DISTRIBUTION OF PDGFRα+ CELLS AND INTERSTITIAL CELLS OF CAJAL IN THE HUMAN FETAL GUT

Goran Radenković¹, Aleksandra Veličkov¹, Vladimir Petrović¹, Miloš Dičić², Marko Gmijović³

INTRODUCTION

The muscular layer of the gastrointestinal (GI) tract is a complex tissue that, in addition to smooth muscle cells, contains a variety of cellular phenotypes. Within the muscular layer of GI tract, there are at least two types of interstitial cells, interstitial cells of Cajal (ICC) and cells called "fibroblast-like" or "ICC-like cells" (1, 2). These interstitial cells of mesenchymal origin form gap junctions with each other and with surrounding smooth muscles thus conducting electrical signal and regulating muscle excitability (3). Interstitial cells of Cajal express c-kit (4), so immunohistochemical labeling of the Kit receptor has enabled the reliable identification and study of ICC function and pathophysiology (5) on human and animal material.

The studies of ICC development suggest that some types of ICC and smooth muscle cells have a common precursor that expresses the c-kit (6). Kit signaling is thought to be necessary for the separation of mesenchymal precursors during differentiation toward ICC or smooth muscle cells, for maintaining the ICC phenotype, and for normal postnatal development (7). The pacemaker role of the ICC around the myenteric plexus (ICC-MY), which spontaneously generate slow waves necessary for peristaltic contraction, has recently been confirmed (3, 8). The ICC within the muscle layer (intramuscular ICC – ICC-IM) have a role as mediators of...
cholinergic and nitrinergic neurotransmission (9-11), participate in afferent signaling and integration of sensory-motor function as an element of the afferent branch of the gastrointestinal reflex (12). ICC also have a sensory role in the transduction of mechanical stimuli, that is, they function as stretch receptors (13). Loss and dysfunction of ICC have been demonstrated in numerous motility disorders (14-18). Contrary to the studies of ICC, the study of “fibroblast-like” cells has only recently been made possible by immunohistochemical labeling of platelet derived growth factor receptor A (PDGFRα) (19). These cells are different from ICC, but they occupy the same anatomical niches in the GI tract in mice, primates, and humans (19-24). ICC and PDGFRα+ cells represent different classes of cells with unique ultrastructure, molecular phenotype and function. Smooth muscle cells are electrically connected to ICC and PDGFRα+ cells via gap junctions and form an integrated unit – smooth muscle cell, ICC and PDGFRα+ cells (SIP) syncytium (3). SIP cells express different receptors and ion channels, and changes in conductivity in any type of SIP cell affect the excitability and reactions of the syncytium. Interstitial cells are also found in various other smooth muscle organs; however, in most cases the physiological and pathophysiological role of these cells is not clearly defined (25, 26).

PDGFRα+ cells are also closely related to the motor neurons varices and are intertwined with ICC around neural processes (19, 27). Immunohistochemical studies have shown that PDGFRα+ cells in the musculature of the GI tract express small conductance Ca2+-activated K+ (SK3) channels, and mediate enteric inhibitory responses to purines in GI muscles (28-30). This observation, together with the findings that PDGFRα+ cells are very closely related to enteric motor neurons and electrically paired with smooth muscle cells (31), suggests that they, such as ICC, may mediate enteric inhibitory neurotransmission. Major mitogens for many cell types of mesenchymal origin, like fibroblasts and smooth muscle cells, are platelet-derived growth factors (PDGFs) (32). During embryogenesis, PDGF signaling is important in organogenesis (33), while adult cells of multiple organs expressing PDGF ligands and receptors often play an important role in the pathophysiology of various disorders, including GI dysmotility (34).

In the available literature, there is no data on the presence and distribution of PDGFRα immunoreactive cells in the human intestine during fetal period.

Aim

The aim of this study was to identify PDGFRα immunoreactive cells in the gut of human fetuses, as well as to determine their distribution in relation to smooth muscle cells, ICC and enteric nerve structures.

Materials and methods

Material

The material consisted of 12 human fetuses, in the gestational age from 10 to 12 weeks. The tissue material was obtained from the Institute of Pathology, Clinical Center Niš, after legal abortions and premature births due to prepartal deaths. Gestational ages were estimated by anatomical criteria according to the crown-rump length, biparietal diameter, and foot length, as well as from the anamnetic data on pregnancy age. There were no gastrointestinal disorders in the specimens, and both sexes were represented in the sample. A macroscopic examination was performed in detail and only specimens that did not undergo post-mortem changes were selected. The study was approved by the Ethics Committee of the University of Niš Faculty of Medicine, and was performed within the internal project no. 22 of the University of Niš Faculty of Medicine.

Tissue preparation

Gut specimens were isolated and fixed in formaldehyde (10%), paraffin embedded, sequentially sectioned at 4 μm, and routinely H&E stained due to histological examination.

Immunohistochemistry

The specimens were exposed to PDGFRα antibodies, anti-c-kit antibodies to investigate ICC, and smooth muscle cells were immunohistochemically labeled with anti-desmin (DES) antibodies. Section deparaffinization was performed in xylol and descending series of alcohol rinses (less than 1 min each) followed by rehydration in distilled water. The tissue sections were incubated after blocking endogenous peroxidase (3% H2O2 for 10 min at room temperature) with the primary antibody in a humidified chamber at room temperature for one hour, followed by rinses in a phosphate-buffered solution (0.1 M PBS, pH 7.4). The primary antibodies were dissolved in Dako antibody diluent (EnVisionTM FLEX DMB830 Code: K8006, Dako, Denmark). After secondary antibody administration (EnVisionTM FLEX SM802, Code: K8000, Dako, Denmark) for 45 min at room temperature, immune complexes were visualized by the Dako REAL EnVisionTM Detection System, Code: k5007 (Dako, Denmark). Mayer’s haematoxylin was used for counterstaining of all immunolabeled sections, and immunoreactivity was absent in negative controls in which the primary antibody was omitted. The primary antibodies used in the research and their respective dilutions are listed in Table 1.
Results

During the development of the human intestine, at week 11, immunoreactivity to desmin is present in all parts of the gut tissue. Desmin immunoreactivity is present in all parts of the intestine in the form of a concentric band of cells (Figure 1), which by their localization correspond to the circular muscle layer, while the longitudinal layer is not yet differentiated. Only in the terminal portions of the foregut and initial portions of the midgut are individual desmin immunopositive cells localized immediately below the serosa, representing the origin of the longitudinal muscle layer.

| Table 1. Antibodies                |
|------------------------------------|
| **Antigen** | **Clone** | **Supplier** | **Dilution** |
| C-kit      | CD-117    | Dako         | 1 : 300      |
| PDGFRα     | Polyclonal| Abcam        | 1 : 50       |
| Desmin     | DE-R-11   | Dako         | 1 : 100      |

PDGFRα immunoreactivity is present in all parts of the human primitive gut (Figure 2) at week 11. PDGFRα immunoreactive cells are present within the circular muscle layer, while they are not present in the myenteric plexus (MP) region, or in the parts below the serosa. PDGFRα+ cells are elongated, spindle-shaped cells, oriented in parallel to the longitudinal axis of the smooth muscle cells within the circular gut muscle layer. In the submucosal region, there are two PDGFRα low immunoreactive cells (Figure 2 arrowhead).

In week 11 of human development, c-kit immunoreactive cells are present in all parts of the gut in the MP region, as continuous rows and nets of cells present around the MP ganglia, at the outer border of the circular muscle layer (Figure 3). C-kit immunoreactive cells lie at the edges of the inception of the MP ganglia but they are not present within them. They are also absent within the circular muscle layer, as well as in the region below the serosa. In submucosa, in the area where submucosal ganglions develop, c-kit immunoreactivity is also absent. C-kit immunoreactive cells are multipolar with large round or oval nuclei, a small body, and numerous thin processes. Their processes form a network around the MP ganglia. In addition to ICC, a large number of c-kit immunoreactive mast cells are present, but they are easily distinguished from ICC on the basis of their shape and granular content.

Figure 1. Desmin immunoreactive cells (dark brown stained) are present in the form of a concentric band and correspond to the circular muscle layer. Desmin immunohistochemistry, x200
Figure 2. The distal portion of the midgut at 11th week of fetal development. PDGFRα immunoreactive cells (dark brown stained) are present within the circular muscle layer. Two PDGFRα low immunoreactive cells in the submucosal region (arrowhead). PDGFRα immunohistochemistry, x200

Figure 3. The distal portion of the midgut at 11th week of fetal development. C-kit immunoreactive cells (dark brown stained) are present in the MP region and clearly limit the onset of the MP ganglia (arrows). C-kit immunohistochemistry, x200
The comparison of desmin, C-kit and PDGFRα immunoreactivity in the distal midgut clearly shows that the localization of C-kit and PDGFRα immunoreactive cells is significantly different (Figure 4).

**Figure 4.** Comparison of desmin, C-kit and PDGFRα immunoreactivity in the distal midgut at 11th week of fetal development. PDGFRα immunoreactive cells are present only within the circular muscle layer, while ICGs do not exhibit PDGFRα immunoreactivity. x200

**Discussion**

Based on the desmin immunohistochemistry results of our research, we can observe that in all parts of the fetal intestine, the circular muscular layer develops first, and only later the longitudinal layer, following the principle of proximal-distal gradient. These results are consistent with the previous studies (35, 36). During 11th and 12th week of development, C-kit immunopositive cells, which correspond to ICC, are present in all parts of the human fetal intestine in the MP region and are surrounding the inception of the MP ganglia, but they are absent within the circular muscle layer. This finding is also consistent with the results of previous studies, which indicate that intramuscular ICC develop only in the late fetal period, while certain ICC subtypes develop after birth (37-39).

Platelet derived growth factor (PDGF) is a major mitogen for many cells of mesenchymal origin, including fibroblasts and smooth muscle cells, which is why PDGF signaling is especially important during embryogenesis (34, 40). PDGF receptor A (PDGFRα) is a receptor present on the surface of a large number of cell types, which binds one of the PDGF isoforms, causing cellular growth and differentiation during organ development and is responsible for the normal functioning of tissues and organs (41, 42). Previously considered mainly a developmental growth factor receptor in the GI tract, the discovery of receptor tyrosine kinase PDGFRα expression in “fibroblast-like” cells within tunica muscularis (19) has opened the door to new trials and definitions of the role of these cells (23, 27). The most significant finding of our study is that PDGFRα immunoreactive cells are present in all parts of the human intestine in the early fetal development period. These cells are localized within the circular muscle layer, on the contrary, no PDGFRα+ cells have been observed in the MP region as well as in parts just below the serosa. An identical localization of PDGFRα immunoreactive cells also exists in the gut of adults, where they form three-dimensional networks. This position of PDGFRα immunoreactive cells is consistent with the fact about their possible role in neurotransmission of signaling from ICC to smooth muscle cells (23, 43). It has been previously reported that PDGFRα+ cells have important roles in the morphogenesis of small intestinal mucosa villus of the mouse (44, 45). PDGFRα+ cells were also found in the subepithelial layer of the adult guinea pig GI tract (46). However, the details of the distribution and functions of PDGFRα+ cells in fetus GI tract have not been reported.
Kurahashi et al. have described a specific type of PDGFRα+ cells in the lamina propria of the human GI tract (47), and suggested that subepithelial PDGFRα+ cells have a role in sensory and secretomotor signaling, proliferation, differentiation, and apoptosis of epithelial cells, and in epithelial cellular pathology, including inflammatory responses and tumorigenesis. Subepithelial PDGFRα+ cells in adults form a sheath just beneath the epithelium and cover the crypts from their base to the luminal surface of the epithelium. It is suggested that these cells may have modulatory functions in immune and sensory responses and in the maintenance of mucosal homeostasis, but the roles of these cells in physiological and pathophysiological processes are still unknown. In our research, there was a low PDGFRα submucosal immunoreactivity during the early period of fetal development, and dominant PDGFRα were present within the circular muscle layer. As already mentioned, due to their close contact with enteric nerve endings and smooth muscle cells within the circular muscle layer, it is assumed that these cells primarily play a role in the neuromodulation of peristalsis.

Another important result of our study is that PDGFRα cells differ from ICC and that they are functionally close but still different cell types of interstitial cells. In adults, PDGFRα cells are widely distributed within the MP region and in circular and longitudinal muscle layers throughout the human colon (23). Blair et al. (21) have shown relationships between enteric neurons and interstitial cells in primates. They have shown that PDGFRα+ cells are closely associated with ICC and occupy the same anatomical niches as ICC-MY and ICC-IM. However, in contrast to the distribution of intestinal cells in adults, we showed in our study that, in the fetal period, PDGFRα activity was not observed in the ICC-MY domains around the ganglia. This finding indicates that PDGFRα cells develop later than the ICC within GI tract. Further, our results show that unlike the region of MP, PDGF cells are present in the circular muscle in the fetal period and there are still no differentiated ICC-IM.

Ultrastructural studies show that enteric neurons do not effectuate any direct contact with smooth muscle cells or synaptic specializations; on the other hand, ICC make close contacts with both cholinergic and nitrinergic neurons, forming synapse-like connections at one end and gap junctions with smooth muscle cells (3, 48). Calcium activated chloride channels (Ano1) are highly expressed and exclusively by ICC throughout the GI tract (21, 49), so the major excitatory neurotransmitter - acetylcholine induces depolarization by the activation of Ano1 currents (50). ICC-IM also respond to inhibitory neurotransmitters between neurons and smooth muscles (51). PDGFRα+ are very similar to ICC-IM in adults, and also form gap junctions with surrounding smooth muscle cells (3, 20, 21). PDGFRα+ cells express guanylate cyclase, purinergic P2Y1 receptors and small conductance Ca2+- activated K+ (SK2) channels (23, 27, 52) which indicates that PDGFRα+ cells mediate enteric inhibitory neurotransmission. It has been confirmed that PDGFRα+ cells, like ICC, generate inhibitory post junctional responses in GI muscles (27). Gap junctions provide electrical coupling between cells, such that induction of a K+ current in PDGFRα+ cells results in hyperpolarization, first of these cells and immediately after that in net hyperpolarization of the SIP syncytium and reduce muscle contraction.

In contrast to our results, PDGFRα+ cells in adults form a network adjacent to ICC around the myenteric ganglia (23). ICC-MY have a pacemaker role to generate spontaneous electrical activity, while the function of myenteric PDGFRα+ is still unknown. Since they are interconnected with ICC-MP, they may have a role in the propagation and modulation of the electrical peristaltic waves.

At present, little is known about the involvement of PDGFRα+ cells in GI motor dysfunction. It is certain that the changes in purinergic neural inputs could have effects on colonic motility. Enhanced activation of PDGFRα in mice, contribute to the development of GI fibrosis and sarcoma (53). In a recent study (54), it has been concluded that colonic transit disorder may be due to the downregulation of the Kit and Ano1 channels and the upregulation of SK3 channels in PDGFRα+ cells, suggesting that the imbalance between ICC and PDGFRα distribution might be a possible reason for gut dysmotility. Furthermore, some stromal tumors (GIST) are positive for PDGFRα (55, 56), so it is possible that these cells, like ICC, can be malignantly transformed.

**Conclusion**

In the fetal period of human development, PDGFRα immunoreactive cells are present in all parts of the intestine, localized within the circular muscle layer, and do not coincide with ICC.

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DISTRIBUTICJA PDGFRα+ ĆELIJA I INTERSTICIJALNIH ĆELIJA KAHALA U CREVU FETUSA ČOVEKA

Goran Radenković1, Aleksandra Veličkov1, Vladimir Petrović1, Miloš Dičić2, Marko Gmijović3

1Univerzitet u Nišu, Medicinski fakultet, Katedra za histologiju i embriologiju, Niš, Srbija
2Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
3Klinika za digestivnu hirurgiju, Klinički centar Niš, Niš, Srbija

Kontakt: Goran Radenković
Bulevar dr Zoran Đinđić 81, 18000 Niš, Srbija
E-mail: goran.radenkovic@medfak.ni.ac.rs

Unutar mišićnog sloja gastrointestinalnog (GI) trakta prisutna su dva tipa intersticijalnih čelija: Intersticijalne čelije Kahala (IČK) i „fibroblastima-slične” čelije, nedavno nazvane receptor za trombocitni faktor rasta pozitivne (PDGFRα+) čelije. IČK i PDGFRα+ čelije predstavljaju različite čelijske klase jedinstvene ultrastrukture i funkcije i zauzimaju iste anatomske niše u GI traktu. Smatra se da PDGFRα+ čelije, kao i IČK, učestvuju u modulaciji inhibitorne neurotransmisije. Trombocitni faktor rasta (PDGF) je glavni mitogen za mnoge čelije mezenhimalnog porekla, kao što su fibroblasti i glatko mišićne čelije, te PDGF signalizacija neophodna tokom organogeneze.

Cilj rada je da identifikuje PDGFRα imunoreaktivne čelije u crevima fetusa čoveka, kao i da odredi njihovu distribuciju u odnosu na glatko mišićne čelije, IČK i strukture enteričkog sistema.

Materijal je činilo 12 humanih fetusa, gestacione starosti od 10. do 12. nedelje. Imunohistohemijsko ispitivanje vršeno je PDGFRα antitelenom, IČK identifikovane su pomoću C-kit antitela, dok su mišićne strukture dokazane desmin antitelenom. PDGFRα imunoreaktivne čelije prisutne su unutar kružnog mišićnog sloja, dok su odsutne u regionu mijenteričnog pleksusa i u delovima ispod seroze. Za razliku od njih, IČK prisutne su samo oko začetaka gangliona mijenteričnog pleksusa.

U fetusnom periodu razvića čoveka, PDGFRα imunoreaktivne čelije prisutne su u svim delovima creva, lokalizovane su unutar kružnog mišićnog sloja i ne podudaraju se sa IČK.

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Ključne reči: PDGFRα, C-kit, intersticijalne čelije Kahala, gastrointestinalni trakt fetusa