose CC cells to TRAIL cytotoxicity by inhibiting IL-6/STAT-3 pathways, with consequent down-regulation of Mcl-1. These findings suggest that the up-regulation of SOCS-3, obtained for example by demethylating agents, could have an important role in inhibiting CC cell growth[39]. The effect of IL-6 has been associated with epigenetic silencing of genes (see other review of this series)[41]. This represents one of the possible links between inflammation and the growth and survival of tumors[41].

Thus, the JAK/STAT pathway is one of the key signaling pathways mediating the resistance of CC cell to apoptosis. In a recent study Blechacz *et al*[42] showed that JAK/STAT signaling can be inhibited by the multikinase inhibitor sorafenib, which could therefore have a therapeutic role in reducing CC growth. The Authors demonstrated that sorafenib induces STAT-3 dephosphorylation by stimulating phosphatase SHP-2 activity and consequently sensitizes CC cells to TRAIL-mediated apoptosis[42].

TGF-β: Transforming growth factor-β, or TGF-β, is a cytokine implicated in a network of functions such as cell growth, differentiation, migration, apoptosis, adhesion, survival and immunity. Several liver cell types synthesize TGF-β[6], whereas cholangiocytes express this cytokine only in pathological conditions such as cholestasis[43]. In general, TGF-β exerts inhibitory effects. For example, it reduces human CC cell proliferation through regulation of the p21 cyclin dependent kinase inhibitor[44]. However, this antiproliferative effect in CC cells is hidden because of the mutations of its receptor (deletion of TβR1) and the alterations of intracellular signaling mediators (e.g. Smad4), together with the intracellular over-expression of cyclin D1[32,45,46]. The lack of TGF-β signaling also stimulates the deposition of fibrotic tissue, abundantly expressed by CC[1]. In summary, the dysregulation of TGF-β signaling leads to an increase of cell proliferation and formation of fibrosis, both hallmarks of CC[46].

DCP4/Smad4: DCP4/Smad4 is a tumor suppressor gene and is also a downstream of TGF-β cascade[47]. It has been postulated that the TGF-β/Smad pathway could play an important role in the regulation of CC growth[48]. In addition, loss of Smad4 correlates with the pTNM stage of intrahepatic CC[48]. Moreover, Smad4 interacts with the other tumor suppressor gene PTEN to maintain a physiologic cellular balance and block the process of cholangiocarcinogenesis[49]. This explains why the lack of functions of these TSGs favors the development of biliary malignancies[49].

c-Met/HGF: *c-Met* is a proto-oncogene located on chromosome 7q that codes for a tyrosine kinase growth factor receptor, highly expressed on the surface of CC cells[50,51]. The ligand for Met is HGF/scatter factor (SF). HGF/SF-Met pathways are implicated in embryonic development. However, abnormal Met signaling has been strongly implicated in the process of tumorigenesis, particularly in the growth of aggressive and metastatic cancers[52].

CC cells express high levels of HGF, both *in vitro* and *in vivo*, together with the up-regulation and hyperphosphorylation of *c-Met*, its specific surface receptor[53]. These data suggest the existence of an autocrine-loop that governs the growth of malignant cholangiocytes[53]. Increased expression of the *c-Met* gene in CC cells is associated with increased cell migration and invasion. Conversely, inhibition of *c-Met* expression is followed by reduced cellular growth and invasiveness[63]. HGF binds to *c-Met* and induces the autophosphorylation of an intracellular tyrosine kinase on the β-subunit of the receptor[52]. This process is followed by the activation of a network of signaling molecules such as Src, P13K, Gab1, SOS, Grb2, and MEK1/2[54], all involved in the regulation of cell invasion, angiogenesis and tumor differentiation/proliferation[55,56].

ErbB-2: Several CC cell lines express large amounts of

the protein ErbB-2[57,58]. This molecule is involved in the development and progression of biliary cancer[59] and its over-expression maintains growth and survival of CC cells. In addition to the direct effect on tumoral cells, ErbB-2 stimulates the production of Cyclooxygenase-2 (COX-2), which forms a complex with a subunit of the IL-6 receptor[60]. This effect suggests a close link between IL-6 and ErbB-2 signaling[1,60].

Recently, Lai and colleagues showed that normal cholangiocytes transfected with the neu (the rat homologue of ErbB-2) oncogene undergo a malignant transformation that closely resembles the molecular features of human CC[61].

GSH: Reduced GSH is the principal intracellular defense against oxidative stress during inflammation[62]. The role of GSH is to favor the reduced state of intracellular molecules and to participate in the detoxification of a multitude of substances[62]. Several studies have demonstrated that GSH is produced by cholangiocytes, but it can also be secreted by hepatocytes and thus be absorbed from bile[9]. An increase of intracellular GSH is associated with decreased cholangiocyte apoptosis and increased Bcl-2 protein expression due to decreased Bcl-2 degradation[62]. Conversely, GSH reduction predisposes cholangiocytes to apoptosis.

Nitric oxide (NO): Inducible nitric oxide synthase (iNOS) is an enzyme found in high concentration during the course of inflammations and malignancies of the biliary tract[63]. Similarly to COX-2, this protein is also involved in a variety of events such as cell proliferation, survival, and angiogenesis[64]. Several pieces of evidence show a strong link between iNOS and COX-2 in the generation of CC[63]. Even if these two proteins could be induced independently[65], iNOS stimulates COX-2 expression through NO production. In particular, iNOS enhances cholangiocyte COX-2 expression, probably through activation of p38 MAPK and JNK1/2[63].

The malignant transformation of cholangiocytes is correlated with an increase of iNOS[13], which stimulates NO. NO counteracts the mechanisms of DNA repair, thus favoring its damage and mutagenesis[9]. Similar to what happens in pancreatic cancer, the effect of NO in promoting CC development is due to the up-regulation of Notch-1[32]. To strengthen this hypothesis, evidence shows that Notch-1 is hyper-expressed in cholangiocytes of patients affected by PSC as well as in CC cells and it co-localizes with iNOS[66]. Notch-1 is stimulated by NO and its expression can be inhibited after cell transfection with iNOS antisense constructs[66]. Inflammatory cytokines represent a stimulus for activation of iNOS. After its activation, the consequent increase of intracellular NO exerts a double effect in the process of cholangiocarcinogenesis: allowing accumulation of DNA mutations by inhibiting DNA repair mechanism[67] and enhancing COX-2 expression[63,66,68].

COX-2: COX is the key enzyme involved in the process of inflammation since it is implicated in the genesis of prostaglandins. COX-1 and COX-2 are the two specific isoforms of COX. COX-1 is constitutively present in several cell types and participates in the homeostatic functions of prostaglandins, while the inducible COX-2 can be stimulated by many molecules, such as cytokines and lipopolysaccharides[69].

COX-2 is increased during the process of inflammation and it stimulates and maintains CC cell growth[70]. Evidence suggests a strong link between COX-2 and the cholangiocarcinogenesis process. For example, in a murine model of biliary adenocarcinoma induced by over-expression of ErbB-2, an increase of COX-2 was initially observed[71] and over-expression of COX-2 enhances rat CC cell growth[72]. Selective COX-2 inhibitors (e.g. celecoxib) have been positively tested to block CC cell proliferation by inducing the apoptotic process[57,72-74]. This antiproliferative effect is accompanied by an inhibition of PDK1 and PTEN, followed by a consequent decrease of Akt phosphorylation[75]. Furthermore, celecoxib treatment also inhibits CC cell proliferation through activation of cyclin-dependent kinase inhibitors p21waf1/cip1 and p27kip1, with consequent cell cycle arrest at G1/S phase[76]. However, not all COX-2 inhibitors are able to inhibit CC cell growth[72].

Recent studies have aimed to codify molecules able to induce the production of COX-2 during the inflammatory process. Oxisterols derive from cholesterol. The involvement of oxisterols in the process of cholangiocarcinogenesis was suggested by the observation that they are present in bile during cholestasis, one of the conditions predisposing to CC development[77]. Moreover, oxisterols enhance COX-2 expression in human CC cells *in vitro*[78].

COX-2 expression was moderately low in cholangiocytes obtained from patients affected by primary biliary cirrhosis (PBC), whereas cholangiocytes from patients affected by PSC showed a very high immunoreactivity[41]. In a recent review, Sirica *et al*[4] speculated that such a discrepancy between PBC and PSC might explain the higher incidence of CC in PSC affected patients[57].

It has been demonstrated that COX-2 is a downstream of iNOS-NO mediated pathway in the promotion of biliary carcinogenesis[43]. In fact, in immortalized murine cholangiocytes, iNOS inhibition markedly diminishes COX-2 mRNA and protein expression[43]. This event is reversed by the introduction of NO donors[43]. These data acquire a major potential clinical relevance since high immunoreactivity of both iNOS and COX-2 has been demonstrated in bile ducts of patients affected by PSC[41,43].

Bile acids: Bile acids accumulate in the course of cholestasis and regulate CC cell growth. Specifically, deoxycholic acid (DA) activates the epidermal growth factor receptor (EGFR), which is known to stimulate pro-survival and pro-proliferative signaling such as the

PI3-kinase[79,80]. The mechanism by which this occurs is very complex as demonstrated by Werneburg *et al*. These Authors showed that the bile acid-dependent activation of EGFR is blocked by Tumor Necrosis Factor-α (TNF-α) antiserum and by metalloproteinase inhibitor[79]. The hypothesis, therefore, is that bile acid induced activation of EGFR is secondary to metalloproteinase activation, which is required for TNF-α release from the membrane and which consequently activates EGFR[79]. In addition to the PI3-kinase signaling, the bile acid dependent activation of EGFR elicits CC cell escape from apoptosis also by promoting the expression of anti-apoptotic molecules[79]. The exposure of CC cells to DA markedly increases the cellular Mcl-1 and Raf-1 inhibitors block this increase of Mcl-1, rendering the cells much more sensitive to Fas-induced apoptosis[37,38].

However, the actions of bile acids on CC development are diverse. For example, bile acid-induced EGFR activation also leads to the stimulation of the MAPK cascade with activation of ERK1/2, p38 and JNK proteins[78] thus having a pro-carcinogenic effect. In addition, the activation of ERK1/2 and p38 is then responsible for COX-2 expression[78]. This effect could suggest that bile acids may play an important role in CC development in a non-inflammatory condition. Therefore, bile acids affect a multitude of intracellular events implicated in CC and members of the wide family of bile acids may exert different, even opposite, effects on malignant cholangiocytes. In fact, we recently demonstrated that tauroursodeoxycholate (TUDCA) inhibits human cholangiocarcinoma growth *via* Ca^{2+}, PKC-alpha and the MAPK-dependent pathways[81]. Previous data showed that TUDCA stimulates hepatocyte regeneration[82]. It is plausible that different cell types could respond to the same stimulus in an opposite way. Similarly, gastrin stimulates the growth of several cells such as colonic epithelial[83] and pancreatic adenocarcinoma[84], but inhibits cholangiocarcinoma cell growth[85].

Dysregulation of apoptosis

Apoptosis is an essential event employed by the organism to eliminate cells not able to repair DNA damage. A reduction or dysregulation of the apoptotic process permits the survival of mutated cholangiocytes, which could accumulate a series of mutations, thus favoring malignant cell transformation. The inhibition of apoptosis is associated with an increase of expression of Bcl-2, mutation of K-ras and/or dysregulation of p53[9].

Bcl-2, the prototype of the homonym family of antiapoptotic proteins[34], is overexpressed in CC cells[36]. These tumoral cells possess an apoptotic threshold significantly higher than normal cholangiocytes[86]. Several studies show that Bcl-2 antiapoptotic activity is exerted by preventing cytochrome-c release from the mitochondria, thereby preventing caspase-3 activation[86].

Many other factors can influence and regulate the apoptotic process of cholangiocytes. Besides being involved in the induction of cholangiocarcinogenesis mechanisms[9], NO inhibits apoptosis of malignant cholangiocytes. Indeed, Torok *et al* demonstrated that nitric-oxide-synthase (NOS)-cDNA leads to a resistance to etoposide-induced apoptosis through the nitrosylation of caspase-9, when transfected to CC cells[39].

Notch-1 and COX-2 reduce TRAIL-mediated apoptosis[16,40]. Recent studies showed that the high quantity of COX-2 in CC cells inhibits Fas-induced apoptosis[40] and the selective COX-2 inhibitor celecoxib enhances cell death by apoptosis, through inhibition of PI3-kinase signaling[24]. However, COX-2 expression correlates with tumor differentiation and is increased in highly differentiated biliary cancers[23].

TRAIL exerts its functions in malignant cholangiocytes[44]. The activation this ligand selectively stimulates apoptosis only in neoplastic and not in normal cholangiocytes[44]. It has been described that high level expression of Mcl-1 in CC cells blocks TRAIL-induced apoptosis[44]. This implies that if Mcl-1 expression is reduced by specific small-interfering mRNA or stable transfection with Mcl-1-small-hairpin-RNA, CC cells become sensitive to TRAIL-induced apoptosis[44]. The expression of Mcl-1 is also modulated by bile acids, which accumulate in the condition of cholestasis. DA, for example, is able to increase the cellular Mcl-1 by blocking protein degradation *via* activation of an EGFR/Raf-1 pathway. Furthermore, Raf-1 inhibitors antagonize the increase of Mcl-1, rendering the cells much more sensitive to Fas-dependent apoptosis[44,87].

Growth and invasion of CC

The high capacity for invasion and metastasis are two other important features of CC cells. Malignant cholangiocytes stimulate the development of a rich vascular structure, which supports the metabolic needs and ensures an adequate source of oxygen and nutrients to the same cells[1]. Tumor angiogenesis is enhanced by high levels of vascular endothelial growth factor (VEGF)[1,7], which is stimulated by β-catenin[53] and TGF-β, and which is expressed by the surrounding mesenchymal cells and the malignant cholangiocytes themselves. This complex system of pathways delineates an autocrine/paracrine mechanism, which supports the production of VEGF necessary for tumor development and growth[54].

A recent study showed that CC expresses a high level of matrix metalloproteinases (MMP) and a correlation between this large amount of MMP and major clinical invasiveness was reported[55].

Human aspartyl (asparaginyl) β-hydroxylase and proteins related to the connective tissue growth factor family are also highly expressed in CC cells[56]. Their concentration is proportional to the increased motility and invasiveness of tumoral cells[56].

"Cell adhesion molecules" represent a network of factors that play a critical role in enhancing cancer invasion and metastasis[57]. Expression of E-cadherin, α-catenin, and β-catenin is reduced in a majority of biliary tumors and their down-regulation correlates with

a high grade malignancy of the tumor. On the contrary, their reduction does not correlate with vascular invasion, metastasis and p53 expression[57].

WISP1v is a member of the connective tissue growth factor family[60]. It has been reported that the expression of WISP1v is significantly associated with high lymphatic and perineural invasion of tumor cells, as well as a poor clinical prognosis[61]. Furthermore, WISP1v stimulates the invasive phenotype of CC cells with activation of both p38 and p42/p44 MAPKs[61].

Cellular senescence

Cellular senescence represents a physiologic process leading to growth arrest due to telomere shortening. Malignant cholangiocytes express high levels of the enzyme telomerase[21], which blocks telomere shortening, thus maintaining chromosomal length. This permits the cells to preserve their replication activity. The expression of human telomerase was homogeneously detected in intrahepatic CC cells, whereas its expression was heterogeneous in the dysplastic biliary lesions[51]. Furthermore, this enzyme was not detected in nondysplastic biliary epithelia, in hepatolithiasis or in normal livers. This indicates that malignant cholangiocytes acquire telomerase activities in the dysplastic condition, thus triggering processes favoring malignant transformation[51]. Recent studies have shown that IL-6 enhances telomerase activity[52].

MICRO-RNA AND CC

Micro-RNAs (miRNAs) are single-stranded RNA molecules of 21-24 nucleotides in length, able to regulate gene expression. miRNAs are encoded by genes from whose DNA they are transcribed but miRNAs are not translated into protein. They are complementary to mRNA molecules, and their main function is to down-regulate gene expression. The miRNA gene expression profile in CC cells[88] has been described only recently. However, only a limited number of genes in CC have been analyzed so far[88]. Interestingly, the oncomiRs miR-141, miR-21, miR-23a, miR-27a, let-7a and miR-200b are up-regulated, while the tumor suppressor miRNAs miR-29b and miR-370 are down-regulated in malignant cholangiocytes[14,89-92].

The miRNAs possess specific functions in CC cells. For example, the up-regulated miR-141 may target the CLOCK gene, which modulates circadian rhythms and suppresses tumor growth[91]. To strengthen this concept, the inhibition of miR-141 reduces CC cell growth[91].

The involvement of other miRNAs in stimulating CC proliferation has also been described. For example, miR-21 modulates PTEN and is an anti-apoptotic and pro-survival factor[14] which is inhibited by gemcitabine, the chemotherapic drug used for CC. When dysregulated, PTPN12 stimulates tumor cell survival and the carcinogenesis process[91]. It has been suggested that PTPN12 could represent a target gene for miR-200b[91].

Moreover, the same MiR-200b could inhibit ZFHX1B, which is involved in the TGF-β signaling pathway and in the processes of EMT *via* regulation of E-Cadherin[93]. Similar to a variety of cancers where the expression of MiR-29b is reduced[94-96], CC express low levels of this molecule[89]. Mott *et al*[89] showed an inverse relationship between miR-29b and Mcl-1 expression in CC cells. In fact, the reduction of miR-29b is accompanied with an increase of the antiapoptotic Mcl-1.

MiR-370 expression is reduced in malignant compared to normal cholangiocytes[90]. Some evidence shows that MiR-370 targets MAP3K8, which is consequently up-regulated in CC cell lines as well as in tumor cell xenografts *in vivo*[14,90]. Epigenetic regulation of miR-370 occurs by hypermethylation and through IL-6[14,90].

IN VIVO ANIMAL MODELS OF CC

The evaluation of therapeutic molecules using *in vivo* animal models of disease is of fundamental importance in testing specific antitumoral drugs[97]. Many studies evaluating the effect of substances or compounds on CC cells have been conducted using xenograft systems in murine animals[98,99]. However, although these studies produced exciting and encouraging results, these results correlate only poorly with clinical outcomes. This finding underlines the need to recreate and use organ-specific *in vivo* cancer models[100-102].

Several *in vivo* animal models of biliary malignancies have been described. Hamsters and rats develop CC after treatment with carcinogenetic chemical compounds such as [*N*-nitrosobis (2-oxopropyl) amine][103], methylazoxymethyl acetate[104], dimethylnitrosamine[105], furan[106] and thioacetamide[107,108]. Furthermore, animals infected with *O viverrini*[109], are able to develop biliary malignancies. Recently, several new genetic CC models have been described. Liver-specific combined deletion of the TSGs *Smad4* plus *PTEN* results in formation of CC in mice[49] and p53-deficient mice treated with carbon tetrachloride develop intrahepatic mass-forming CC[110]. Moreover, Sirica and colleagues have developed two models of CC in which malignant transformation of explanted rat cholangiocytes followed by direct biliary inoculation of these cells result in CC formation in 56% to 100% of animals[61,111].

In summary, models of CC have been developed in recent years which resemble human CC in many characteristics. The majority of these models represent intrahepatic CC, but genetic models of hilar CC are still missing[97].

CONCLUSION

The multitude of factors released in the environment during the course of cholestasis and chronic inflammation trigger genomic and epigenetic damage leading to malignant transformation and uncontrolled proliferation of cholangiocytes. CC is a highly lethal disease with an

extremely poor response to conventional anticancer therapies and a poor survival rate. In the last few years, research has made important steps in clarifying the intracellular pathways of malignant cholangiocytes. Only the complete identification of molecular pathways involved in the pathogenesis of malignant changes of cholangiocytes will permit the discovery of novel tools for early diagnosis, and the detection of specific molecular targets for therapies.

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Meetings

Events Calendar 2010

January 25-26
Tamilnadu, India
International Conference on Medical Negligence and Litigation in Medical Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology Conference

January 28-30
Hong Kong, China
The 1st International Congress on Abdominal Obesity

February 11-13
Fort Lauderdale, FL, United States
21th Annual International Colorectal Disease Symposium

February 26-28
Carolina, United States
First Symposium of GI Oncology at The Caribbean

March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 09-12
Brussels, Belgium
30th International Symposium on Intensive Care and Emergency Medicine

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 23-26
Cairo, Egypt
14th Pan Arab Conference on Diabetes PACD14

March 25-28
Beijing, China
The 20th Conference of the Asian Pacific Association for the Study of the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology and Hepatology Conference, EGHC 2010

April 14-17
Landover, Maryland, United States
12th World Congress of Endoscopic Surgery

April 14-18
Vienna, Austria
The International Liver Congress™ 2010

April 28-May 01
Dubrovnik, Croatia
3rd Central European Congress of surgery and the 5th Croatian Congress of Surgery

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

May 06-08
Munich, Germany
The Power of Programming: International Conference on Developmental Origins of Health and Disease

May 15-19
Minneapolis, MN, United States
American Society of Colon and Rectal Surgeons Annual Meeting

June 04-06
Chicago, IL, United States
American Society of Clinical Oncologists Annual Meeting

June 09-12
Singapore, Singapore
13th International Conference on Emergency Medicine

June 14
Kosice, Slovakia
Gastro-intestinal Models in the Research of Probiotics and Prebiotics-Scientific Symposium

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

June 20-23
Mannheim, Germany
16th World Congress for Bronchoesophagology-WCBE

June 25-29
Orlando, FL, United States
70th ADA Diabetes Scientific Sessions

August 28-31
Boston, Massachusetts, United States
10th OESO World Congress on Diseases of the Oesophagus 2010

September 10-12
Montreal, Canada
International Liver Association's Fourth Annual Conference

September 11-12
La Jolla, CA, United States
New Advances in Inflammatory Bowel Disease

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

September 16-18
Prague, Czech Republic
Prague Hepatology Meeting 2010

September 23-26
Prague, Czech Republic
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October 07-09
Belgrade, Serbia
The 7th Biannual International Symposium of Society of Coloproctology

October 15-20
San Antonio, TX, United States
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting

October 23-27
Barcelona, Spain
18th United European Gastroenterology Week

October 29-November 02
Boston, Massachusetts, United States
The Liver Meeting® 2010--AASLD's 61st Annual Meeting

November 13-14
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December 02-04
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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

Statistical expression

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