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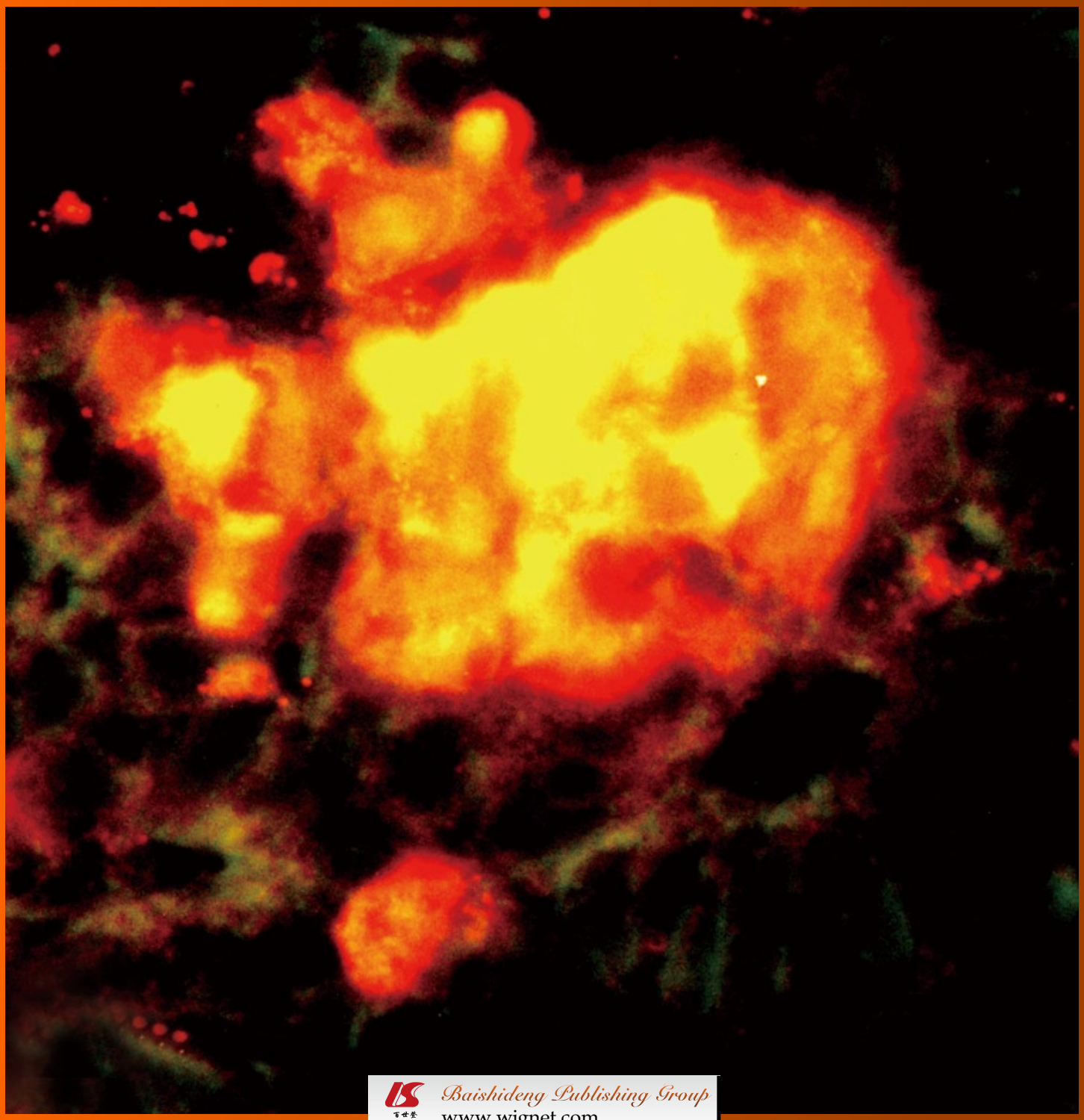

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EDITORIAL

Age related changes in pancreatic beta cells: A putative extra-cerebral site of Alzheimer's pathology

Magdalena Maj, Aysegul Ilhan, Dashurie Neziri, Wolfgang Gartner, Tord Berggard, Johannes Attems, Wolfgang Base, Ludwig Wagner

Magdalena Maj, Aysegul Ilhan, Dashurie Neziri, Wolfgang Gartner, Wolfgang Base, Ludwig Wagner, Department of Internal Medicine Ⅲ, Division of Nephrology and Dialysis, Medical University of Vienna, 1090 Vienna, Austria
Tord Berggard, Alligator Bioscience AB, SE-223 70 Lund, Scheelevägen 19, Sweden
Johannes Attems, Newcastle University, Institute for Ageing and Health, Newcastle upon Tyne, NE4 5PL, United Kingdom
Author contributions: Maj M, Berggard T, and Wagner L wrote this manuscript; Ilhan A and Neziri D were responsible for manuscript correction and discussion; Gartner W and Base W participated in manuscript correction and critical review; and Attems J was responsible for consultation and discussion.
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Correspondence to: Ludwig Wagner, MD, Department of Internal Medicine Ⅲ, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna,
Austria. ludwig.wagner@meduniwien.ac.at
Telephone: +43-1-40400-4319 Fax: +43-1-40400-7790
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Abstract

Frequent concomitant manifestation of type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) has been recently demonstrated by epidemiological studies. This might be due to functional similarities between β-cells and neurons, such as secretion on demand of highly specific molecules in a tightly controlled fashion. An additional similarity represents the age-related alteration of hyperphosphorylated tau in AD patients. Similarly, alterations have been identified in β-cells of T2DM patients. The islet amyloid polypeptide has been associated with β-cell apoptosis. As a consequence of increasing age, the accumulation of highly modified proteins together with decreased regenerative potential might lead to increasing rates of apoptosis. Moreover, reduction of β-cell replication capabilities results in reduction of β-cell mass in mammals, simultaneously with impaired glucose tolerance. The new challenge is to learn much more about age-related protein modifications. This can lead to new treatment strategies for reducing the incidence of T2DM and AD.

Key words: Type 2 diabetes mellitus; Pancreatic beta cells; Age; Alzheimer's disease; Hyperphosphorylated tau; Islet amyloid polypeptide

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INTRODUCTION

Prevalence of impaired glucose tolerance and type 2 diabetes mellitus (T2DM) is increasing among the elderly in humans. The absolute number of T2DM patients is rising worldwide, particularly in industrialized countries. This is not only because of the higher incidence of obesity and reduced physical exercise, but is also due to the longer life expectancy in these countries, as well as superior food quality, and the availability of highly effective medication. However, a longer life span brings with it age-associated

diseases such as diabetes mellitus type 2, cognitive disorders, and Alzheimer's disease (AD). Primarily, one would not expect that these two very different forms of age related complications (TD2M and AD) to have any connection. Yet, epidemiological studies have demonstrated that, when compared with age matched individuals with absence of cognitive dysfunction[1,2] , impaired glucose tolerance and T2DM is more prevalent among AD patients. The link between these entities with different loci of pathological processes might be found in similar key mediators or signaling pathways. In both, the pancreatic beta cell and neurons of the central nervous system, secretion on demand of highly specific molecules represents a genuine task. This is mediated *via* a tightly controlled exocytosis process. The SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor) protein complex gears transmitter and insulin secretion at neurons and beta cells, respectively. The SNARE complex exerts its function at the neuronal synapse and in the β-cell, using already pre-primed mature granules. Three so called SNARE proteins participate in fusing the vesicles to the plasma membrane: the vesicle-associated membrane protein (VAMP, also called synaptobrevin); syntaxin, an integral plasma membrane protein; and the synaptosomal-associated protein of 25 kDa (SNAP-25), anchored to the plasma membrane *via* a palmitoyl group. Together, these three proteins form a helical bundle consisting of four amphipathic helices, or SNARE motifs, two of which are contributed by SNAP-25[3]. It is believed that assembly of the SNARE complex proceeds in a zipper-like fashion from the N-terminal end of the interacting helices toward the C-terminal membrane anchors. In this way, assembly of the proteins in the opposing membranes pulls the membranes together[4]. At the beginning of membrane fusion, the SNARE proteins are located in still separated membranes (so-called *trans*-complexes) and, after fusion, the trans-membrane segments of the SNAREs are present in the same membrane (*cis*-complexes). To restore the cell for new exocytosis events, the *cis*-complexes are then disassembled by NSF (N-ethylmaleimide-sensitive factor) and additional cofactors[5], and vesicles containing VAMP are recycled. Secretagogin, a novel hexa EF-hand calcium-binding protein was recently found to interact with SNAP-25[6]. Further complex interdependencies will be demonstrated in establishing inter-actoms[7].

ALZHEIMER'S PATHOLOGY IN PANCREATIC β-CELLS

As most cellular processes are regulated by multi-protein complexes, abolishing or enhancing a protein-protein interaction may have a profound impact and possibly manifests in distinct diseases. Since protein-protein interactions are critical events for a wide range of physiological and pathological processes, the precise control of these interactions and their biological consequences present a major challenge and opportunity for modern drug design[8]. Hyperphosphorylation and glycosylation might induce impairment of the protein interaction machinery. As protein expression deficiencies of SNARE members have been demonstrated in the brain at the Lewy body variant of AD patients[9], there might exist forms of T2DM in which pancreatic β-cells undergo similar expression deficiencies, but this is still a matter of investigation.

In contrast to neuronal transmitters, insulin does not undergo a reuptake into β-cells. The premature insulin granules[10] have to be transported to the cell periphery along microtubules *via* an energy-consuming process using kinesin[11]. In this respect, microtubular dynamics as well as microtubule-associated protein tau (MAPT), also named tau, play an important role. Abnormalities in tau protein structure such as tangles and hyperphosphorylated tau aggregates were identified in the brains of AD patients[12,13] about 30 years ago. This has led to the technical term tauopathy and has been defined as detergent insoluble tau aggregates forming tangels and neuritic plaques[14]. Very recently, hyperphosphorylated tau, representing a factor responsible for the inhibition of microtubule assembly and microtubule disruption[15], has been identified in pancreatic islets of Langerhans of T2DM patients[16]. In contrast, this was not found in pancreatic islets of healthy individuals. Such data have been confirmed by in vitro studies using insulinoma tissue and cell lines from rodents[17]. At least six individual tau isoforms have been identified in these rodent β-cell lines, of which two are of higher molecular weight than the brain derived isoforms. Insoluble aggregates were isolated and demonstrated. Most interestingly, a slight but not significant up-regulation of tau[18] expression could be defined at the gene level using expression screens when comparing normal age matched donor islets with pancreatic islets from T2DM patients.

Although tau has become an important molecule in defining AD pathology, it is not solely responsible for disease development[19]. In the brain, extracellular beta amyloid deposits are the second main hallmark of AD pathology. Interestingly, a homologous protein[20] named islet amyloid polypeptide (IAPP)[21] is present in beta cells, which is intriguing in this respect. It is co-expressed and secreted with insulin by pancreatic beta cells[22,23]. The IAPP has a propensity to misfold and aggregate into cytotoxic oligomers, which result in islet amyloid deposits found in T2DM patients[24]. Oligomers of human IAPP are known to cause membrane disruption[25], and are therefore involved in the mediation of β-cell apoptosis in T2DM. Interestingly, the single amino acid mutation (proline substitution) in rodent IAPP hinders the formation of IAPP deposits[22], and rodents do not spontaneously develop diabetes characterized by islet amyloid deposits[26]. This, in turn, has led to the development of transgenic rats expressing the human variant of IAPP[27]. The transgenic rat model indeed resembles the T2DM of humans closely, and provides proof that this molecule is involved in derangement of β-cell function. It is of note that, using these models, it has been shown that the toxic effect of human IAPP on β-cell apoptosis is initiated by a threshold-dependent effect[26].

MODIFICATIONS OF INSULIN SECRETION

Insulin secretion from pancreatic beta cells has been monitored in a pulsatile mode under physiological conditions[28,29]. The frequency of pulses changes depending on the blood glucose level, and can be influenced by drugs such as sulfonylurea[29]. Most interestingly, impairment of this mode of secretion has been observed much earlier than the abnormal glucose tolerance could be measured[30]. Each pulse of insulin release is preceded by an increase of intracellular calcium[29]. This tightly controlled mechanism as reviewed by Tengholm and Gylfe[31] is deranged in individuals with impaired glucose tolerance and diabetes[32,33]. Additionally, an age-dependent change in pulsatile insulin secretion has been demonstrated in animal models[34] as well as in humans[35].

β-CELL REPLICATION AND AGE

It has been demonstrated in rodent models that the beta cell mass is the result of a balanced mode of replication and apoptosis[36,37]. An adjusted increase in replication has been found in obesity of rodents[38] and humans[39]. Moreover, the adaptive increase in beta cell mass has been shown to have important biological relevance for the increased insulin demand in pregnancy[40,41,42]. Furthermore, as depicted in rats, these adaptations are necessary to balance the age-related insulin resistance building up within 12 mo of birth[34]. Data beyond this age are not available from rats, though it has to be speculated that this β-cell replicative potential decreases in rodents in an age-dependent manner[43] as it has been shown for humans[44]. In younger individuals, β-cell mass can adapt to increase in body mass in order to maintain glucose tolerance within the normal range, this seems not to be the case in older individuals.

β-CELL APOPTOSIS AND AGE

Although T2DM has been associated with increased β-cell apoptosis[44,45,46], it does not necessarily mean that there is an increase in apoptosis going along with the age. However, there exists clear evidence that islet amyloid polypeptide increases with age at the islet of Langerhans[47,48]. This physiological peptide can cause apoptosis in its oligomeric form[25,45,49,50]. In addition to this, hyperphosphorylated tau protein can accumulate within the islet of Langerhans, as mentioned above[16]. Rodent models suggest that increased apoptosis might be responsible for the decrease in β-cell mass[26,27,51,52].

PROTECTIVE EFFECTS

It has been suggested that several protective effects exist to prevent neuronal death[53,54]. The former author describes that the calcium-binding protein secretagogin might exhibit a neuro-protective effect. This protein is highly expressed at the pancreatic islet of Langerhans, and might indeed exert important sensing capabilities at the calcium spikes preceding each pulse of insulin secretion[55]. Some preliminary data have been suggested in recent work, which might indicate that this protein provides more resistance to β-cell stressors under *in vitro* conditions[17]. Similarly, chaperon proteins have been implicated in refolding proteins back into their normal structure, following derangement from their native structure due to exposure to toxins or disease-mediated changes in body temperature[56,57].

Further basic research work will be necessary to teach us how age-related changes in β-cells can be reduced, prevented and counteracted.

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Insulin-producing cells are bi-potential and differentiators prior to proliferation in early human development

Kai-Ming Yang, Wang Yong, Ai-Dong Li, Hui-Jun Yang

Kai-Ming Yang, Wang Yong, Department of Anatomy and Histology and Embryology, Dali University, Dali 671000, Yunnan Province, China
Ai-Dong Li, Hui-Jun Yang, Chengdu Medical College, Chengdu 610083, Sichuan Province, China
Author contributions: Yang KM designed and supported the study; Yong W was responsible for literature collection and manuscript drafting; and Li AD and Yang HJ revised the paper critically.
Correspondence to: Kai-Ming Yang, Professor, Department of Anatomy and Histology and Embryology, Dali University, Dali 671000, Yunnan Province, China. kming1965@163.com
Telephone: +86-0872-2257140 Fax: +86-0872-2257106
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Abstract

AIM: To investigate the differentiation and migration of endocrine cells to form the pancreatic islets of Langerhans in early human development.

METHODS: Embryonic pancreas of 6-14 wk gestation was observed using immunocytochemistry methods in early human development.

RESULTS: Insulin and glucagon are expressed in the same epithelium cells in the pancreas. In addition, insulin-producing cells also secrete somatostatin in early human embryonic development and these insulin-producing cells also express nestin.

CONCLUSION: Pancreatic duct epithelial cells that can produce insulin in early human development are precursors and still have the potential to differentiate other endocrine cells. These progenitors have differentiated before migration from primary ductal epithelium to form the pancreatic islets.

INTRODUCTION

For decades, investigators have been studying pancreatic development in the hope of isolating a stem cell that could be induced to generate new β-cells. Recent clinical trials indicate that transplantation of isolated islets, combined with immune suppressive therapy, can cure type I diabetes[1]. This further raises the hopes of patients and researchers that a stem cell therapy for this disease is feasible. The problem is where to find such stem cells and how to control their differentiation. We know that the islets of Langerhans in the pancreas are specialized endocrine micro-organs composed of four distinctive cell types; insulin-producing β-cells, glucagon-producing α−cells, somatostatin-producing δ-cells and pancreatic polypeptide producing pp cells. During embryonic development, four endocrine cells of the pancreatic islet derive from a common set of epithelial cells that originate in the early gut endoderm[2]. The pancreas derives from two patches of epithelium that bud dorsally and ventrally from the gut epithelium, between the stomach and duodenum, beginning (in the mouse) at approximately embryonic day 9 (E9). Prior to and during budding, the organ primordium expresses the homeodomain protein Pdx1/Ipf1; all pancreatic cell types derive from *Pdx1*$^+$progenitors[3,4].

After budding, the pancreatic primordia begin dramatic growth and branching while reorienting and fusing into a single bipolar organ. As we know, all adult pancreatic cells derive from *Pdx1*-expressing progenitors. During bud outgrowth, *Pdx1* expression shifts from uniform to biphasic at high levels in β-cells and lower levels in undifferentiated precursors[5,6]. Inactivation of *Pdx1* after bud formation, using the tTA system[7], prevents both islet and acinar differentiation; this general function in development may reflect a role in multipotent progenitors or stem cells. A key regulator of endocrine development is the bHLH protein Neurogenin3 (*Ngn3*), which is expressed exclusively in endocrine precursor cells and subsequently downregulated during differentiation[4,8]. Its absolute requirement for islet cell development[9] suggests that *Ngn3* promotes endocrine fate in cells descended from *Pdx1*C progenitors. Moreover, misexpression of *Ngn3* is sufficient to induce endocrine differentiation throughout the gut epithelium[10]. *Ngn3* is ordinarily expressed in scattered cells of the epithelium; broader misexpression of *Ngn3* in the early pancreas, using the *Pdx1* promoter, results in complete diversion of the organ to an endocrine fate[10,11].

Lineage studies[12] illuminate additional aspects of endocrine development. Previously, cells co-expressing glucagon and insulin in the early pancreatic bud were suggested to represent bi-potential progenitor cells[13]. However, using the glucagon or insulin promoter to drive Cre-dependent lineage marking, it was found that adult β-cells derive from progenitors that had never expressed glucagon and vice-versa for α-cells. Surprisingly, it was also found that β-cells, but not α-cells, derive from progenitors that did express pancreatic polypeptide, although PP expression is not maintained in β-cells. Expression data, therefore, can be an unreliable guide to lineage.

During embryonic development, a cascade of transcriptional factors control β-cells formation in the pancreas. Different transcription factors control distinct checkpoints along the pathway to the differentiated β-cells. The first step of pancreatic epithelial cells towards an endocrine fate is controlled by neurogenin 3. Loss of neurogenin 3 function in mice results in a complete absence of endocrine cell differentiation[9]. β-cell competence factors likely include the NK-homeodomain genes *Nkx2.2* and *Nkx6.1*[14,15]. Both of these factors act either downstream of or in parallel to *Ngn3*, as *Ngn3* expression is normal in mice lacking *Nkx2.2* or *Nkx6.1*[11]. *Nkx2.2* mutants completely lack insulin expression; in place of normal β-cells, islets contain a large population of cells apparently arrested "just short" of β-cell fate. *Nkx6.1* mutants have a phenotype that is both more and less dramatic: a small number of insulin-producing cells are generated during early pancreatic development but the normally exponential increase in β-cell generation that initiates during the secondary transition is completely absent and no immature β-like cells are formed.

There is considerable evidence suggesting that the differentiation of *Pdx-1*$^+$ progenitor cells into pancreatic islet cells occurs by a multi-step process, involving successive changes in the antigenic profile of the stem cells. *Pdx-1* cells co-expressing insulin and glucagon appear at E 9.5 in the pancreatic bud of mice before full morphogenesis of the pancreas[5]. From an embryonic development study, we found that at 6 wk gestation, the pancreatic primordium has branched and there is a lack of secreting role in the epithelial cells. At 10 wk gestation, the pancreatic ductal epithelial cells begin expressing insulin glucagon and somatostatin before migration from the duct. Interestingly, a few epithelial cells simultaneously express insulin and glucagon or insulin and somatostatin. Moreover, these insulin-producing cells also express nestin, which continues until the 14 wk when the process of islet formation begins. We conclude that endocrine cells differentiation prior to the migration and insulin-producing cells in pancreatic epithelium are endocrine progenitors, with bipotential in early human development.

MATERIALS AND METHODS

Human embryonic tissue

Embryo samples of 6 cases (6 to 14 wk gestation, based on menolipsis and the size of the fetus) were obtained from donors at the department of Jiming Obstetrics hospital from first trimester spontaneous abortions. The investigation complies with the principles of the Declaration of Helsinki and has been approved by the Ethics Committee of SiChuan University where it was performed. The subjects gave informed consent to the work.

Tissue preparation

The embryonic tissues were fixed with 4% paraformaldehyde in phosphate buffer and 5 μm thick paraffin sections were mounted on silanized slides for immunocytochemical labeling.

Immunocytochemistry

For immunocytochemical labeling, we chose the streptavidin-biotin-peroxidase kits (Beijing Zhongshan Biotechnology Co. Ltd., Beijing, China). Mouse anti-insulin, glucagons, PCNA and rabbit anti-nestin and somatostatin were purchased from Santa Cruz Co. Ltd. (America). Immunocytochemistry sections were first blocked with 10% normal goat serum for 20 min at room temperature and incubated with primary antisera (Insulin: 1:300, Glucagon: 1:300, Somatostatin: 1:300, PCNA: 1:500, Nestin: 1:1000) overnight at 4℃. Then primary antisera were washed with phosphate-buffered saline (PBS) and incubated with the biotin labeled secondary mouse or rabbit antisera (1:100) for 2 h at 37℃, followed by a second PBS wash. Finally, slides were incubated with streptavidin for 1 h at 37℃, or streptavidin conjugated immunofluorescence FITC/Cy3, and washed extensively with PBS. After treatment with Streptavidin Affinity Complex (SABC), the tissues were oxidized by diaminobenzidine (Sigma, America) and counterstained with hematoxylin for microscopic observation. Fluorescence was visualized with a Nikon microscope. Double staining was according to the details of Histostain^{TM}-DS kits (BeiJing Zhongshan Biotechnology Co. Ltd., Beijing, China). During the whole procedure, PBS was applied as a negative control in place of a primary antibody. Microscopy and imaging of

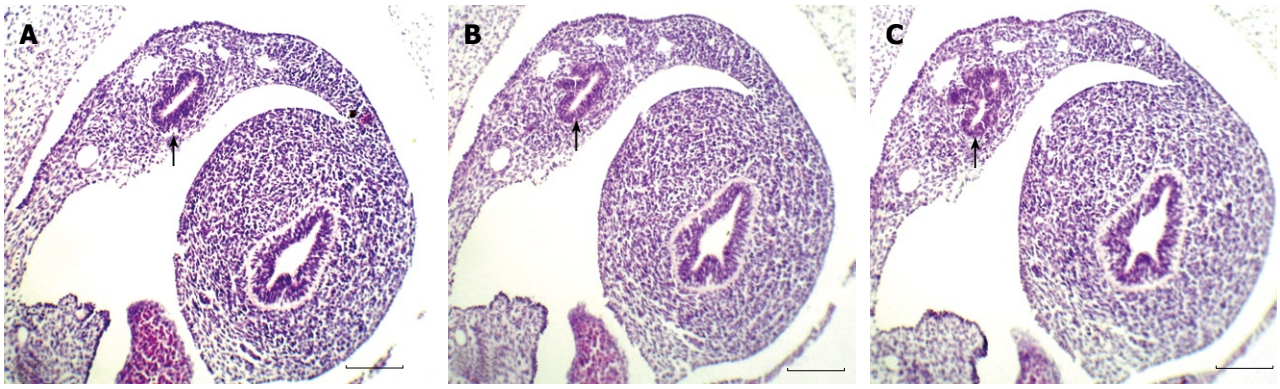

Figure 1 Staining by hematoxylin-eosin in embryonic pancreas exhibited pancreatic primary duct at 6 wk gestation. A: The first-order bifurcation; B: The second-order bifurcation; C: The third-order bifurcation. Arrows show the duct beginning bifurcation. Bars = 200 μm.

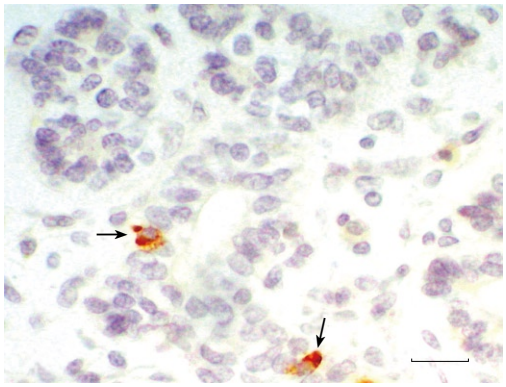

Figure 2 Expression of insulin in fetal pancreas. Immunohistochemical staining is shown for insulin in pancreatic duct epithelium of 11 wk gestation. Arrow shows β-cell has differentiated before migration from duct. Bars = 50 μm.

the immunostained sections and the sections with haematoxylin and eosin (H&E) was done with an Olympus microscope (Model CX4IRF, Olympus Optical Co. Ltd.). At least 10 sections were observed from each pancreas. Images were recorded with a digital camera (C-5050zoom, Olympus Optical Co. Ltd.). The final images were assembled with Adobe Photoshop 7.0. The reaction results for the SABC method was yellow-brown adjacent to the top of cell or in cytoplasm of pancreatic tissues of human embryo. The immunofluorescence reaction is red or green but the cross-reaction was yellow. The reaction results of double staining (DS) were indigo and scarlet. The tissues of the control were all negative.

RESULTS

The pancreas derives from two patches of epithelium that bud dorsally and ventrally from the gut epithelium, between the stomach and duodenum. At 6 wk gestation, the pancreatic primordia begin to grow and branch. The growing epithelia cells concentrate and have polarity (Figure 1). The growing epithelium was surrounded by mesenchymal cells. At this stage, no insulin-positive cells and nestin$^+$ cells were detected. Pancreatic epithelia cells begin to auto-secrete at 11 wk gestation. A large number of primary pancreatic duct and insulin (Figure 2), glucagon and somatostatin (data not shown) positive cells could be seen located the pancreatic epithelium. The positive reaction epithelia cells still remained in the duct and did not migrate. At this stage, the pancreatic premordia began dramatic growth with curvature and branching. With double staining, we detected some epithelial cells that co-express insulin and glucagon and somatostatin (Figure 3). The same insulin –producing epithelial cell co-expressed glucagon and somaotostaitin lasting up to 12 wk gestation. At this stage endocrine cells began to aggregate. To our surprise, we did not detect the proliferation of pancreatic epithelial cells to express antigen PCNA in this stage of epithelium differentiation but PCNA show the strong reaction in the liver (data not shown).

For the immunofluorescence, we observed that the insulin-producing epithelial cells not only co-express glucagon and somatostatin but also express nestin (Figure 4). The expression of nestin occurred in pancreatic epithelium (11 wk) and lasted until the formation of islets (14 wk). The insulin reaction is stronger than nestin. Meanwhile, a few insulin negative epithelia cells also express nestin. However, the endocrine cells began to differentiate and move from pancreatic ductal epithelium to form the islets. When the islet began to form, the insulin-positive cells still can be seen in ductal epithelial cells.

DISCUSSION

Pancreas development is a complex process that requires the timely expression of numerous factors; among them, the neurogenin3 and neuroD1/BETA2 drive endocrine differentiation. But the cell fate induced by ectopic *Ngn3* expression is predominantly or exclusively α-cells. The ability to identify endocrine precursor cells was based on neurogenin3 expression. Previous studies suggested the cascade of transcription factors controlling islet cell differentiation[5,6-14]. It has been suggested that the early insulin/glucagon co-expression cells represent a transition state as cells differentiate from the early glucagon expressing cells into mature β-cells[11,12]. However, the investigation in mouse development does not support this view[14] because they do not detect significant replication of insulin-expressing cells. Maturation of the small early population of insulin/glucagon co-expressing cells can not explain

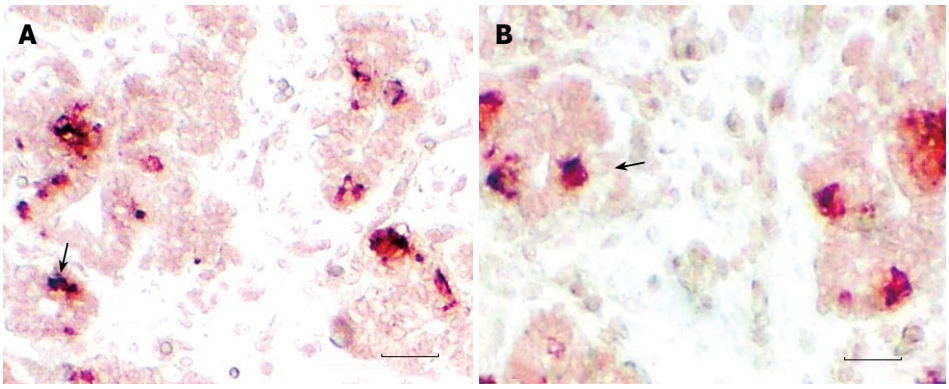

Figure 3 Immunohistochemical double staining in fetal pancreas. A: For insulin glucagon (indigo) and insulin (scarlet); B: For somatostatin (indigo) Insulin (scarlet). Arrows showing the duct epithelia cell co-expressing insulin, glucagon or somatostatin at 11 wk gestation. Bars = 200 μm.

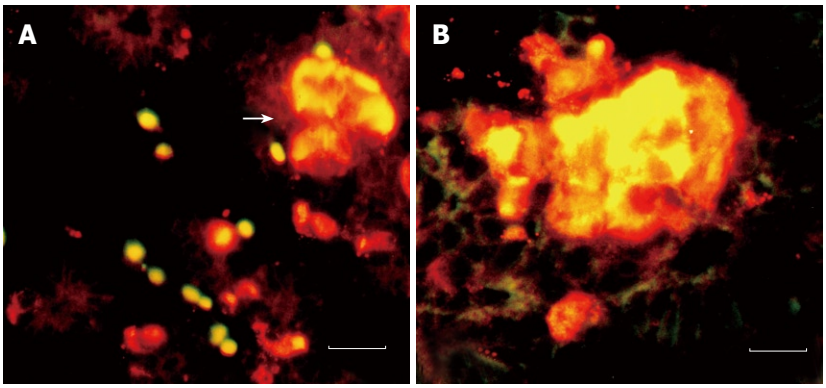

Figure 4 Double immunofluorescence staining in fetal pancreas insulin (FITC label, green) and nestin (Cy3 label, red). A: At 11 wk, bar = 100 μm; B: At 14 wk, bar = 100 μm. Arrow shows that islet has formed and insulin-producing cells express nestin.

the much larger number of mature β-cells that appear after E13. Furthermore, Herrera and colleagues have used lineage tracing in transgenic mouse model to show that the glucagon promoter is not active in the progenitor cells for mature β-cells[11,16].

In our study, we determined the expression of insulin, glucagon, somatostatin and nestin in early human embryonic pancreas development. We found that at 10 wk gestation, a few epithelial cells in pancreatic duct begin expressing insulin, glucagon and somotostaitin. It suggested that endocrine cells differentiated prior to migration. Double staining shows that a few epithelial cells co-express insulin/glucagon or insulin/somatostatin. But we could not detect the cells co-expressing glucagon/somatostatin. This suggested that insulin producing cells also have the potential to secrete glucagon or somatostatin. In addition, at the same gestational age, immunofluorescence staining showed that insulin-producing epithelium also expresses nestin, a marker that generally is considered as pancreas stem cell labeling[17]. All of this suggests that insulin producing epithelia cells are endocrine progenitors and bipotential cells. In contrast, we detected endocrine cells differentiation instead of proliferation in gestational age 6-14 wk, showing differentiation prior to proliferation. Therefore, we support that most of the β-cells in the late fetal pancreas must develop from non-hormone-expressing progenitor cells.

The above mentioned reminds us of mouse studies[17,18]. They identified that mature islets contain a stem cell population that can be induced to differentiate into insulin-producing cells following islet injury. Fernandes and his colleagues identified two subsets of PDX-1$^+$ β precursor cells in islet in mouse studies: the IN$^+$/GLU$^+$/PDX-1$^+$ cell type which can lead to monospecific β-cells in embryo. Whereas IN$^+$/SOM+/PDX-1$^+$ β cells, a cell type at a more advanced stage in the cellular hierarchy, are proposed to generate IN$^+$ cells in regenerating islets of adults. It may be a hypothesis that progenitor from embryo set aside in islets lasts till the late postnatal life of adult. The transcription factor PDX-1 is expressed in pancreatic precursor cells and becomes restricted to β-cells in the mature islet where it controls insulin transcription [19].

A similar transient expression of nestin was proposed to occur in the human insulin-producing β-cell precursors[17,20]. In our study we found the β-cells which secreted hormone in the duct prior to moving from duct, suggesting insulin may be a vital cytokine to promote the migration of β-cells and formation of islets. In addition, these insulin producing cells not only co-express glucagon or somatostatin but also express nestin and last up to the formation of islets. It indicated that insulin-producing cells are pancreatic stem cells and always remain in islets themselves. So we support the view that regeneration of β-cells after β-cells impaired showing the stem cells remaining of islets themselves[18]. Recently, to determine whether nestin can be used to identify β-cell progenitors in the developing human pancreas, nestin$^+$ cells were purified by using an enhancer/promoter-driver selection plasmid to determine whether nestin$^+$ cells differentiated into β-cells[21]. The experimental result suggested that nestin is not a specific marker of β-cell precursors in the developing human pancreas. According to our investigation, the expression of nestin may not be a specific marker of β-cell precursors but insulin-producing cells co-expressing glucagon or somatostatin in pancreas of

early human development show that these cells have bipotential. Simultaneously, these insulin-producing cells with nestin-positive reactions suggest that these cells are progenitors of islet. Nestin is localized specifically to the mesenchyme of the developing human pancreas but not to any epithelial cell population. Furthermore, after isolation, nestin$^+$ cells do not differentiate into β-cells, neither *in vitro* nor *in vivo*[21,22]. We hypothesize that expression of nestin shows the cells have the power of differentiation which can be differentiated under certain times and certain conditions, other than multipotential stem cells.

COMMENTS

Background

Differentiation of early gut endoderm cells into the endocrine cells forming the pancreatic islets of Langerhans in human embryonic development still needs to be clarified. To observe how gut endoderm cells differentiate into endocrine cells and migrate from epithelium was significant for the investigation of pancreatic stem cells.

Research frontiers

In this article, embryonic pancreas of about 6-14 wk gestation was observed using immunohistochemistry methods in early human development. We found that insulin and glucagon are expressed in the same epithelium cells in the pancreas. In addition, insulin-producing cells also secrete somatostatin in early human embryonic development.

Innovations and breakthroughs

Insulin and glucagon are expressed in the same epithelium cells in the pancreas. In addition, insulin-producing cells also secrete somatostatin in early human embryonic development. Moreover, these insulin-producing cells also express nestin, generally considered the pancreatic stem cells marker.

Applications

These experiments revealed that pancreatic duct epithelial cells that can produce insulin in early human development are precursors and still have the potential to differentiate other endocrine cells. These progenitors have differentiated before migration from primary ductal epithelium to form the pancreatic islets.

Peer review

This is an interesting descriptive study concerning an investigation about endocrine cells differentiation for pancreatic development in China.

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Moshira Abdel Hakim Rateb, MD, Assistant professor of Human Physiology, Faculty of Medicine (Kasr Al Aini.), Manial El Roda, Cairo University, Cairo, Egypt

Nadja Herbach, DVM, Institute of Veterinary Pathology, 13 Veterinaerstr. Munich 80539, Germany

Nigel Hoggard, PhD, Senior Research Scientist, Aberdeen Centre for Energy Regulation and Obesity (ACERO), Obesity & Metabolic Health Division, University of Aberdeen Rowett Institute of Nutrition and Health, Greenburn Road, Bucksburn, Aberdeen, Scotland, AB21 9SB, United Kingdom

Arulmozhi D Kandasamy, PhD, Cardiovascular Research Centre, 4-62 Heritage Medical Research Centre, University of Alberta, Edmonton T6G 2S2, Alberta, Canada

Reema Mody, PhD, MBA, Principal Scientist, Global Health Economic and Outcomes Research (HEOR), Takeda Pharmaceuticals International, Inc., 33976 Wooded Glen Dr. Grayslake, IL 60030, United States

Greg Tesch, PhD, Department of Nephrology, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia

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World J Diabetes 2011 April 15; 2(4): I
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Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami
FL, United States

January 28, 2011
Diabetes UK and External Conferences
Diabetes Awareness Training
London, United Kingdom

January 28-29, 2011
9. Gastro Forum München
Munich, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference
Sydney, NSW, Australia

February 16-19, 2011
The 4th International Conference on Advance Technologies & Treatments for Diabetes
London, United Kingdom

February 24-26, 2011
2nd International Congress on Abdominal Obesity
Buenos Aires, Brazil

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver
British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A Whole-system Strategic Approach
Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine
Gainesville, FL, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham
England, United Kingdom

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology
Jacksonville, FL , United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform
Sacramento, CA, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research
Istanbul, Turkey

March 28–30, 2011
The Second World Congress on Interventional Therapies for Type 2 Diabetes
New York, United States

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition
Riyadh, Saudi Arabia

May 7-10, 2011
Digestive Disease Week
Chicago, IL, United States

June 2-5, 2011
The 1st Asia Pacific Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension
Shanghai, China

June 11-12, 2011
The International Digestive Disease Forum 2011
Hong Kong, China

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer
Barcelona, Spain

August 3-6, 2011
AADE 38th Annual Meeting
Las Vegas, United States

October 16-18, 2011
ISPAD Science School for Health Professionals
Miami, Unites States

October 19-22, 2011
ISPAD 36th Annual Meeting
Miami, United States

October 22-26, 2011
19th United European Gastroenterology Week
Stockholm, Sweden

October 26-29, 2011
CDA/CSEM Professional Conference and Annual Meetings
Toronto, Ontario, Canada

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course
Washington, DC, United States

November 10-12, 2011
The Second International Diabetes & Obesity Forum
Istanbul, Turkey

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INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a monthly, open-access (OA), pe er-reviewed journal supported by an editorial board of 323 experts in diabetes mellitus research from 38 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results.

Maximization of personal benefits

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. So, to realize these desired attributes of *WJD* and create a well-recognized journal, the following four types of personal benefits should be maximized. 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. (1) 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; (2) Maximization of the benefits of authors: Since *WJD* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJD* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) 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; and (4) 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. 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.

Aims and scope

The major task of *WJD* is to report rapidly the most recent results in basic and clinical research on diabetes including: metabolic syndrome, functions of α, β, δ and PP cells of the pancreatic islets, effect of insulin and insulin resistance, pancreatic islet transplantation, adipose cells and obesity, clinical trials, clinical diagnosis and treatment, rehabilitation, nursing and prevention. This covers epidemiology, etiology, immunology, pathology, genetics, genomics, proteomics, pharmacology, pharmacokinetics, pharmacogenetics, diagnosis and therapeutics. Reports on new techniques for treating diabetes are also welcome.

Columns

The columns in the issues of *WJD* 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 Article: To report innovative and original findings in diabetes; (9) Brief Article: To briefly report the novel and innovative findings in diabetes research; (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 *WJD*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of diabetes mellitus; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in diabetes mellitus.

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In the interests of transparency and to help reviewersassess any potential bias, *WJD* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

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Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

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Title page

Title: Title should be less than 12 words.

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Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

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Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

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Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

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The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for

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Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

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Format

Journals

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1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

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/index.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 200201 03498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107145507.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

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