IMMUNOHISTOCHEMICAL STUDY

Telocytes (interstitial Cajal-like cells) in human Fallopian tubes

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ABSTRACT

BACKGROUND: Telocytes represent a relatively newly discovered population of cells found within the various tissues and organs, including Fallopian tubes. It is presumed that telocytes could serve as a sensor of hormone levels or regulate activity of muscle peristaltic movement.

METHODS: Tissue sections from anatomically different parts of Fallopian tubes of 48 women (age 48.8±9.1) were stained for the expression of five different antigens: c-kit (CD117), CD34, vimentin, podoplanin (D2-40) and Dog-1.

RESULTS: Telocytes form a network associated with the smooth muscle cells. From the mentioned antibodies, only anti-c-kit (CD117) seems to be relatively selective specific to the telocytes, others react also with numerous other cells and tissue structures. Our results when using antibodies against podoplanin and Dog-1 are in dissonance with recent literature – with regards to our results, they are not suitable for detection of telocytes.

CONCLUSION: Methods of immunohistochemistry are suitable for identification of telocytes in Fallopian tubes. C-kit (CD117) antigens are useful for routine identification of telocytes in histological sections. This antigen can be combined with CD34 or vimentin in cases of double staining immunohistochemistry (Tab. 1, Fig. 6, Ref. 44).

Key Words: telocytes, interstitial Cajal-like cells, human Fallopian tubes, muscle peristaltic movement, tissue structures.

Introduction

The Spanish neuro-histologist, Santiago Ramón y Cajal, discovered in 1893 a new cell type within the muscle layer of the gut, which he named “interstitial neurons”. These cells were located within the loose connective tissue between nerve endings and smooth muscle cells in the muscularis externa of the gut. Cajal gave them the name “interstitial neurons” due to specific staining characteristics after staining with methylene blue and silver impregnation led to the assumption that these cells were primitive neurons (Popescu and Faussone-Pellegrini, 2010). After about half a century, thanks to electron-microscopic examinations of the wall of digestive tube, rare cells, probably corresponding to Cajal’s “interstitial neurons” had been rediscovered there. It was immediately clear that these cells were not “real” neurons and these cells got the new name “interstitial cells of Cajal” (Faussone-Pellegrini et al, 1977; Thuneberg, 1982).

From that time, several other cell populations, morphologically and functionally mimicking interstitial cells of Cajal (and therefore called interstitial Cajal-like cells, ICLCs) were described also in other organs. They can be found in most tissues and organs of human body. Some of them probably contribute to tissue repair and regeneration (Bei et al, 2015), especially inside the liver (Liu et al, 2015), skin (Caefalan et al, 2012), or heart (Tao et al, 2015). In other organs, ICLCs have an important signaling function among different cell populations (Edelstein and Smythies, 2014) and they work as “pacemaker” cells, such as in gallbladder (Matyja et al, 2013), urinary bladder (Rusu et al, 2014) or exocrine pancreas (Nicolescu and Popescu, 2012). ICLCs were also described in some of the female reproductive organs, for the first time in the wall of the uterus (Duquette et al, 2005; Ciontea et al, 2005) and Fallopian tubes (Popescu et al, 2005; Shafik et al, 2005a), later also in the vagina (Shafik et al, 2005b) and placenta (Suciu et al, 2007; Bosco et al, 2015).

Obviously, the ICLCs are morphologically and functionally different from the “original” interstitial Cajal cells in gut. For this reason Popescu and Faussone-Pellegrini (2010) proposed a new term for these cells – telocytes. Telocytes are distinct cells that possess very long and slim cytoplasmic processes called telopodes, which are not visible on light microscopic level (probably this is the reason why telocytes hadn’t been described for centuries). The length of the telopodes ranges between dozens and hundreds of micrometers, some of them having secondary and tertiary branches, which form a three-dimensional network. This network surrounds capillaries and connects neighboring telocytes or other cell types,

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such immune reactive cells, epithelial cells, dendritic cells, smooth muscle cells, etc. (Gherghiceanu and Popescu, 2005; Niziaeva et al., 2014). On the other hand, recently introduced term “telocyte” is not generally recognized by all scientists. Despite the fact that when entering the term „telocytes“ into the database Medline/PubMed, more than 150 different articles appear, there is no reference about it in internationally accepted Terminologia Histologica (Federative Committee on Anatomical Terminology, 2008), which contains all accepted terminology for cellular structures, tissue and organs at the microscopic level (Allen, 2009).

Generally, it is accepted that transmission electron microscopy is fundamental in identification and recognition of telocytes (Cantrarero et al., 2015). Yet, methods of transmission electron microscopy are technically and also financially demanding. When using methods of immunohistochemistry, instead of electron microscopy, the identification of telocytes doesn’t seem so difficult. Several different antigens, less or more characteristic for telocytes, were described in recent years. The aim of our study was the identification of telocytes – ICLCs within the wall of human Fallopian tubes with monoclonal antibodies against five different antigens previously described in literature. We were looking for answers about the usefulness of these antibodies against different antigens in order to visualize telocytes.

Patients and methods

The specimens from the Fallopian tubes were taken from 48 women (age from 21 to 75, mean age 48.8 ± 9.1) who underwent trans-abdominal or laparoscopic surgery – salpingectomy and/or hysterectomy in the Department of Gynecology and Obstetrics in General Hospital in Komárno, Slovakia, with diagnoses of myomatous uterus, ectopic tubal pregnancy or inflammatory diseases of the pelvis. The study protocol was approved by the ethical committee of local hospital, and informed consent was obtained from all patients.

Tissue samples from anatomically different parts of the Fallopian tubes were fixed in formalin for 24 h, embedded in paraffin, and 5μm thick sections were used for immunohistochemistry. Sections were stained for the expression of five different antigens: c-kit (CD117), CD34, vimentin, podoplanin (D2-40) and Dog-1 (Tab. 1). The final reaction product was visualized with diaminobenzidine as a brown chromogen. For better orientation within the slide, cell nuclei were stained with Mayer’s hematoxylin in dark blue. Histological examination was performed on LEICA DM2500 microscope and images were captured with LEICA DFC290HD digital camera.

Results

From the antibodies used, anti-c-kit (CD117) proved to be the most useful and reliable specific marker of telocytes. Antibodies c-kit (CD117) visualized telocytes and mastocytes only. Telocytes’ cytoplasm showed strong c-kit (CD117) positivity. Thanks to this staining, we were able to describe their morphology and arrangement within the wall of Fallopian tube. They were localized mainly in tunica muscularis externa, where they had a close spatial relationship with smooth muscle cells (which are c-kit negative) (Figs 1 and 2). Their bodies were spindle-shaped or star-shaped and the beginnings (proximal portions) of their cytoplasmic projections (telopodes) had been described as well. Within the connective tissue of the Fallopian tubes, c-kit positive mastocytes were detected. It did not diminish the informative value of this approach, as mastocytes could be excluded from the analysis easily, thanks to their conspicuous ovoid shape of the body and well depicted centrally localized nucleus while lacking cytoplasmic projections (Fig. 3).

Vimentin and CD34 belong to the group of markers of telocytes with a reduced specificity. Together with telocytes, they react with many other structures. Vimentin in our specimens visualized cytoplasm of the epithelial cells, of the smooth muscle cells and of the fixed cells of connective tissue, mostly fibroblasts (Fig. 4). Antibodies against CD34 reacted with endothelial cells, some fibroblasts, and they cross-reacted with some of the basement membranes located in the tissue (Fig. 5). All this results reduced the information value of protocols using these two antibodies, as they were not specific enough.

Podoplanin (D2-40) and Dog-1 proved to be inappropriate for telocyte identification purposes. Podoplanin antibodies reacted with endothelium of lymphatic vessels only, none of other structures in the wall of Fallopian tubes were positive (telocytes including) (Fig. 6). When using anti-Dog-1 antibodies, the reaction was negative across the whole thickness of the wall.

Discussion

Telocytes represent a relatively newly discovered cell population within the various tissues and organs. In our immunohisto-
In a chemical study, we studied specimens from human Fallopian tubes and described the localization and the distribution of telocytes. Telocytes inside the Fallopian tubes are described not only in human (Popescu et al, 2005; Shaﬁk et al, 2005a), but also in different animal species such as mice (Dixon et al, 2010), rats (Yang et al, 2015a, b), turtles (Ullah et al, 2014) or poultry (Gandahi et al, 2012). The exact functions of telocytes are still not well defined.

Inside the Fallopian tubes, telocytes are found in the lamina propria of the mucosa and within the tunica muscularis. Electron microscopy is the “gold standard” for identification of telocytes in Fallopian tubes. Morphological features typical for telocytes on ultrastructural level encompass numerous and large mitochondria, abundant rough and smooth endoplasmic reticulum, dispersed intermediate filaments, presence of surface caveolae and discontinuous basal lamina. Contacts between telocytes and nerve fibers and/or smooth muscle cells are also often found (Popescu et al, 2005). Currently, when using methods of immunohistochemistry, the identification of telocytes is not so difficult. Different markers are described in telocytes, such as c-kit (CD117), CD34, vimentin, S-100 protein, smooth muscle actin or vascular endothelial growth factor (Popescu et al, 2005; Shaﬁk et al, 2005; Ardeleanu and Bussolati, 2011). Especially in Fallopian tubes’ telocytes, progestosterone- and estrogen- receptors are also present (Cretoiu et al, 2009).

C-kit (CD 117), a protein transmembrane protein kinase receptor, is essential for the function of telocytes. Another c-kit-dependent cell types include mast cells, some hematopoietic stem cells, germ cells, melanocytes, and Cajal cells of the gastrointestinal tract (Miettinen and Lasota, 2005). In histopathological practice, the main application of c-kit detection by immunohistochemistry is identification of gastrointestinal stromal tumors, but rare subsets of other soft tissue tumors can be positive (Miettinen, 2014). As one can see in our microphotographs, the development of antibodies against c-kit has allowed also a routine identification of telocytes in histological sections. It is sensitive and specific enough as it is expressed (according to our data) only in telocytes and mast cells. The differentiation between telocytes and mast cells is conspicuous – telocytes have spindle-shape or multipolar cell body with cytoplasmic projections, while mast cells are ovoid or spherical in shape. For this reason the anti-c-kit antibodies are the best choice for routine identification of telocytes.

Similarly, telocytes readily react with antibodies to vimentin. Vimentin, the major constituent of the intermediate filament family of proteins, is ubiquitously expressed in mesodermal-originated cells and is known to maintain cellular integrity and provide resistance against stress (Satelli and Li, 2011). In our slides, vimentin-positive cells were also fibroblasts, smooth muscle cells or the cytoplasm of surface-lining epithelial cell, and not only telocytes.
For this reason, detection of telocytes via single antibodies again vimentin is not possible.

*CD34* is a commonly used marker to identify human hematopoietic stem/progenitor cells, and is also another non-specific marker of telocytes (Zhou et al., 2015). In general, CD34 is believed to be important in the regulation of cell recognition and trafficking and also is involved in the adhesion of stem cell to specific tissues. It is surprising that this antigen is expressed significantly higher in the Fallopian tube, than for example in bone marrow (Indumathi et al., 2013). In our slides, anti-CD34 antibodies stained mostly the cytoplasm of endothelial cells of blood vessels, some fibroblasts also with some cross reactivity to basement membrane collagen. For this reason the selective identification of telocytes via single antibodies again CD34 is not possible.

*Podoplanin* is a mucin-type transmembrane glycoprotein which is recognized by the monoclonal antibody D2-40, is considered a specific lymphatic endothelial cell marker. It is not found in the endothelium of blood vessels (Herwig et al., 2014). But according to Povýšil et al. (2014), the anti-podoplanin antibodies are also markers of telocytes, especially in human urinary bladder. According to our result, the inside the wall of Fallopian tubes the podoplanin is expressed only in the endothelial cells of lymphatic vessels and no other cells (include telocytes) were positive.

*Dog-1* is a calcium-activated chloride channel protein expressed strongly in the gastrointestinal interstitial Cajal cells and gastrointestinal stromal tumors, which arise from interstitial Cajal cells (Hemminger and Iwenofu, 2012; Miettinen, 2014). Nevertheless, the interstitial Cajal cells of the gut are very similar to telocytes (“Cajal-like cells”). In our study, we did not find positivity of telocytes in Fallopian tubes.

Fallopian tubes perform several important functions. They capture the oocyte after ovulation, maintain and control the migration of spermatoozoa to the site of fertilization. They provide special microenvironment for fertilization; they nourish the early embryo while this is being carried to the uterus and amplify signals from embryo to the mother (Kajanová et al., 2012). These processes are controlled by changing levels of ovarian steroid hormones in the blood, which consequently significantly change the morphology and activity of (not only) the surface epithelial cells of Fallopian tubes. So, all above-mentioned actions have a common denominator - regulation by hormones, the most important, estradiol, progesterone, prostaglandins, oxytocin (Wånggren et al., 2008; Nutu et al., 2009; Kowalik et al., 2013). In the light of this information, the several years old discovery of possible “sensory and pacemaker” function of telocytes inside Fallopian tubes is remarkable. The telocytes are widely believed to be neuroeffector cells responsible for smooth muscle activity in Fallopian tubes (Gandahi et al., 2012). They express estrogen and progesterone receptors (ER-alpha and PR-A verified) on their surface and therefore serve as steroid sensors (Cretou et al., 2009). Finally, telocytes are in close contact not only with one another but also with smooth muscle cells, nerve fibers and capillaries (Popescu et al., 2007). All findings heretofore offer us plausible explanation that telocytes could serve as a sensor of hormone levels, which, with the help of its intercellular junctions, perhaps even by paracrine pathway, controls smooth muscle contraction and movement of cilia of Fallopian tube (Cretou et al., 2009).

It is important to emphasize, that, as well as in the digestive tube (where “genuine” Cajal cells are responsible for this), also in muscular layer of Fallopian tube slow waves of electrical activity (depolarization) occur (Dixon et al., 2012). Telocytes form a dense network associated with the smooth muscle cells along the entire length of Fallopian tubes. This network may be damaged by activity of macrophages in response to Chlamydia infection (Dixon et al., 2010) or during other pelvic inflammatory disease which could affect Fallopian tubes (Yang et al., 2015a). Telocytes also degenerate in endometriosis-affected Fallopian tubes (Yang et al., 2015b). Therefore, destruction or dysfunction of telocytes might provide a new explanation for dysregulation of tubal transport leading to tubal infertility or tubal pregnancy.

**Conclusion**

Methods of immunohistochemistry are suitable for identification of telocytes in Fallopian tubes. C-kit (CD117) antigens are useful for routine identification of telocytes in histological sections. This antigen can be combined with CD34 or vimentin in cases of double staining immunohistochemistry.

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