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INTERSTITIAL CELLS OF CAJAL – PACEMAKERS OF THE INTESTINAL MUSCULATURE

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Summary. *The paper reviews the present-day knowledge on the origin, location, morphology, function, and identification of interstitial cells of Cajal (ICC). ICC include several types of mesenchymal cells, spindle-shaped or stellate, found within the musculature of the gastrointestinal tract. Some types of ICC act as pacemakers of the intestinal musculature, while others are implicated in the modulation of enteric neurotransmission. Current methods for ICC identification rely on a combination of ultrastructural characteristics as defined by transmission electron microscopy and immunohistochemical analyses with antibodies to the Kit receptor. ICC play a crucial role in the regular intestinal activity, which is the reason why changes in these cells may lead to various intestinal disorders.*

Key words: *Interstitial cells of Cajal, enteric nervous system, peristaltic contractions*

Introduction

The term "interstitial cells of Cajal" (ICC) refers to several types of cells located in the musculature of the gastrointestinal tract (GIT) and, morphologically and functionally, intercalated between the segments of the enteric nervous system and smooth muscle cells. Some ICC groups act as sources of spontaneous, electric slow waves responsible for paced contractions of the intestinal musculature ("intestinal pacemakers"), whereas other ICC groups are involved in the modulation of enteric neurotransmission (1, 2).

As early as 1893, Santiago Ramon y Cajal, a Spanish neuroanatomist (2), used the Golgi technique and methylene blue staining to describe spindle-shaped or stellate cells associated with autonomic nerve endings of the intestine; he termed them "cellules interstitielles". Because they had long ramified cell processes and oval nuclei with sparse perinuclear cytoplasm, Cajal believed them to belong to a distinct type of neurons. In the century to follow, a heated debate was led concerning the origin, function, distribution, and identification of ICC. While a number of scientists claimed that ICC originate from neural structures, some other authors suggested smooth muscle cells, fibroblasts or glial cells. ICC reached its historical renaissance with the analyses of Thuneberg, who hypothesized that ICC act as intestinal pacemakers and participate in the transmission of impulses to the intestinal musculature, a transmission analogous to that in the heart muscle. Some researchers have provided abundant evidence that ICC are implicated in the generation of slow waves that act as an electrical basis for pacemaking functions (3). Today, ICC are given a central place in research studies aimed at understanding gastrointestinal contractions and elucidating the pathogenesis of various motility disorders.

Localization

In the human digestive tract, the presence of ICC has been confirmed within and around the muscle layer of the esophagus, stomach, small and large intestine. ICC are arranged in two- or three-dimensional networks and bundles of mutually associated cells that form close contacts with the nerve plexus and smooth muscle cells (4, 5). According to the terminology of Thuneberg, ICC can be divided into several groups on the basis of their location: 1) ICC-AP (Auerbach's plexus), located between the circular and longitudinal smooth muscle layer, and around the ganglial cells of the myenteric plexus; 2) ICC-DMP (deep muscular plexus), present in the thin, yet well-defined, connective tissue space between the thin inner and thick outer sublayer of the circular muscle layer of the small intestine; 3) ICC-SMP (submuscular plexus), located on the submucous margin of the circular muscle layer; 4) ICC-CM (circular muscle), located within the circular muscle layer; and 5) ICC-LM (longitudinal muscle), located within the longitudinal muscle layer (Fig. 1) (6).

A detailed analysis of the distribution of ICC along the esophagus has not to date been provided. ICC have been demonstrated around and within the muscle bundles in both muscle sublayers of the abdominal segment

of the esophagus (7). However, ICC have not been found either around the myenteric plexus or on the submucous margin. In the esophagus, ICC form synapse-like contacts with nerve endings and nexal contacts with smooth muscle cells. ICC are particularly numerous in the lower sphincter of the esophagus, especially in its esophageal part (7).

In the stomach, ICC are more numerous in the corpus and the antrum than in the fundus. They have been observed within the muscle layers, especially the circular ones, appearing as long cells parallel to the longitudinal axis of the muscle bundle, but failing to group on the submucous margin, except as single, scattered cells (3, 8). Around the myenteric plexus ICC form a thick network of ramified cells surrounding ganglial cells. Particularly numerous are ICC found in the pylorus region, in both of its muscle sublayers and around the myenteric plexus. No ICC have been identified on the submucous margin.

In the small intestine, ICC have been identified in all the locations described by Thuneberg. Of all ICC types, ICC-AP are the most numerous. They are organized in groups or bundles of five or more cells, arranged in a continual layer around the ganglial cells of the myenteric plexus and nerve fascicles (9, 10). ICC-AP arrays are spread along the inner longitudinal muscle sublayer and along the interlamellar connective septum of the upper circular muscle sublayer. Grouped and single ICC-AP are in contact with the nerve cells of the myenteric plexus, but no formation of contacts is apparent with other ICC types or smooth muscle cells. The identical distributional pattern has been observed in the duodenum, jejunum, and ileum, with the greatest density of ICC network being in the ileum. Some differences have been noticed in the duodenum, where the distribution is similar to that in the stomach, which is possibly a consequence of their both originating from the anterior gut. In the small intestine, numerous ICC-DMP are found in the form of small bundles containing 2-3 cells that form synapse-like contacts with DMP nerve endings (9). In addition, the presence of ICC has been identified in the connective tissue septa spiral to the inner circular muscle sublayer (10). These septa organize smooth muscle cells into lamellae and are in continuity with the connective tissue of the submucosa and the one located between the circular and longitudinal muscle layers. ICC-CM that are located inside the septa make contacts with nerve cells but not with other ICC and smooth muscle cells. ICC are also present between the smooth muscle cells of the circular muscle layer (11). These ICC-CM form contacts with ICC-DMP and smooth muscle cells, but no contacts have been noticed with ICC inside the connective septa.

A great number of ICC have been identified in the large intestine, where they are arranged along the circular muscle sublayer, as well as in the tenias of the longitudinal muscle sublayer. The longitudinal sublayer between tenias, however, contains a considerably smaller number of ICC. In the circular muscle sublayer, ICC are present in the connective septa between lamellae and between smooth muscle cells (12). ICC-AP in the large intestine are not numerous and are arranged in the way similar to that in the small intestine. Also present, but not numerous, are ICC-SMP, arranged around the ganglial cells of the submucous plexus (13, 14). In the large intestine, the deep muscular plexus is lacking.

Morphology and Functions

Some ICC types exert morphological differences with respect to their location. A number of characteristics, however, have been recognized to be typical of these cells. ICC are similar in size or slightly smaller than the adjacent smooth muscle cells. They are spindle-shaped or stellate, having a different number of ramified cell processes. The cells located between smooth muscle

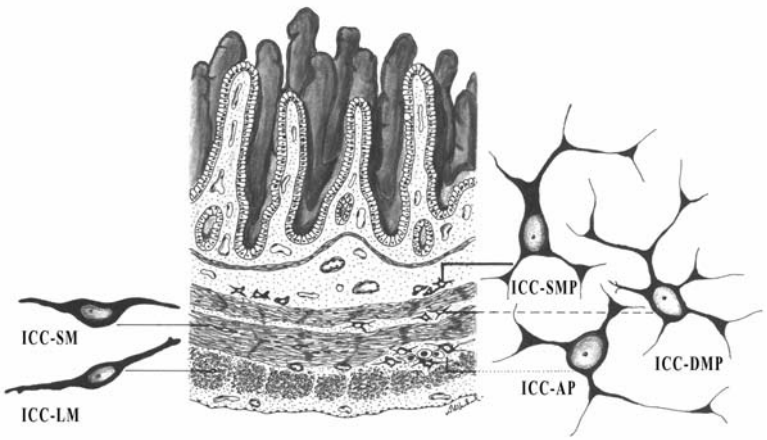

Fig. 1. Distribution of interstitial cells of Cajal (ICC) in the intestinal wall. ICC-AP- cells are located between the circular and longitudinal smooth muscle layer, and around the ganglial cells of the myenteric plexus; ICC-DMP-cells are present between the thin inner and thick outer sublayer of the circular muscle layer of the small intestine; ICC-SMP-cells are located on the submucous margin of the circular muscle layer; ICC-CM-cells are located within the circular muscle layer; and ICC-LM-cells are found within the longitudinal muscle layer.

cells are spindle-shaped with several long cell processes parallel to the axis of the muscle bundles. The cells surrounding the nerve plexus are more stellate in shape, containing numerous ramified cell processes. At the sites of ramification, a somewhat more abundant cytoplasm can be noticed. ICC contain a large, oval core surrounded by scarce euchromatic cytoplasm with one to two nuclei (15, 16).

The ultrastructure of ICC is mainly myoid, most similar to that of smooth muscle cells, with several fibroblastic features. Cytological properties identified by transmission electron microscopy (TEM) include: ramified profile, high electronic density of the cytoplasm, a well-developed perinuclear Golgi complex and centrioles, an independent basal lamina (although some ICC types have a discontinuous basal lamina or lack it completely), numerous, irregularly distributed caveolae of the plasma membrane, membrane-associated dense bands, cytoplasmic dense bodies, abundant subsurface smooth endoplasmic cisternae (sER), numerous mitochondrias, and the presence of bundles of thin and intermediate filaments (frequently associated with dense bodies and dense bands) (10, 11). The cell processes of ICC contain a well-developed smooth ER of the large mitochondria, as well as a thick network in the intermediate filaments organized in bundles (13). They differ from smooth muscle cells in having a poorly-developed filament complex, lacking in thick myosin filaments, the presence of irregularly distributed caveolae, dense bodies and bands, and a better-developed secretory complex (granulated ER and the Golgi complex). The general consensus today is that these principles serve as a basis for distinguishing various ICC types, although an accurate identification of ICC is impossible without a careful observation.

After numerous hypotheses on possible functions of ICC, it is now generally recognized that some ICC groups act as sources of electric activity by generating slow waves (17, 18). Slow waves synchronize circular muscle contractions (paced contractions) along the longitudinal axis of the GIT and drive the propagation of contractions in the aboral direction. Experiments on animals have shown that generation of slow waves is impossible without ICC-AP, although it is difficult to determine the exact contribution of ICC-AP to the intestinal electric activity (19). Namely, ICC are part of a network of mutually associated both smooth muscle and nerve cells, so the electric activity has been registered in any cell being influenced by strong activity in the adjacent cells. The absence of or damage to ICC-AP in experiments leads to disorders in paced contractions (20). On the other hand, a treatment of the intestine with tetratoxin has been demonstrated to block the propagation of nerve action potentials; however, it does not eliminate the slow-wave activity. The electric rhythmicity in the GIT has thus been confirmed to be independent of the enteric neuron inputs; rather, it is developed and maintained irrespective of the enteric nervous system (19, 21). The evidence supporting the ICC "pacemaking" role also includes the finding that, at 30-39^{0}C, cultured ICC exert spontaneous rhythmic contractions with a frequency of 5-30 contractions per minute, often engaging contractions of cell processes irrespective of the cell body (22).

Other ICC types are involved in the modulatory processes of nerve control of the GIT muscle activity, although the hypothesis on a possible role of mechanoreceptors is still under debate (22, 23).

Identification

Considerable differences between some ICC types with respect to their location, as well as the absence of a specific ultrastructural feature that would allow for their distinction, make the identification of ICC most difficult. Nevertheless, a series of ultrastructural criteria that allow for distinguishing ICC from smooth muscle cells, fibroblasts, and other types of interstitial cells, have been generally recognized.

Recently, several new attempts have been made to identify ICC using the immunohistochemical method, including NADPH-diaphorase histochemistry, Chorela toxin b-subunit marking, cyclic GMP immunoreactivity and NO synthase immunoreactivity (6, 24). However, none of the methods could be considered ICC specific.

The ZIO method (Zinc-Iodide-Osmic acid) is not ICC specific staining. It better stains nerve fibers and specific interstitial cells probably involving ICC-like cells. This specificity of the ZIO method is common to both the Golgi technique and methylene blue staining.

Recently, it has been shown that some populations of ICC express the proto-oncogene c-kit, so the antibodies to its gene product, the Kit (or c-kit) receptor, which is a membrane receptor tyrosine kinase, are now available for use as an immunohistochemical label for ICC in some species (25, 26). Mature smooth muscle cells are c-kit negative and, in the intestine mast cells, seem to be the only significant c-kit expressing cell type besides ICC that should be taken into account during ICC identification.

The gold standard for identification of ICC remains a combination of structural characteristics as defined by TEM and immunohistochemical analyses with antibodies to the Kit receptor.

Embryology

ICC are of non-neuronal, mesenchymal origin, sharing precursors with smooth muscle cells. For ICC development, the signalization via Kit receptor is mandatory. Kit is activated by means of the stem cell factor (SCF), but the source of SCF in the small intestinal wall is still unknown. Various controversial opinions exist on whether the enteric neurons generate SCF (27). Given several well-known cases of ICC (with normal functions) developing in the intestine with an as yet undeveloped enteric nervous system, the possible SCF source is considered to be smooth muscle cells that develop prior to ICC (28, 29, 30). Other types of cells in

the intestinal wall can also be an SCF source, the fact that does not exclude the possibility that even nerve cells can generate SCF.

Clinical implication

ICC play a key role in the normal function of the intestine, due to which changes in these cells can be a trigger for various intestinal disorders. In Hirschsprung's disease, the number of ICC is significantly smaller in the aganglial segments than in the ganglial intestine (31, 32). The absence or presence of a number of c-kit immunoreactive cells has also been observed in the infantile, hypertrophic pyloric stenosis (33, 34). Recently, Isozaki et al. (35) have reported two cases of myopathic chronic intestinal pseudo-obstruction with a decreased number of cells associated with the myenteric plexus.

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INTERSTICIJALNE ĆELIJE CAJALA - PEJSMEKERI GASTROINTESTINALNE MUSKULATURE

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Kratak sadržaj: *U radu je dat pregled dosadašnjih saznanja o poreklu, lokalizaciji, morfologiji, funkciji i identifikaciji intersticijalnih ćelija Cajala (ICC). ICC obuhvataju više tipova ćelija mezenhimskog porekla, vretenastog ili zvezdastog oblika, koje su lokalizovane unutar muskulature gastrointestinalnog trakta. Pojedini tipovi ICC predstavljaju pejsmekere crevne muskulature, dok su drugi tipovi uključeni u modulaciju enterične neurotransmisije. Savremene metode identifikacije ICC podrazumevaju kombinovanje ultrastrukturnih karakteristika opisanih pomoću TEM i imunohistohemijskih ispitivanja pomoću antitela na Kit receptor. ICC imaju ključnu ulogu u normalnom funkcionisanju creva, zbog čega promene u ovim ćelijama mogu biti uzrok raznih intestinalnih poremećaja*.

Ključne reči: *Intersticijalne ćelije Cajala, enterični nervni sistem, peristaltičke kontrakcije*