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C-Kit Expression as a Feature of Functional Differentiation of Progenitor Cells.

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ABSTRACT

Identification of regional stem cells and progenitor cells remains one of the key challenges of the developmental biology, the solution of which will promote progress of regenerative medicine. Recently, a receptor of stem cells factor - CD117 (C-kit) - has been treated as one of the most successful and widely used marker of stem and progenitor cells. Objective of this study was to analyze the possible affinity of C-kit-positive cells of the pancreas to the pool of regional stem cells. The study was conducted using whole embryos and human fetal organs derived during the period of week 4 to 28 of gestation, and pancreas autopsy of 2 months children and adults up to 50 years old. Paraffin histological sections were immunohistochemically stained with antibodies to stem cell factor receptor C-kit, insulin, and glucagon. To identify the proinsulin mRNA, the hybridization reaction was conducted in situ. During human prenatal development, the earliest (starting from week 4 of gestation) and pronounced expression of C-kit was observed in the originating nervous system (spinal cord, dorsal root ganglia, and Schwann cells). Since week 5 of gestation these cells appeared in the mesenchyme of the dorsal mesentery, and then in the derivatives of splanchnic mesoderm of the digestive tube where they spread over the smooth muscle cells of the muscle membrane. Expression of C-kit in the endodermal epithelium of the stomach occurs from week 13.5, and week 8.5 of gestation in some cells of the pancreatic ducts. C-kit-positive cells formed islets in the pancreas and started synthesizing hormones, such as glucagon and insulin from 8.5 and 11.5 week of gestation, respectively. C-kit-positive islet cells, expressing both insulin and glucagon, were found both in prenatal and postnatal pancreas. The resulted findings and the analysis of C-kit expression during human prenatal development allow referring the receptor of stem cells factor to the markers of committed precursor cells, the expression of which coincides with the functional differentiation of precursor cells. In the human pancreas there is a common C-kit-positive progenitor cell of α- and β-cells of the islets of Langerhans, which remains in the organ after birth.

Keywords: prenatal development, pancreas, progenitor cell, C-kit, islet cells, nervous system, intestinal tube.

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INTRODUCTION

Stem cells are undifferentiated or poorly differentiated cells that are capable of maintaining their own population and generating at least one type of committed progenitor cells. One of the main reasons hampering the development of regenerative medicine is the lack of reliable markers of stem and progenitor cells. One of the most successful markers of such cells being known is C-kit (CD117) - a receptor of stem cell factor.

C-kit is a transmembrane receptor of tyrosine kinase protein (TKPR), which is encoded by the dominant allele in *white-spotting (w)* rodents, located on chromosome 5 [5], and the human homolog is located on chromosome 4 (4 qll-12) [17]. This receptor is also known as CD 117 - a stem cell factor receptor (SCF-R), Kit/SCF-R, and mast cell growth factor (MGF) receptor. Interaction of C-kit receptor with SCF triggers a chain of intracellular reactions through intracellular proteins, which triggers a variety of cellular processes, including increase in transcription of the genes, the rapid growth and differentiation of cells [10, 16, 28]. The interaction of SCF/C-kit plays a key role in hematopoiesis [3, 32], gametogenesis [7,9,19], neurogenesis [13] and melanocytes development [8] and induces a number of other biological effects in mammalian development [4, 30]. Nearly 1-5% of hematopoietic stem cells have a SCF receptor.

In 2006, Li et al. described C-kit-positive cells in the islets of the pancreas of 14-16 week human fetuses. These cells were able to synthesize insulin when being isolated and cultivated [15].

There have been publications in recent years reporting the detection of Cajal cells - namely C-kit-positive cells - in the pancreas [23]. At the same time it was shown that such C-kit-positive cell can be considered a regional stem progenitor cell of pancreatic islet cells [15]. Also, there was determined a role of pancreatic C-kit-positive cells in the regulation of carbohydrate metabolism disorders in case of experimental alloxan diabetes [22].

Apparently, C-kit is not a unique marker of either stem or progenitor cells. C-kit helped to identify a specific type of cells located in the wall of the hollow internal organs, which are pacemakers and named interstitial cells of Cajal [6, 18, 25, 26]. Their origin remains still controversial. One of theories supposes that Cajal cells are derivatives of mesenchyme [31], another - that they are derivatives of the ventral part of neural tube [24].

One of the methodological approaches that establish the nature of C-kit-positive cells in human pancreas is the study of the dynamics of the expression of this marker during human prenatal histo- and organogenesis. In addition to prenatal pancreatic development, it was necessary to study the development of the intestinal tube, as the pancreas develops from its epithelium, as well as liver, which develops from a single source like a pancreas. Furthermore, since nervous system is considered as one of the main potential sources of Cajal interstitial cells in the gut [24], it was necessary to study the formation of population of C-kit-positive cells in the tissues of this system.

Our study was aimed at temporal and spatial relationships of populations of C-kit-positive cells in the human internal organs during prenatal development. Objective of this study was to analyze the possible affinity of C-kit-positive cells of the pancreas to the pool of regional stem cells.

MATERIALS AND METHODS

Research Materials

The study was conducted on whole embryos and isolated organs of human fetuses obtained in the result of miscarriage or legal abortions with the voluntary consent of patients, as well as on autopsy material of infantile and adult human pancreas. The study has been approved by the Republic Committee for Ethics in clinical trials of drugs under the Ministry of Health of the Republic of Tatarstan. Total 42 specimen of week 4 to 28 of gestation and pancreas autopsy of 2 months children and adults up to age 50 were used.

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Research Methods

Human embryos and fetuses up to 12 weeks of gestation were fully embedded in paraffin. Single organs of late ontogenesis were cut into 3x4x5 mm pieces, placed into 10% neutral formalin with 0.2 M phosphate buffer (pH = 7.4) for 24 hours for fixation and embedded in paraffin using standard techniques. Immunohistochemical staining of paraffin sections was performed with C-kit antibodies (1:20, T595 clone, "Novocastra", UK), insulin (1:75, 2D11-H5 clone, "Novocastra", UK), and glucagon (1:50, rabbit polyclonal, "Novocastra", UK). Studies were conducted using the indirect immunoperoxidase streptavidin-biotin methods, the method of labeled immune complexes, the method of amplification with tyramide-biotin CSA and their combinations. We also conducted a double immunohistochemical staining to detect the simultaneous expression of C-kit and insulin, glucagon and insulin, C-kit and glucagon using streptavidin-biotin method with alkaline phosphatase and CSA with horseradish peroxidase. To identify the proinsulin mRNA, the hybridization reaction was conducted in situ (“Novocastra”, UK) in compliance with the manufacturer protocol. Histological sections were further studied under the microscope (Leica DM 1000, Germany), and photographed through the photoconverter using photo camera (Leica DFC 290, Germany).

We also carried out morphometric study of dimensions of C-kit-, insulin- and glucagon-positive parts of human pancreatic islets for assessing the population dynamics of C-kit-, insulin- and glucagon-positive cells in the islets at different stages of ontogenesis. The morphometric analysis was performed by three researchers using ocular morphometric grid in the microscope “Микмед-5” (Micmed-5) at 400x magnification. From the findings obtained by the researches we received mean values, which were further statistically processed with Microsoft Excel statistical graphic package.

RESULTS

Expression of stem cell factor receptor in the pancreas

C-kit expression in the pancreas was first detected at week 8.5 of gestation in the epithelial cells of the ducts, while no C-kit was detected in the pancreatic mesenchyme cells at this and further periods (Figure 1A).

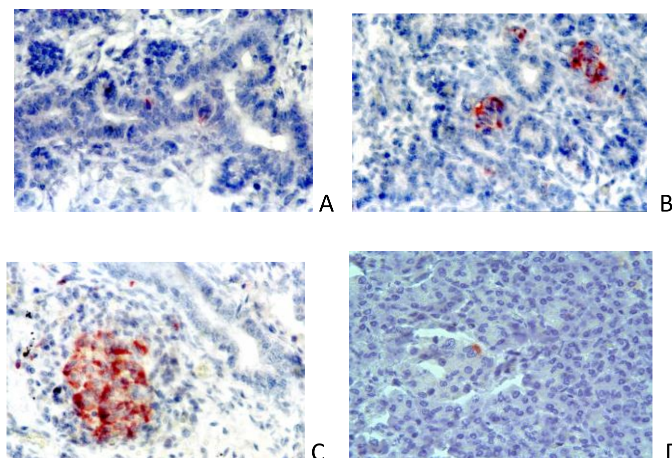

Figure 1: Red C-kit-positive cells in a human pancreas, 400x. A. A human embryo, week 8.5 of gestation. Red C-kit-positive cells in a ductal epithelium; B-D Red C-kit-positive cells in an islet; B. A human fetus, week 11.5 of gestation. C. A human fetus, week 20 of gestation. D. An adult, age 50.

Starting from week 11.5 of gestation, C-kit-positive cells differentiated from the ductal epithelium and began to form round, islet-like clusters (Figure 1B). Since that stage and up to week 20 of prenatal development there was an increase in both the number of C-kit-positive cells in the islets and the number of islets (Figure 1C). C-kit-positive cells disappeared shortly after the C-kit-positive islets appeared from ductal epithelium.

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In the second half of gestation (after week 20) the number of these cells in the islets reduced, however, C-kit-positive cells were observed in single islets even in the first few months after birth (Table 1).

Moreover, individual C-kit-positive cells were detected in adult human pancreas (Figure 1D).

In 2005, Popescu et al described the C-kit-positive cells of Cajal in the exocrine pancreas. They are described as individual, branched cells in the pancreatic acini [23]. It should be noted that no staining for C-kit was observed among the pancreatic acini at any of the stages studied.

Differentiation stages of C-kit-positive cells of the endocrine human pancreas during prenatal and early postnatal development

The number of insulin- and glucagon-positive cells in the islets increased during the pancreas prenatal development and was maximum in 2 months after birth (Table 1). A comparative study of sections stained with antibodies against C-kit, insulin and glucagon revealed that during all periods investigated the C-kit-positive islets resembled in shape and arrangement the islets of Langerhans positively stained for insulin and glucagon.

Table 1: Changes in area of C-kit-, insulin- and glucagon-positive parts of human pancreatic islets at different stages of gestation

Gestation periods, weeks	Area of C-kit-positive islets, μm^2	Area of insulin-positive islets, μm^2	Area of glucagon-positive islets, μm^2
8.5	Single cells in the ducts	none	Single cells in the ducts
11.5	0.20±0.09	0.16±0	0.16±0
12.5	0.57±0.09*	0.29±0.05*	0.35±0.08*
15	0.85±0.15*	0.48±0.14*	0.86±0.37*
20	1.54±0.57*	1.54±0.57*	1.51±0.09*
23	0.94±0.39*	1.56±0.68*	1.76±0.09*
25	0.57±0.08*	2.24±0.56*	1.97±0.09*
26-27	0.41±0.01*	2.66±0.20*	2.15±0.20*
2 months after birth	0.3±0.66*	3.18±0.81*	2.22±0.81*

(*) - $p<0.05$.

Since the number of C-kit-positive cells in the islets decreased starting from the 20th weeks of gestation (Table 1), we hypothesized the possibility of differentiation of C-kit-positive cells in either β-cells or α-cells. To test this hypothesis we performed staining of serial histological sections for insulin and C-kit. We found that C-kit- and insulin-positive islets have the same localization and similar morphology in the serial sections. The results of double staining also confirmed our hypothesis, when we found cells expressing both c-kit and insulin (Figure 2A). Moreover, we identified such cells not only in human fetuses, but also in newborn pancreas (Figure 2B).

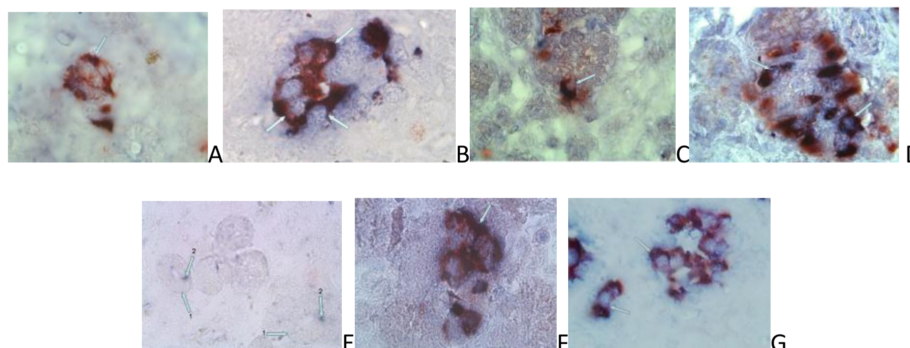

Figure 2: A, B - Human pancreas, islet cells containing insulin (blue) and C-kit (red), 1000x; A - week 11.5 of gestation, B - 2 months after birth; C, D - Human pancreas, islet cells containing glucagon (blue) and C-kit (red) x 1000. C - week 8.5 of gestation, D - 2 months after birth. E. A human fetus, week 8.5 of gestation. Blue proinsulin-positive cells (2) are observed in the ductal epithelium (1) of the pancreas, 1000x. F, G. Human pancreas, islet cells containing insulin (blue) and glucagon (red), 1000x; A - week 11,5 of gestation, B - 2 months after birth.

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Using double immunohistochemical staining, we also confirmed the assumption that the C-kit-positive cells can differentiate not only into β-cells, but also into α-cells, and the differentiation begins in ductal epithelia, where glucagon-coexpressing C-kit-positive cells were detected at week 8.5 of gestation (Figure 2C). During subsequent periods, including one after birth, there were glucagon-containing C-kit-positive cells in the islets (Figure 2D).

Here should be noted that during week 8.5 of gestation we detected a proinsulin mRNA in ductal epithelial cells together with glucagon-expressing C-kit-positive cells. At the same time, we found the insulin expression during week 11.5 of gestation (Figure 2E).

We decided further to investigate, whether β-cells and α-cells can develop from a single progenitor cell. For this purpose we performed double staining for insulin and glucagon. During week 11.5 of gestation we revealed the cells in the islets simultaneously synthesizing insulin and glucagon (figure 2F). These cells persisted in the islets even after birth (Figure 2G). These results suggest that a population of C-kit-positive cells can serve as a source of both β- and α-cells, which synthesize glucagon first, and then insulin.

No double staining for insulin and glucagon was observed in the C-kit-positive cells of the islets of adult human pancreas.

Expression of stem cell factor receptor in the nervous system, intestinal tube and liver

Nervous system

The results of our study showed that, starting from the first gestation periods investigated (4 weeks), C-kit is expressed in the cells of the developing nervous system and the derivatives of neural crest, namely in the cells of spinal ganglia as well as cells surrounding the fibers of neural ganglia. We hypothesized that these cells may be Schwann cells. C-kit expression persisted in these cells in the later periods (Figure 4A).

Figure 3: Red C-kit-positive cells in the developing human nervous system 100x. A. A human embryo, week 4 of gestation. C-kit in the trigeminal ganglion neurons (1), the Schwann cells (2) and the marginal fogging (3). B. A human embryo, week 5-6 of gestation. Red C-kit-positive cells in the rostral (1) and caudal (2) spinal ganglia. B. A human embryo, week 5-6 of gestation, spinal cord. C-kit-positive staining (red) in the ventral part of the marginal fogging (1), the cells of the ventral part of mantle layer (3) and the adjacent ependymal layer (6), the lateral part of the mantle layer (4), the dorsal part of the mantle layer and the adjacent ependymal layer (5). There is no staining in the dorsal part of marginal fogging (2).

C-kit-positive cells in the central nervous system during week 4 of gestation were observed only in the cerebral vesicles and rostral regions of neural tube. Only the ventral part of marginal fogging was stained in the caudal regions of neural tube. At the further stages, C-kit-positive cells were observed in more caudal regions of neural tube. We observed a similar situation in the spinal ganglia, where sensitivity to SCF decreased from head to caudal regions (Figure 3B).

During all the stages investigated, the developing central nervous system showed staining for C-kit in the mantle zone, where the differentiation of progenitors of neurons and glial cells occurs, and in the marginal fogging, which consists of processes of these cells. At the same time, the ependymal layer, which is the source of the neural stem cells [2], showed a small area staining. The main part of the ependymal layer had no staining (Figure 3C).

Intestinal tube

During week 4 of gestation, staining for C-kit was observed in some cells of the head mesenchyme first only in the stomodeum zone, and then in the dorsal mesenchyme of the cervical and thoracic regions (Figure 4 A, B).

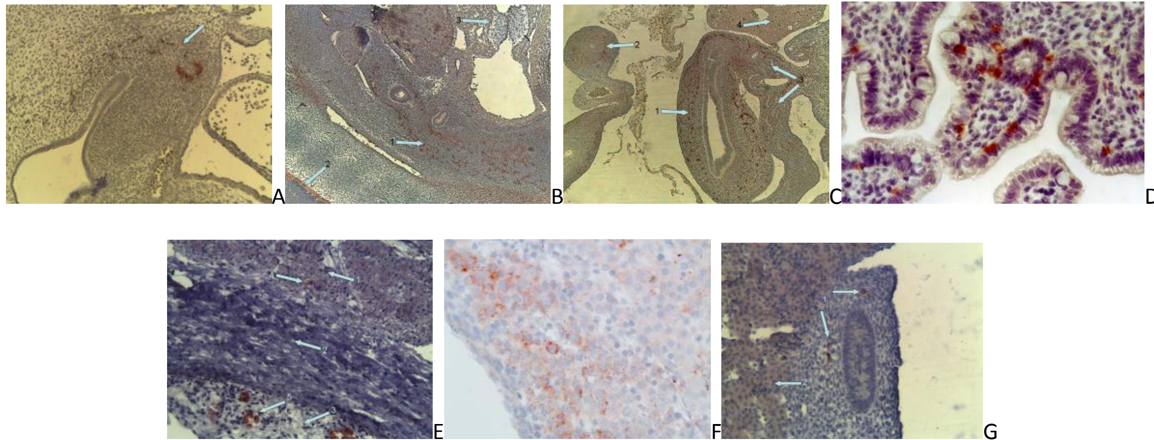

Fig. (4) - Red C-kit-positive cells in the developing human digestive system. A. A human embryo, week 4 of gestation. Red C-kit-positive cells in a mesenchyme of stomodeum, 100x. B. A human embryo, week 5 of gestation. Red C-kit-positive cells in the thoracic mesenchyme (1), a visible colored marginal fogging of spinal cord (2) and the heart (3), 50x. C. A human embryo, week 5 of gestation. Red C-kit-positive cells in the mesenchyme of the stomach (1), colon (2) and dorsal mesentery (3), a visible liver (4), 50x. D. A human fetus, week 13.5 of gestation. Red C-kit-positive cells in a gastric epithelium, 400x. E. A human fetus, week 20 of gestation. Stomach: muscular tunic (1), submucosa (2) and epithelium (3). Red C-kit-positive cells in the gastric muscular tunic (4) and epithelium (5), 200x. F. A human embryo, week 5-6 of gestation. C-kit-positive hepatoblasts in the liver, 200x. G. A human embryo, week 7 of gestation. C-kit-positive cells in the mesenchyme of the gall bladder (1) and hepatoblasts (2), 400x.

During week 5 of gestation, C-kit-positive cells were located in the mesenchyme of the dorsal mesentery (Figure 4B) and in the mesenchyme of the intestinal duct in the form of single elongated cells. Later, these cells were located in the muscular tunic (Figure 4C). This staining pattern allowed us to conclude that the C-kit-positive cells migrate to the intestinal tube from the head regions.

During week 13.5 of gestation, a stem cell factor receptor first occurred in single cells of gastric epithelium (Figure 4D).

Later (20th week of gestation) C-kit-positive cells were arranged in groups at the bottom of fundic glands in the gastric epithelium. At the same time, single elongated C-kit-positive cells persisted in the muscular tunic (Figure 4E).

Liver

During week 4 of gestation, we observed staining for C-kit hepatoblasts (Figure 4F). Some of them were colored more brightly. This pattern maintained up to week 7 of gestation. Further, the intensity of hepatoblast staining decreased gradually, which may indicate a reduction in sensitivity of hepatoblasts to stem cell factor in this period and their differentiation. In addition to hepatoblasts, we observed staining for C-kit in single elongated cells starting from week 5 of gestation. Moreover, we detected C-kit-positive cells around the gallbladder epithelium during week 7 (Figure 4G).

DISCUSSION

Our study of prenatal and postnatal pancreatic organogenesis showed that the C-kit-positive cells appear first in the epithelium of the pancreatic duct during week 8.5 of gestation, and further differentiate into cells of the islets of Langerhans and persist there after birth until adulthood. An additional evidence of differentiation of endocrine cells of the ductal epithelium is the proinsulin mRNA detected in these cells prior

to synthesis of insulin. Obviously, C-kit-positive ductal cells are progenitor cells of the islet cells. Thus, we traced the origin of the development and further evolution of C-kit-positive cells described in 2006 by Li et al. in the islets of the pancreas of 14-16 week human fetuses [15].

We suppose the following way of development of α- and β-cells of the pancreas. At a certain stage of development, C-kit appears on the membrane of ductal epithelium. It begins further to interact with its SCF ligand. This interaction triggers the processes of cell differentiation of ductal epithelial cells into endocrine ones [15]. SCF/C-kit interaction activates the same of intracellular proteins that induce growth and differentiation of cells by increasing the PDX-1 [15], which causes activation of the insulin genes [20], somatostatin [14] and others. As a result, hormones are synthesized in the epithelial cells, the cells begin to form islands which are separated from ductal epithelium into independent formation, while the cells of the islets begin first to synthesize glucagon, and then insulin. Furthermore, it may be a C-kit required for migration of cells from ducts and formation of islets. Thus, the results of our study refute the information on the independent development of α- and β-cells from different sources [11]. They confirm on the contrary that there is a common precursor cell of pancreatic α- and β-cells [12]. Later, islet cells differentiate into α- and β-cells that synthesize the relevant hormones. This differentiation takes place with the growth of the islet, that is, this islet has the progenitor cells that differentiate into α- and β-cells. Moreover, such cells persist in the islets after birth. As the endocrine cell differentiate, the number of stem cells factor receptors on the membrane decreases, and the differentiated endocrine cells are formed capable of synthesizing a particular hormone. While the presence of C-kit-positive cells in adult human pancreas may indicate the maintenance of a population of progenitor cells in the islet of Langerhans at least up to age 50.

Detecting one progenitor cell for pancreatic α- and β-cells that synthesizes glucagon first, and then insulin, requires further study and review of the pathophysiological bases of the development of pancreatic diseases, particularly type I diabetes. Thus, we may suppose that, in case of type I diabetes, hyperglycemia is a consequence of both the lack of insulin, and the excess of glucagon: the lack of β-cell makes precursor cells differentiate to compensate for the deficiency of insulin. They first start to synthesize glucagon, which further exacerbates the situation. Moreover, we may assumed that some of cells may differentiate into α-cells. We observed a similar situation when studied diabetes in rats after administering alloxan, causing the death of β-cells, when the authors observed an increase in the number of α-cells along with a decrease in the number of β-cells [22].

The resulted findings and the analysis of C-kit expression during human organogenesis allow referring the receptor of stem cells factor to the markers of committed precursor cells, which C-kit expression is observed during their differentiation. Thus, the lack of staining of neural stem cells located in the ependymal layer [2] and positive staining of cells, which migrate from the ependymal layer to the mantle layer, where their differentiation into neurons and glia is likely to occur, allow us to suggest that C-kit in the nervous system is a marker of committed progenitor cells rather than of stem cells. Positive staining of only the ventral part of the marginal fogging in the caudal regions of neural tube allow us to suggest that these cells differentiate into motor neurons of the anterior horns, although we cannot exclude a differentiation into the glial cells.

During week 4 of gestation, we also observed C-kit-positive cells in some cells of the head mesenchyme. These mesenchymal C-kit-positive cells appeared first in the head regions in the stomodeum, and then in the cervical and thoracic regions of dorsal mesentery with their further distribution into the mesenchyme of the intestinal tube. Thus, the cells appeared in the caudal direction, then colonized the mesenchyme of intestinal tube, and later appeared among the muscle membrane cells. The fact that the single elongated C-kit-positive cells were located in the muscular tunic of the intestinal tube led us to the conclusion that these cells can be progenitors of interstitial cells of Cajal, and the presence of C-kit at the early stages in neural crest derivatives (buds of spinal ganglia in Schwann cells) and the similarity of the developmental processes of C-kit-positive cells of mesenchyme and intestinal tube with development and migration of vegetative neurons (neural crest derivatives) may suppose that the C-kit-positive progenitor cells of Cajal interstitial cells of intestinal muscle tunic are also derived from the neural crest. However, there is no migration of C-kit-positive cells into the liver and pancreas. At the early stages of gestation, we observed staining for C-kit in hepatoblasts, some of which were stained brighter than others. After week 7, the intensity of hepatoblast staining decreased gradually, which may indicate a reduction in sensitivity of hepatoblasts to stem cell factor in this period and their differentiation. C-kit in the liver is probably a marker of committed progenitor cells of hepatocytes, which entered into a process of differentiation. Moreover, we detected C-kit-

positive cells around the gallbladder epithelium during week 7 of gestation. We assume that these C-kit-positive cells are progenitors of the cells of Cajal described in the gallbladder [29], which migrated here from the mesenchyme of head regions of the stomodeum.

The literature describes the results of studies of C-kit-positive cells in the gastrointestinal tract within the intestinal duct after week 9 of fetal development [27]. The results obtained by the authors coincide in general with the ones represented in this article. However, our discovery of C-kit-positive cells first derived from the neural crest during week 4 of gestation, and then in the gut mesenchyme at the later stages allowed us to get a more complete idea and to confirm the hypothesis that the source of interstitial cells of Cajal of the intestinal tube is a neural tube [24] rather than the mesenchyme [31]. It is interesting that C-kit-positive cells in the intestinal tube were not only in the muscle tunic. Starting from week 13.5 of gestation, we observed temporary expression of C-kit among the gastric epithelial cells. Since the C-kit is a receptor of stem cell factor, its expression in the gastric epithelium may indicate the beginning of the differentiation of progenitor cells of the glandular epithelium during this period when there is a formation process of gastric pits and glandular cells [1].

SUMMARY

The resulted findings and the analysis of C-kit expression during human prenatal development allow referring the receptor of stem cells factor to the markers of committed precursor cells, the expression of which coincides with the functional differentiation of precursor cells. In the human pancreas there is a common C-kit-positive progenitor cell of α- and β-cells of the islets of Langerhans, which remains in the organ after birth.

CONCLUSION

In summary, it is arguable that there are at least two populations of C-kit-positive cells in the intestinal tube such as interstitial cells of Cajal and committed progenitor cells of the glandular epithelium, while C-kit-positive cells in the nervous system, liver and endocrine pancreas are committed progenitor cells of certain cell types. The question of whether there are C-kit-positive interstitial cells of Cajal in human pancreas remains open. At the same time, the C-kit expression in progenitor cells coincides with the period of their functional differentiation, and the C-kit-positive cells are those where expression of glucagon and insulin in the pancreatic islet cells starts.

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Conflict of Interest

The author declares that the provided information has no conflicts of interest.

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