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Interstitial Cells of Cajal (ICC) and Gastrointestinal Stromal Tumor (GIST): facts, speculations, and myths

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Abstract

Interstitial cells of Cajal (ICC) is a peculiar cell network composed of cells having processes described by the eminent Spanish neuroanatomist of the 19th century, S. Ramon y Cajal. ICC became a fascinating subject to many investigators and it is estimated that there are over 100 publications yearly on the subject related to ICC, in the last three years. Now it is widely accepted that ICC are pace maker cells of the gut and probable progenitor cells of gastrointestinal stromal tumors (GIST). Lately, interstitial Cajal-like cells (ICLC) are being found in various organs and their physiological role is still to be defined. We have reviewed the literature trying to evaluate the validity of the current concept and found that there are a few salient points to be considered. 1) There has been some important departure in defining the identity of ICC from the original criteria of Cajal. In particular, ICC with myoid feafures in intestinal smooth muscle layers (ICC-DPM) do not seem to fit to the original description of interstitial cell network by Cajal. We have also pointed out that the current reports assigning a pace maker role to ICC vastly depend on the scientific data on "ICC with myoid features", not on "fibroblast-like ICC", which are more abundant and easier to identify. 2) There seem to be an overwhelming amount of data proving the relationship between ICC and GIST. Both are known to express c-Kit and the ultrastructural characteristics seen in GIST roughly parallel those of ICC including minimal myoid differentiation seen in the majority of GIST, supporting the current concept that GIST are ICC tumors. 3) According to the original description of Cajal, ICC was not limited to the gut, suggesting an existence of ICC in other organs. The list of organs reported to contain ICC (currently identified by immunohistochemistry and electron microscopy) is ever growing and further studies are needed to define their identity and pathophysiologic role. 4). Recent data concerning gut development suggest that both c-Kit expressing ICC (fibroblasts-like as well as muscle-like) and gut muscle cells

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derive from the common progenitor cells of the embryonic gut unifying the histogenetic concept of all GIST with heterogeneous cytomorphologic features. In this review we attempted to incorporate recent information on interstitial Cajal-like cells (ICLC) found in other organs to broaden our understanding of ICC in general in terms of their ultrastructure, physiology, and neoplasia.

Keywords: interstitial cell of Cajal (ICC) • GIST • pacemaker cell • extra-gastrointestinal stromal tumor • interstitial Cajal-like cells (ICLC)

Introduction

A peculiar cell network of interstitial cells with processes was a fascinating subject for the eminent Spanish neuroscientist of the 19th century, S. Ramon y Cajal [1, 2]. Using methylene blue dye or heavy metal impregnation techniques, he demonstrated that interstitial cells stained differently from nerve cells with which they kept a close spatial relationship. Nowadays, these interstitial cells were found in a wide variety of organs [3] including pancreas [4], urinary tract [5–7], uterus [8, 9], fallopian tube [10], mammary gland [11, 12], heart [13–15], blood vessel wall [16, 17] and, indeed, gastrointestinal tract [18].

Their physiologic role was not clear at that time and even today, but Cajal speculated on a possible ancillary neural function, because of their anatomic proximity and morphologic similarity to nerves. In fact, interstitial cells of the gut are neither neural nor muscular, but are interposed *between* nerve and muscle cells. He considered that they might play a controlling function of the gut motility, and he thereby stimulated many investigators to study his concept. Consequently, a vast amount of data has been accumulated on **1**) their existence, **2**) the ultrastructural characterization of a possible pacemaker role in the gut, **3**) electrophysiologic studies of peristalsis, **4**) c-Kit as a molecular marker of ICC, and **5**) as progenitor cells of gastrointestinal stromal tumors (GIST). The data thus far accumulated is vast and complex, but has promulgated the definition and identification of ICC [19].

In recent years, much interesting molecular information has accumulated, and some authors are tempted to accept it uncritically. Therefore, there is a strong need to clarify some of the current concepts about ICC. According to the currently prevailing concept, ICC are heterogeneous cells, composed of fibroblast-like, and/or muscle-like cells interposed between nerve and muscle cells, but recently close appositions to capillaries and immunoreactive cells were reported for ICC [12, 20]. ICC are considered as pacemaker cells, expressing CD117, and serving as progenitor cells of GIST.

Cytomorphology

The cytomorphology and anatomic relationships of ICC in the intestine have been amply confirmed [21–23]. However, it is important to remember that ICC are located along the nerves in the interstitium. According to Cajal's drawing (as well as the drawings and photomicrographs of subsequent investigators), ICC are numerous in the submucosal interstitium along the inner surface of the circular muscle layer of the intestines, and between layers of muscularis propria. ICC form a loosely arranged network linked by long cytoplasmic processes. Indeed, the number of ICC within the muscular bundle is limited. A similar pattern has also been also confirmed on ICLC distribution in other organs in terms of gradient in density, and morphological interrelationships with various tissue structures [10]. These features suggest an important role for ICC, or ICLC in tissue physiology and pathologies, including GIST.

Ultrastructural characterization of ICC

Electron microscopy has been used to study ICC, yielding a large amount of information. However, it has to be pointed out that there was no reliable marker for recognizing ICC ultrastructurally. Identification of ICC as well as ICLC under elec-

tron microscopy needs a set of criteria. The cells depicted as ICC have been heterogeneous and initially confusing [24]. The earliest investigators used their light microscopic experience, and Cajal's description to identify ICC ultrastructurally. The cells they considered to be ICC were fibroblast-like cells which appeared to be too plain to have a significant function assigned to them [25–31]. Later, investigators reported cells with features of muscle-like cells, intermediate cells, and with features of fibroblasts to be ICC [32, 33]. These cells were similar to the modified muscle cells found in the esophagus by Faussone-Pellegrini *et al*. [34] who did not accept them as ICC at first, because it was thought highly unlikely that silver impregnation methods and vital methylene blue methods (as used by Cajal and others for the identification of ICC) would stain cells with the characteristics of smooth muscle cells. The authors suggested that they might have a pacemaker role.

Later investigators used additional information from electrophysiologic studies of the gut [35–37], the techniques which were expanded further to correlate electrophysiology and cell morphology by immunofluorescence [38, 39]. With additional information of electrophysiological data defining the intestinal motility, it was thought to be reasonable to include cells with smooth muscle appearance in the group of ICC [40, 41]. These cells contained impressive numbers of cytoplasmic organelles and filaments. They had gap junctions with smooth muscle cells and varicosities of nerve cells deemed adequate for a signal transmitting [40–42]. These findings corresponded well with the electrophysiological studies [35, 36]. Isolated gut specimens separated from myenteric nerve control were found to generate spontaneous rhythmic electric activity termed as "slow wave" consistent with peristaltic activity. Furthermore, the origin of slow waves was thought to be located in the submucosal border of the circular muscle and muscles around the myenteric plexus. These two sites were regarded as pacemaker regions so that pacemaker cells were expected in these regions. Not surprisingly, smooth muscle-like ICC were most frequent in the inner circular muscle. They focused their attention on these regions to identify ICC, and found elongated cells along the inner border of circular muscle bundles. Their topographic set-up was appropriate for signal transmitting. These cells contained bundles of filaments, caveolae and external lamina similar to smooth muscle cells [41, 42]. This was the beginning of the new era in which smooth muscle-like cells were added to the ICC. Now, it is widely accepted that ICC consist of a heterogeneous group of cells (as listed previously).

In 1982, Thuneberg published his monograph; "Interstitial cells of Cajal: pace-maker cells?" [42]. He presented a comprehensive review of previous findings and his own data, which included detailed ultrastructural analysis of cells assumed to be ICC. He asserted that different types of ICC were found in different layers of the intestinal wall. ICC-I were found around Auerbach's plexus, and ICC-II were found in the serosal interstitium, ICC-III in the *plexus muscularis profundus*, and ICC-IV accompanying nerves in the interstices of the main (outer) circular muscle layer. ICC-I, II and IV of Thuneberg stained positively with methylene blue, and exhibited ultrastructural features similar to those described as fibroblast-like ICC by previous investigators. In this author's judgment, these cells fit the criteria of ICC according to Cajal. These ICC had branching cytoplasmic processes, and were found external to the external lamina of the enteric nerves, and only rarely exhibited direct contact with muscle cells. In contrast, ICC-III were similar to smooth muscle cells, and were most frequently found within the inner circular muscle (*plexus muscularis profundus*). These cells did not stain with methylene blue, but were identified as ICC "only by electron microscopy". The author stated that the characterization of an interstitial cell at the level of the *plexus muscularis profundus* as a separate type of ICC was justified by ultrastructural and topographical peculiarities of this cell with similarities to ICC of other locations.

We note that the significant deviation of ICC-III from other ICC by their failure to stain with methylene blue. ICC-III were very similar to smooth muscle cells including "similar nuclear morphology and orientation with respect to apparent length, shape, distribution of condensed chromatin, and the characteristic arrangement of nucleoli in a longitudinal row, filaments of mainly the actin type to form bundles only in the smaller processes, abundance of caveolae, and distinct basal lamina". Now, it is not

clear, however, how the author justified his inclusion of these smooth muscle-like cells into ICC. He stated that "these cells were unstained by methylene blue, under any conditions of incubation tried". Obviously they were not ICC interstitial cells as Cajal defined by methylene blue staining. It is also very significant that they do not appear to be a part of an ICC network which led to the hypothesis of ancillary neural function of ICC. Despite evidence, smooth muscle-like cells of the *plexus muscularis profundus* were added to the group of ICC. The cells depicted as ICC-III maintained close cellular contacts with nerve fibers as well as muscle cells. With this finding he proposed a pacemaker function for ICC. These authors did not mention that they were departing from Cajal's original criteria in regard to the location and anatomic distribution of the ICC. Clearly the authors proposed that ICC have a pacemaker function, and that smooth muscle-like cells with a peculiar shape and topographic arrangement were included. Subsequent investigators confirmed what was suggested by Thuneberg and his group [43–48].

Currently smooth muscle-like features are required to confirm ICC identity by electron microscopy [49]. They have departed from Cajal's criteria defining and identifying his interstitial cells. These cells are unstainable by methylene blue, and are parenchymal cells. Therefore, they should not be designated as ICC in our opinion. Kobayashi, who applied extensive ultrastructural studies in his investigation, raised the same question as to the true nature of ICC-III [50]. Admittedly, the data accumulated through electrophysiological studies indicates that the so-called ICC-III may have a pacemaker function in the gut [35, 36]. Thus, the authors may be describing pacemaker cells of the gut, but not ICC.

According to our own study of ICC in human small intestinal specimens, ICC defined following Cajal's criteria, were limited to fibroblast-like cells [51]. Interestingly, there were frequent nerve endings forming synapse-like junctions with smooth muscle cells without intervening ICC, as could be noted by electron microscopy. Furthermore, smooth muscle cells having synaptic contacts with nerve endings showed the irregular shapes of multipolar cells in contrast to bipolar smooth muscle cells. The electron density of the cytoplasm of these cells was usually less than that of bipolar cells, suggesting loss of cytoplasmic filaments. However, on closer examination, these cells had all the features of smooth muscle cells. These cells may represent a sentinel cell for receiving neural signals. It might be speculated that the different staining characteristics of these cells could reflect a different state of depolarization or perhaps a fixation artifact. These smooth muscle cells presented some of the features of ICC-III, and were interpreted as smooth muscle-like ICC or ICC-III, by other investigators [52, 53]. It has to be reiterated that the location and pattern of distribution of smooth muscle-like ICC do not coincide with those described by the methylene blue stained crushed tissue preparations by Cajal or with ICC demonstrated by CD117 staining. It is now well known that CD117, the product of the *c-Kit* gene, is normally expressed in ICC, and serves an excellent marker of ICC [54–56] and can be used for the identification of ICC. However, ICLC expressing on a less consitstent basis this marker have been reported [9, 13–15].

Recently, a comprehensive review on ICC in regard to their identification by ultrastructure, light microscopy, and c-Kit as a light microscopic marker has been published [57]. The authors made detailed analysis of data from over four hundred ninety articles, and concluded that transmission electron microscopy is the "gold standard" for the identification of ICC. Moreover, ultrastructural identification of ICC were newly detailed and improved by defining a "platinum standard" [10].

Electrophysiology of gut motility

Electrophysiology studies yielded influential information in our understanding of the pace making process of the gut and they influenced morphologists in identifying ICC-III by electron microscopy. The cytomorphologic appearance, cellular organelles, and relationships kept with other cells provided the morphologic and functional basis for assigning a pacemaker function to ICC. As with other studies, these cells do not comply with Cajal's criteria. Many recent articles deal with "slow wave" generation of isolated cells, giving additional support to the claim that ICC are pacemaker cells [58–61]. However, data in the literature in regard to cells capable of generating slow waves are some-

what controversial. Some investigators recently state that "slow wave" generation is specific and limited to ICC-III, while others have observed "slow wave' generation within muscle cells of the gut in general [35, 36]. Therefore, further studies are needed to clarify this question.

Molecular biology of ICC

One of the most significant advances in the study of ICC was the discovery of the proto-oncogene, *c-Kit*, which encodes a receptor tyrosine kinase in the PDGF-receptor family. Initially, *c-Kit* was known to be involved in developmental processes of three cell lineages; hematopoietic cells, melanocytes and germ cells. In 1992, Maeda *et al.* performed an interesting experiment to further define the *in vivo* role of *c-Kit*. They injected ACK2, a monoclonal antibody of the *c-Kit* gene product, to BALB/c mice neonates. They found that small numbers of cells in the intestine expressed c-Kit, and antibody injection caused a lethal paralytic ileus in these animals [54]. The authors speculated that c-Kit expressing cells might represent ICC, and they concluded that lethal ileus in these animals was due to an anti-c-Kit effect. Subsequently, a plethora of reports appeared in the literature claiming that *c-Kit* expressing cells are indeed likely to be ICC [*e. g.* 55, 56]. It is interesting to note that their spatial distribution and cytomorphologic features are comparable, if not identical, to those of methylene blue stained preparations Cajal originally used. Thus, it is now widely accepted that c-Kit expressing cells in the gut represent ICC and *c-Kit* expression is being used as an immunohistochemical marker for ICC.

c-Kit is allelic with the dominant white spotting (*W*) locus of mutant mice. A number of *W* mutant mice have been identified with various degrees of intestinal motility disturbances, suggesting the importance of c-Kit expressing cells in the autonomic control of intestinal muscular contractions. However, it is important to note that even with such a severe mutational disorder, most mouse strains with mutations at the (*W*) locus manage to maintain defecation, and avoid lethal paralytic ileus. This suggests that other regulators may also be involved in motility [54].

Obviously, the discovery of *c-Kit* expression of ICC as a usable marker for facilitating various research efforts related to ICC and for expanding our general knowledge and understanding of ICC. It is still not known whether ICC-III express *c-Kit*.

Another important development in relation to the cells expressing *c-Kit* was that tumor cells frequently express the *c-Kit* gene product in the so-called GIST [62–64], and many of these tumors were shown to have a gain-of-function mutation of the *c-Kit* gene [65, 66]. These findings led to the current concept that GIST are ICC-originating tumors. Recently STI571 (Glivec, Novartis, Basel, Switzerland), a tyrosine kinase inhibitor, was found to have a favorable therapeutic effect on metastatic GIST and *c-Kit* expression in tumor cells became an important criterion in the diagnosis as well as the management of GIST [67–69]. Moreover, Glivec was proved to inhibit spontaneous rhythmic contractions of human intestine and also in human uterus, perhaps *via* ligand-independent c-kit/CD117 tyrosine-kinase signaling [70].

Pacemaker function of ICC

There have been numerous reports indicating that the ICC control rhythmic gut motility [58, 59]. The majority of studies have used sophisticated research techniques such as isolated cells, cultured cells of purified populations of ICC, electrophysiologic use of super- or ultrafine electrodes probing into single ICC, and electron microscopy [60, 61]. The data presented by these reports are overwhelmingly convincing. These studies are concerned about "slow wave" generation in isolated cells, CD117 positivity, and ultrastructural demonstration of myoid features as confirmation of their identity as ICC. Whether ICC-III are a part of ICC, or whether they express *c-Kit* remain to be shown. Concerning the so-called "slow wave" activity (the best marker of peristaltic activity of the gut), it does not seem to be limited to cells in the pacemaker region, or to *c-Kit* expressing cells. Generation of slow-wave activity has also been recorded in isolated portions of muscle wall. It is, therefore, not conclusive whether the cells are *bona fide* ICC.

In the report of Maeda *et al.* [54], the animals developed fatal paralytic ileus quite dissimilar to the findings in *c-Kit* mutated animals. In the later, animals can still survive and defecate, suggesting

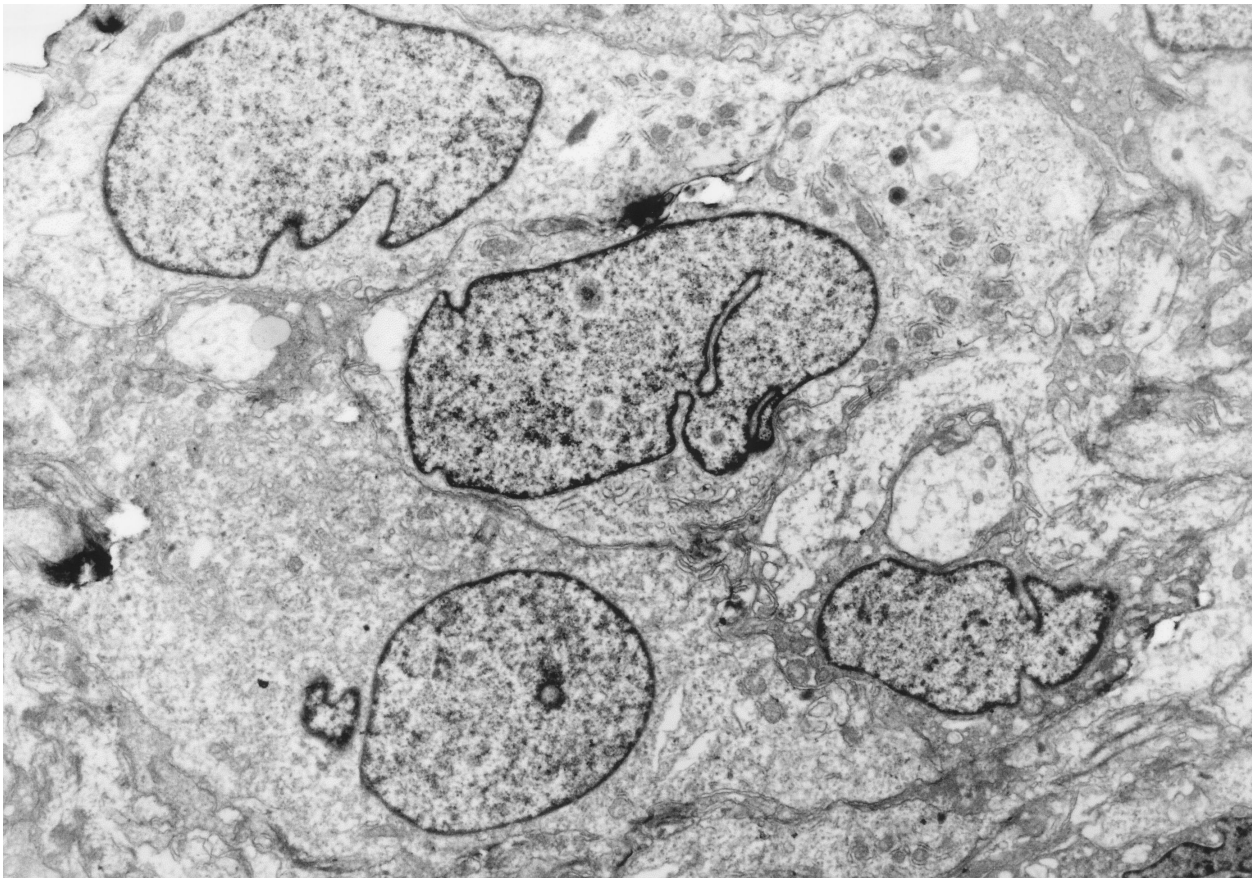

Fig. 1 A gastric GIST in a 54 year old female composed of non-specific mesenchymal cells forming closely packed fascicles.

that other regulators of gut motility may compensate for the defect [54]. Although the authors did not comment, the reason for the fatality of animals in their study might have been due to another reason. Perhaps, they may have died due to the sudden impact of ACK2 not only from *c-Kit* expressing ICC but from the immature musculature of new-born.

Gut musculature is endowed by a rich network of autonomic nerves and a high concentration of ganglia along the submucosal border of the inner circular muscle, and the myenteric plexus, both pacemaker regions [51]. Granted, "slow wave" activity can be observed without the influence of the autonomic nerves, the afore-mentioned concentration of ganglia near the pacemaker regions may not just be coincidence. Indeed, there may be a close collaboration between enteric nerves and ICC in the control of the gut motility (as Cajal speculated). Which one plays the primary role remains to be evidenced by future studies.

In addition to a concentrated presence of enteric ganglia near the known pacemaker region, our study has also shown that a rich network of enteric nerve fibers is present throughout the entire muscular wall of the intestines. Indeed, direct synaptic contact between muscle cells and nerve endings of myenteric nerves were frequently found [51]. Fibroblast-like ICC were found around nerves, interposing between nerve and muscle cells. These smooth muscle cells in direct contact with nerves may be responsible for passing signals to other muscle cells for coordinated rhythmic contractions of gut musculature. If this is true, the role of ICC according to Cajal's definition may have a limited role in the rhythmic control of gut motility. In this regard, it is also interesting to note that the majority of patients receiving Glivec for chronic myelogenous leukemia and metastatic GIST experienced no significant gastrointestinal symptoms, except dysfunctions of digestive motility [71]. It seems

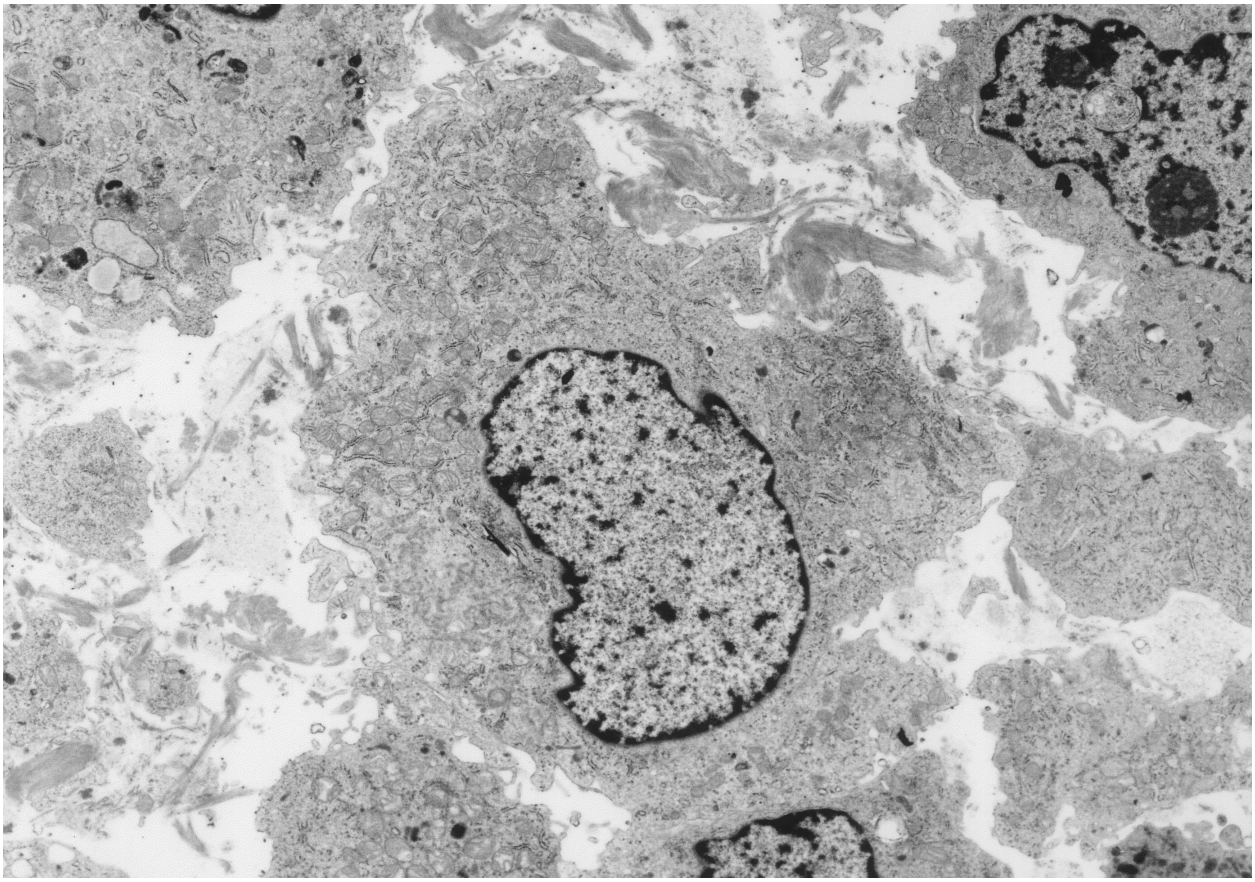

Fig. 2 A gastric GIST composed of epithelioid mesenchymal cells having prominent cytoplasmic organelles including RER.

that only a small number of patients (perhaps less than 5%) of treated patients had documented nausea and vomiting and non-specific abdominal pain when treated with a relatively large daily dose.

GastroIntestinal Stromal Tumors (GIST)

We owe the term "gastrointestinal stromal tumor" to Mazur and Clark [72] who pointed out that the majority of spindle cell tumors of the gastrointestinal tract were not smooth muscle tumors, and therefore, deserved a new designation. GISTs were thought to be tumors of uncertain cellular origin. GIST exhibit heterogeneous ultrastructural features [62, 73–75]. Several different types of GIST have been characterized, including plexosarcomas [76], gastrointestinal autonomic nerve tumors (GANT) [77, 78], myenteric plexomas [79], and generic GIST. These different designations were justified mostly by ultrastructural features, which pointed toward an origin from ganglion cells in approximately one half of all GIST [78]. The tumors designated as generic GIST frequently present minimal myoid differentiation including focal presence of external lamina (EL) and focal accumulation of cytoplasmic filaments suggestive of actin filaments [62, 74, 75]. In others, the tumor cells resemble uncommitted mesenchymal cells with no myoid or neuronal features. In spite of the differences in ultrastructural features, the majority of GIST express *c-Kit* protein in the cytoplasm of tumor cells. These findings led us to believe that GIST are ICC tumors [62–64]. In most tumors, the cells usually show a uniform line of cellular differentiation. Conversely, GIST exhibit ultrastructural features ranging from minimal myoid (Fig. 1, 2), to uncommitted mesenchymal (Figs. 3, 4), to

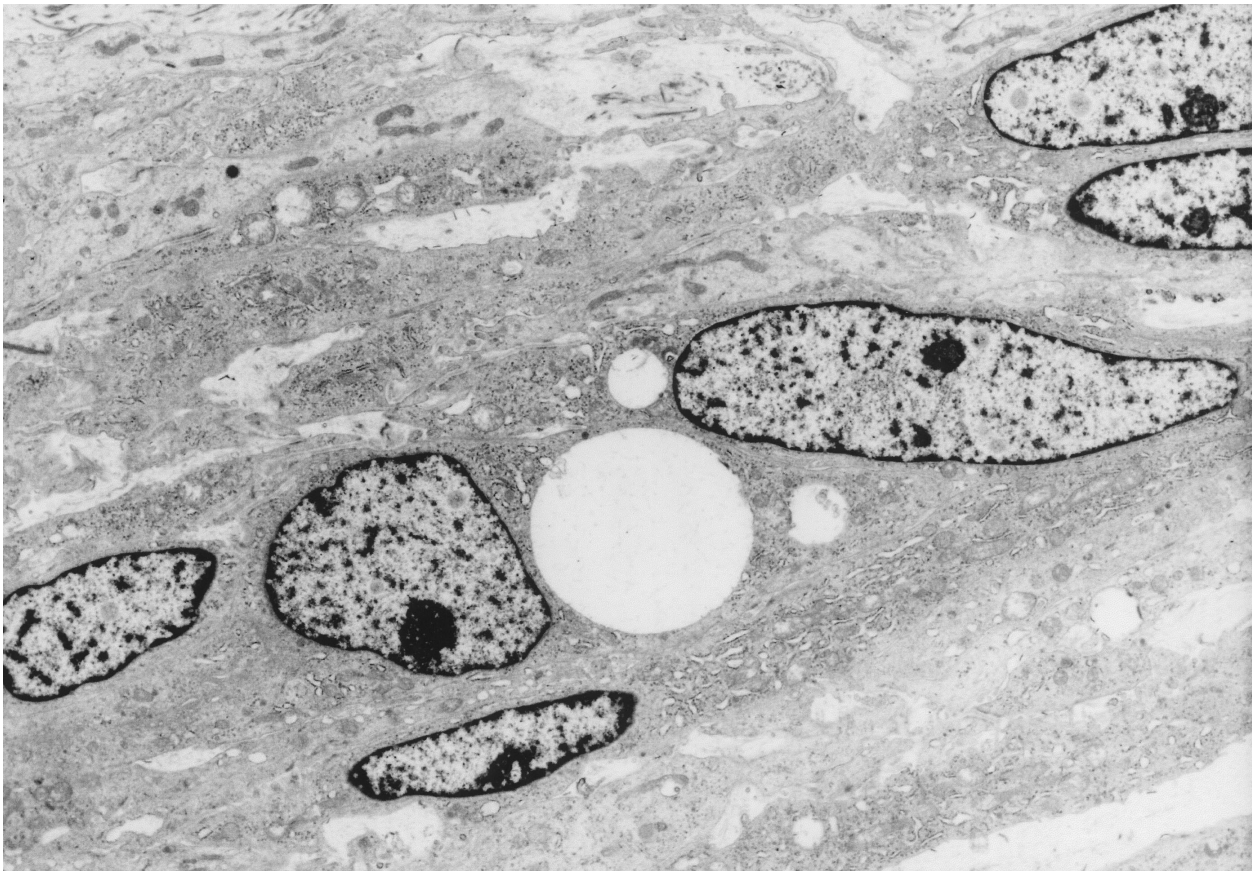

Fig. 3 A gastric GIST composed of closely packed spindle cells with elongated cytoplasm. Note the presence of rich RER. Note a perfectly round empty cytoplasmic vacuole. At this magnification, the presence of EL is not evident.

neuronal (Fig. 5–7), and even to mixed neuronal and myoid features (Fig. 7).

Despite this morphologic variability, the majority of these cells can express CD117, identical genetic and molecular characteristics [80]. Thus, *c-Kit* is the unifying marker of GIST, which is important for diagnosis and management of GIST in the present time [65, 66, 68, 81, 82]. The morphologic variety of GIST at the ultrastructural level has been partly explained by heterogeneity of ICC.

Other interesting clinical features in patients with GIST include familial occurrence [81, 83], presentation with neurofibromatosis [84, 85], and Carney's triad [86, 87]. It is particularly interesting that GIST in patients with NF1 and familial examples are usually multiple, and may show varying stages of development of microscopic tumors arising from the myenteric plexus. It is also significant that multiple GIST have been experimentally produced in *c-Kit* gene knock-in experimental animals [82, 88].

Another aspect of GIST is the presence of peculiar collagens in the stroma including skeinoid fibers (Fig. 8), tram-track-like precipitated collagen (Fig. 9), and centriole-shaped crystallized collagen (Fig. 10) [89]. It is notable that GIST with these special crystallized collagens are those with the least myoid features. The overall ultrastructural features in these cases are closer to gastrointestinal autonomic nerve tumors (GANT). Also, some of the tumor cells contain frequent dense-core granules in the perikarya as well as in the bulbous cytoplasmic processes similar to nerve endings (Fig. 5, 6). In spite of these features of neuronal differentiation, some cells in the same tumor may exhibit focal accumulation of filaments with foci of increased density (Fig. 6, 7) suggestive of myoid differentiation. In this instance, tumor cells appear to differentiate toward more than one line of cellular differentiation [62, 74, 75, 90]. Thus the ultrastructural features of GIST correlate well with that

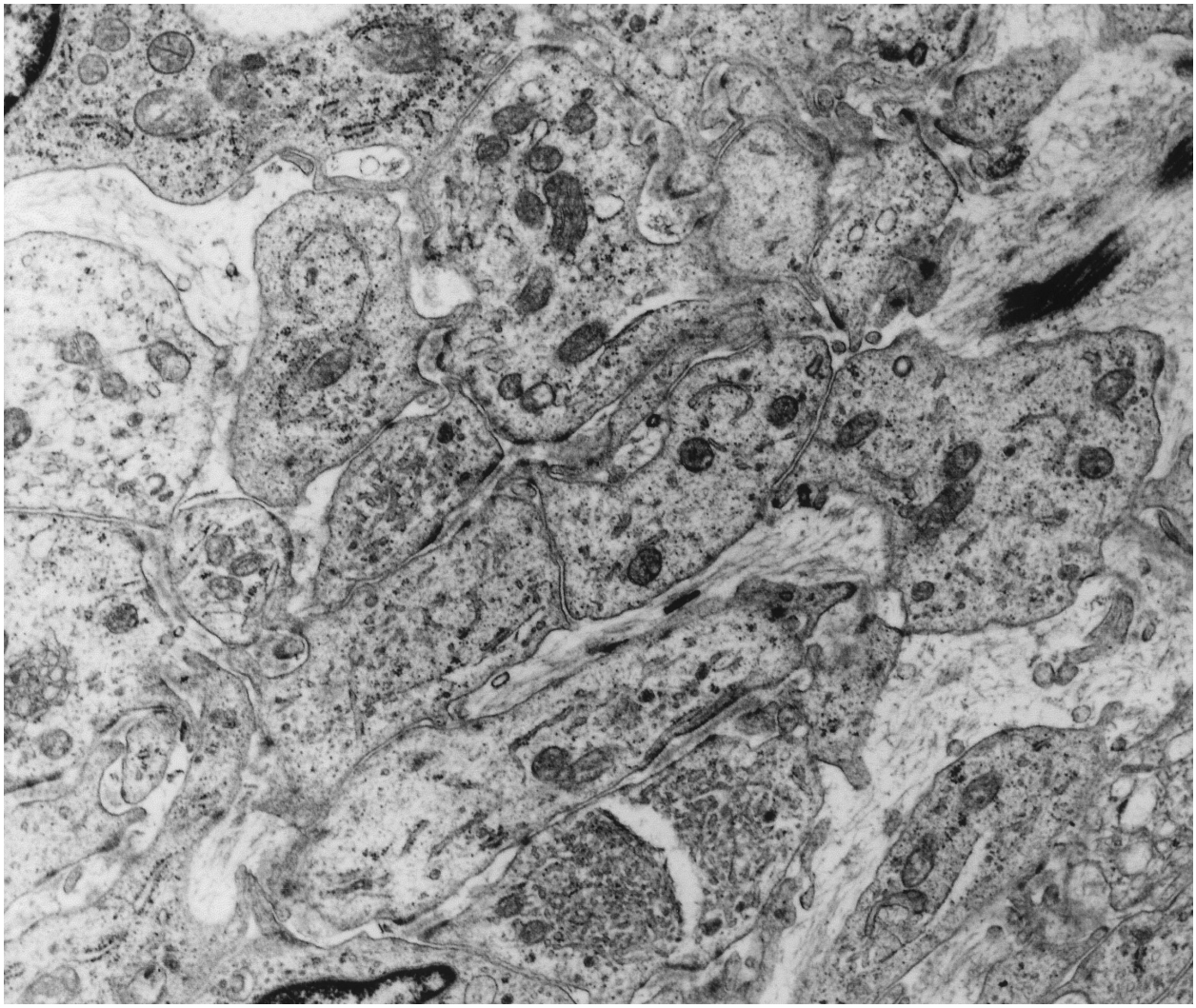

Fig. 4 Higher magnification of the area of Fig. 3 showing frequent presence of EL on which tumor cells are anchoring with hemidesmosomes.

of ICC, other than the latter, since no neuron-like features have ever been reported in ICC. Again, there is no proof that muscle-like ICC (ICC-III) can qualify as true ICC according to their tinctorial properties or CD117 expression. Thus, it is essential to clarify the nature and origin of *c-Kit* expressing cells in the gut.

Origin of ICC

The embryonic origin of ICC was unknown and remained speculative until Lecoin *et al.*, using chimeric animals, demonstrated that they derive from the embryonic mesenchyme, not from neuroectoderm [91]. This was confirmed by Young *et al.* [92] using slightly different experiments. In recent years, many investigators have utilized new molecular markers and advanced molecular techniques to follow developmental sequences of ICC. These techniques indicate that smooth muscle cells and *c-Kit* expressing ICC develop from common progenitor cells [93, 94]. Kluppel *et al.* [94] analyzed cells expressing mRNA of *c-Kit* and smooth muscle myosin heavy chain (SMMHC) in the gastrointestinal tract throughout various stages of embryonic development of wild type, *W*57 and *W*bd mutant mice. They found that both smooth muscle cells and ICC derive from common pro-

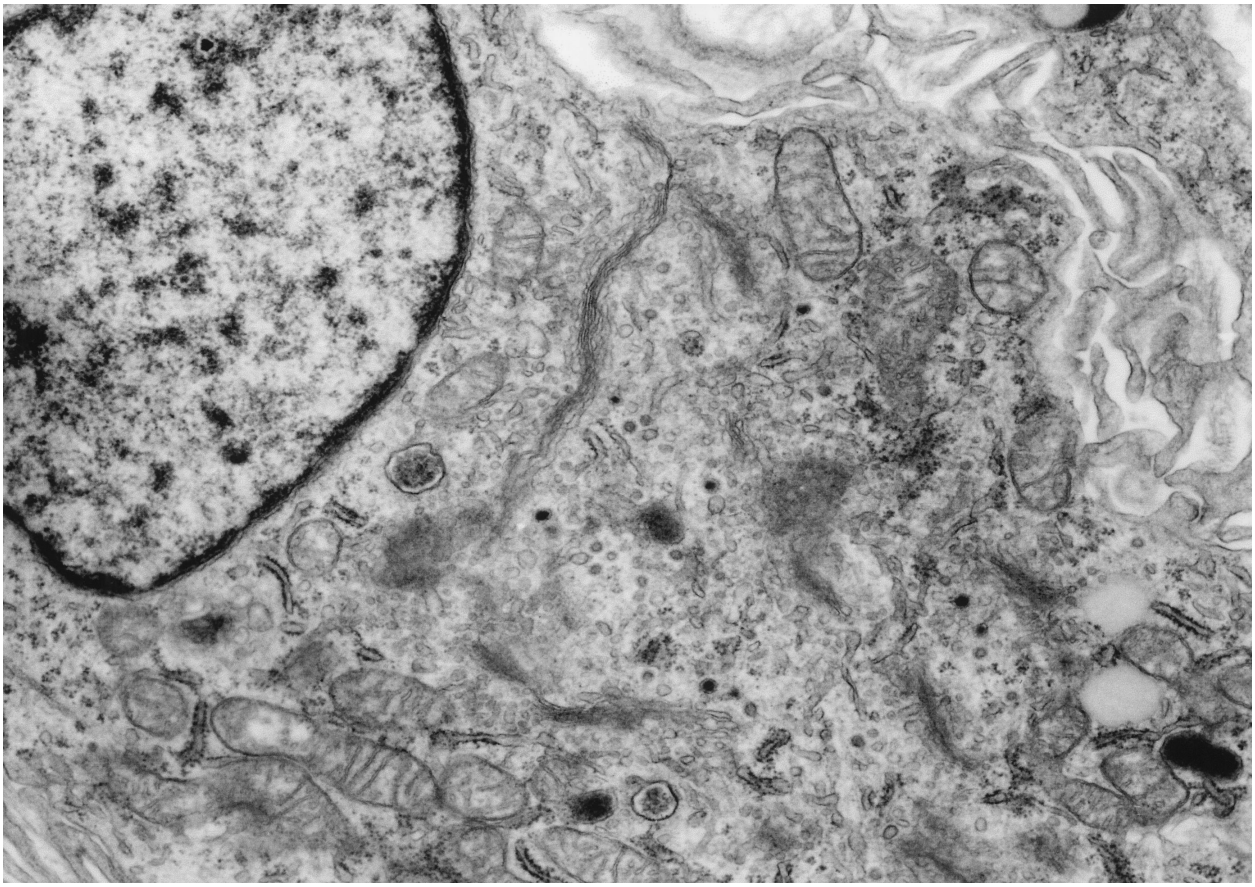

Fig. 5 A gastric GANT from a 26 year old female showing numerous dense core granules in the perikarya.

genitor cells expressing both mRNA of *c-Kit* and SMMHC until day 14.5 in both animals. After day 16, the distribution pattern of *c-Kit* and SMMHC expressing cells gradually changes to the pattern seen in adults in both murine strains. These findings indicate that the majority of progenitor cells expressing both markers gradually lose *c-Kit* to become smooth muscle cells. Other cells, particularly those around Auerbach's plexus, maintain *c-Kit* expression, lose SMMHC, and become ICC. Thus, it is likely that the developing gut contains immature cells with different combinations of these two proteins until full development. Maybe some of these cells may remain in adult tissues functioning as organ-specific stem cells. If this is true, then it is logical that such stem cells are progenitor cells of GIST. Indeed, SMMHC has been demonstrated in a majority of GIST [95] despite the lack of demonstrable muscle markers, supporting our hypothesis. Furthermore, Torihashi *et al* [96] demonstrated that blockage of *c-Kit* signaling induced transdifferentiation of ICC into a smooth muscle cells. Therefore, a uniform histogenesis for all GIST can be posited.

A few issues must still be addressed. The first is the neuronal features in some GIST. Neuronal features of GIST known as GANT have been well documented. Bidirectional differentiation of GIST was also suggested by immunohistochemical studies which detected muscle markers in some GIST with neural features [62, 74, 75, 90]. Neuronal features described in GANT are usually detected by electron microscopy and the diagnosis of GANT largely depends on electron microscopic studies [78]. Ultrastructurally, dense core granules seen in cases of GANT and GIST with dual differentiation appear to be true neuroendocrine granules, but this cannot be adequately confirmed by immunohistochemistry in the majority of cases raising questions as to their true nature. Similar dense core granules of uncertain nature have mimicked the appearance of neuroendocrine granules in some tumors (Figs. 5, 6).

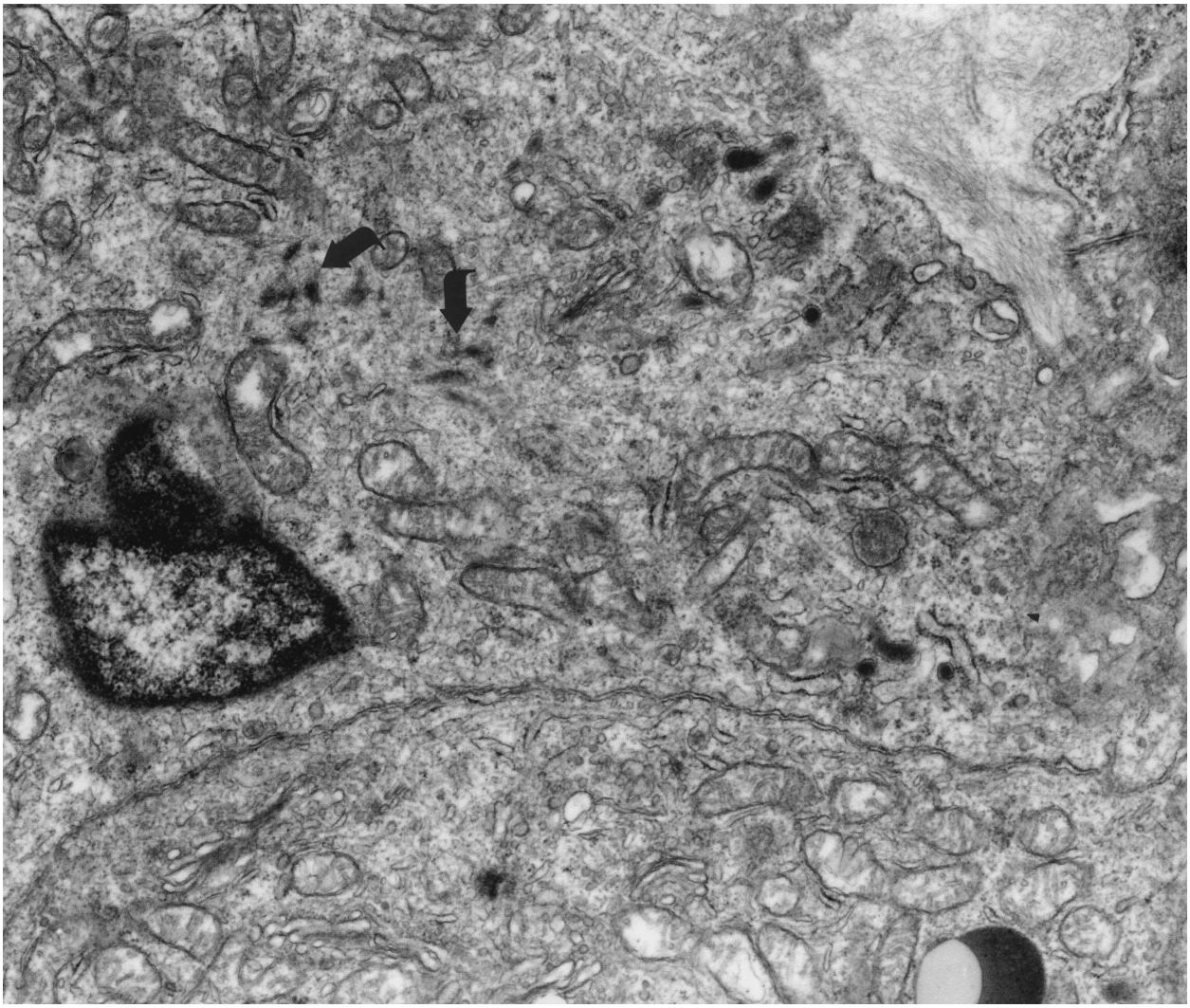

Fig. 6 A gastric GANT from a 16 year old female showing dense core granules and aggregates of intermediate filaments with increased densities suggestive of actin filaments in the same cell.

Furthermore, some tumor cells in GANT may show focal cytoplasmic accumulation of filaments suggestive of actin filaments in addition to dense core granules in the same cell (Fig. 7). These findings have been interpreted as evidence of bidirectional differentiation by some [62]. However, it is highly unusual and difficult to explain two different lines of differentiation in a given cell. Neuronal features seen in GIST may be non-specific and may represent an aberrant feature as seen in some other tumors such as extraskeletal chondromyxoid sarcomas [97]. It should also be pointed out that ICC, the putative precursor cell of GIST, have never been shown to express neuroendocrine features by electron microscopy or immunohistochemistry.

The second is the presence of abnormal collagen in some GIST. In GIST classified as non-myoid or neuronal, no convincing EL can be found. Instead, peculiar collagen fibers known as skeinoid fibers, crystallized forms of tram-track appearance, and centriole-like fibers can be seen. They are found along the cytoplasmic borders of tumor cells or intercellular spaces where EL may normally be expected (Figs. 8–10). It is interesting that the presence of EL and crystallized collagen seem to be mutually exclusive in a given tumor, suggesting the possibility that the abnormal collagen seen in some GIST may represent altered collagen of lamina forming protein. It is further interesting that GIST with abnormal collagen are the ones more frequently associated with

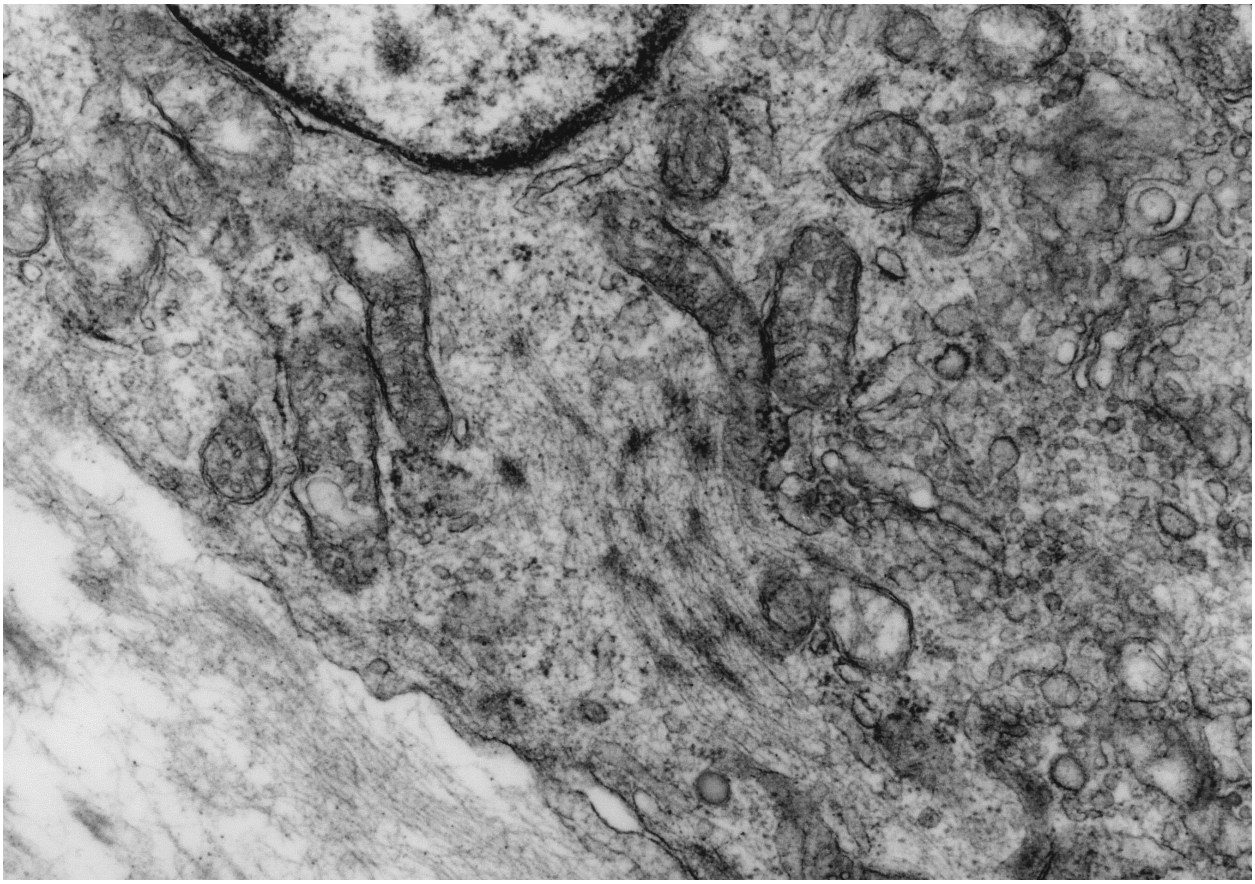

Fig. 7 Detailes of actin-like filaments in a tumor cell of GANT.

multiple tumor syndromes such as NF1 and Carney's triad. In these syndromes, complex multiple mutations may be an underlying condition. The abnormal collagens in these syndromes may be the result of other as yet unknown genetic change. In such cases, it is not unreasonable to conclude that the majority of GIST are composed of cells differentiating toward smooth muscle cells and might derive from the progenitor cells which retain potential to differentiate toward c-Kit expressing smooth muscle cells and mesenchymal cells.

The third is the presence of interstitial Cajal-like cells (ICLC) in the extra-gastrointestinal sites and the occurrence of so-called extra-gastrointestinal stromal tumors (eGIST). The presence of ICLC has been reported in many organs (urinary bladder, gallbladder, omentum, and mesentery; see ref. [98] for a short overview). A partial list of organs proved to contain ICLC includes pancreas [3], urinary tract [4, 5], uterus, fallopian tube [6, 7], atrial and ventricular myocardium [9–11] and the list is growing. Moreover, spatial distribution of ICC or ICLC and interrelationships with nerves, blood vessels, and various resident or migrating cells in interstitium hypothetically suggest a role in local integration of cells in tissues and tissues in organs by either gap junctions or paracrine and/or juxtacrine mechanisms.

The finding of ICLC in the organs other than the gut is not surprising, and is rather expected. According to the original concept of interstitial cell system of Cajal, ICC are supposed to be present in other organs (*e.g.* pancreas) [1, 2]. While Cajal has used methylene blue staining techniques to demonstrate ICC, investigators in these reports used immunostain techniques using antibodies against CD117 for the identification of ICLC. We would like to advice caution on staining technique to evaluate CD117 expression. In our experience and others' [99–101], interpretation of staining results of CD117 can be complicated. Intensity and sensitivity of stain depends on the staining techniques applied (methods of antigen retrieval, and strength of antibodies, and length of incubation of primary anti-

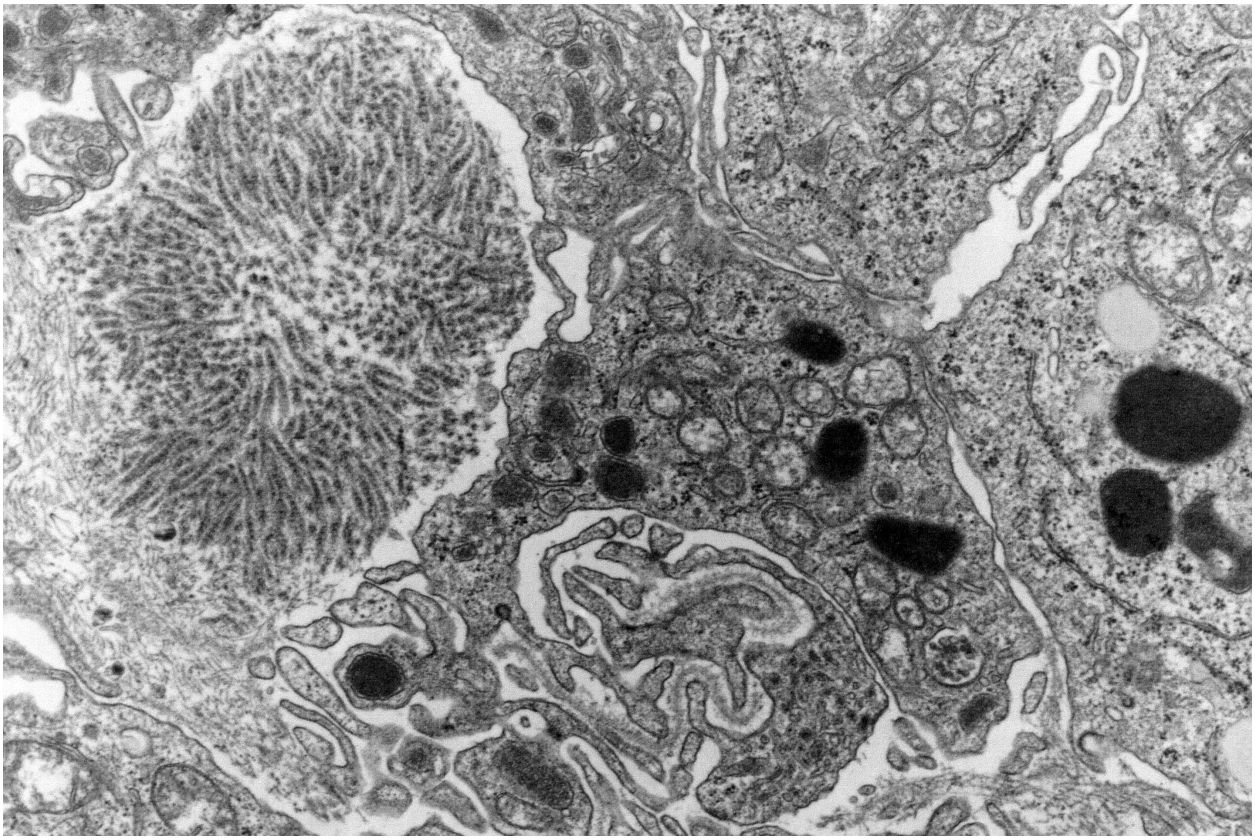

Fig. 8 A small intestinal GANT from a 78 year old female showed frequent skeinoid fibers. Note EL-like lamina material nearby.

body). With application of antigen retrieval procedure and increased strength of antibody, spindle cells in various fibromatosis and other sclerosing lesions could be stained positively for CD117 without background staining, thus appearing as true specific expression [102]. Yet, no one would accept these lesions as ICC related lesions. Currently, we use a section of small intestine containing myenteric plexus and ICC-AP as positive control and sections of fibromatosis as negative control to set the optimum staining condition for CD117 immuno-stains. ICLC seem to have cytologic and immunohistochemical characteristics similar to ICC and express c-Kit. Judging by the illustrations included in the reports, ultrastructural characteristics of ICLC are quite similar to ICC and more closely resemble to those fibroblast-like ICC found around the myenteric plexus [51] than ICC with myoid features. The physiological role of ICLC is not clear. They are not only found in muscular organs similar to the gut but also in the organs in which no pacemaker function is expected. Such ICLC may have other physiologic role. Thus, further clarification of identity and function of these cells may provide additional insight into our understanding of ICC [102].

extra-GastroIntestinal Stromal Tumors (eGIST)

The presence of ICLC in other organs could explain the development of eGIST [98]. Spindle cell tumors similar to GIST have been reported in the extra-gastrointestinal sites such as omentum, mesentery, urinary bladder, gallbladder, retroperitoneum, and pancreas [103–110] and their incidence is frequent enough to be separately categorized as extra-gastrointestinal stromal tumors (eGIST). They seem to be indistinguishable from GIST in their cytomorphology, *c-Kit* expression and genetic changes. This similarity led to the speculation that eGIST, particularly those arising

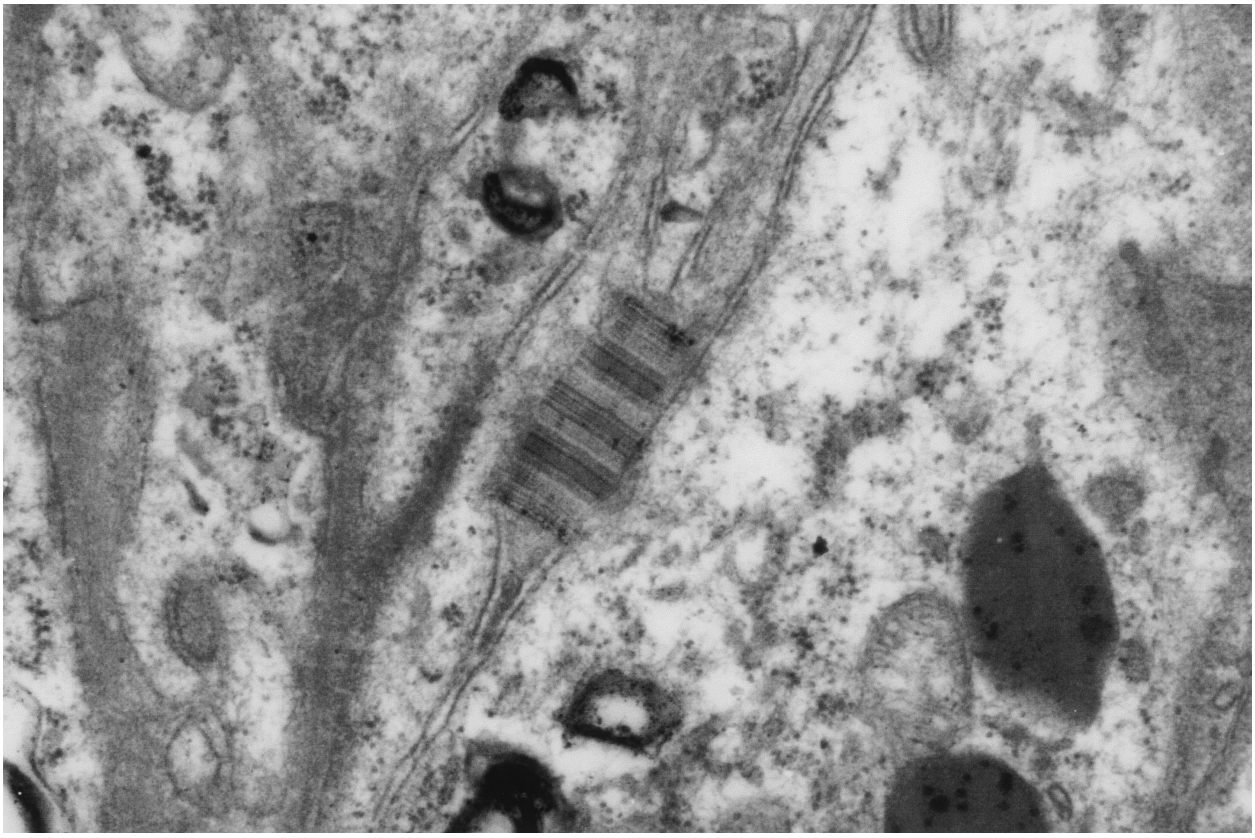

Fig. 9 A gastric GANT in a 17 year old girl having classical Carney's triad showing tram-track-like precipitated EL. Note the normal looking EL adjacent to the precipitate.

in the adjacent area of the gut, were presumed to arise from ICC or ICLC [103, 104]. Recent documentation of the wide presence of extra-gastrointestinal ICC in the sites eGIST occur suggests that ICLC may be the progenitor cells of eGIST. In a case of pancreatic eGIST reported by Yamamura *et al.* [109], *c-Kit* expressing cells were found in the adjacent normal pancreas and interpreted as the possible progenitor cells of the pancreatic eGIST they have described.

Conclusion

It appears that ICC with myoid features are not the ICC originally depicted by Cajal. It was pointed out that most of data assigning pacemaker function to ICC is based on the observations related to ICC with myoid features. The interstitial cells which express CD117 and are abundant around the myenteric ganglia more likely represent the ICC described by Cajal. Their physiologic role has not been adequately documented. In this regard, it is very encouraging to note that there have been recent efforts to investigate the relationship of various types of ICC, particularly ICC found around the myenteric plexus (ICC-AP) [111]. New data are forthcoming and are expected to clarify some of these questions.

GIST derive from a progenitor cell, most likely a committed stem cell residing in the muscular wall of the gut. These cells maintain some characteristics of developing embryonal stem cells of the gut, expressing both the mRNA of *c-Kit* and SMMHC protein, and are capable of differentiating into *c-Kit* expressing mesenchymal cells and/or actin containing smooth muscle cells.

The acummulating data on ICLC and a more precise description of eGIST will highly improve our knowledge about interstitial cells in both physiology and pathology.

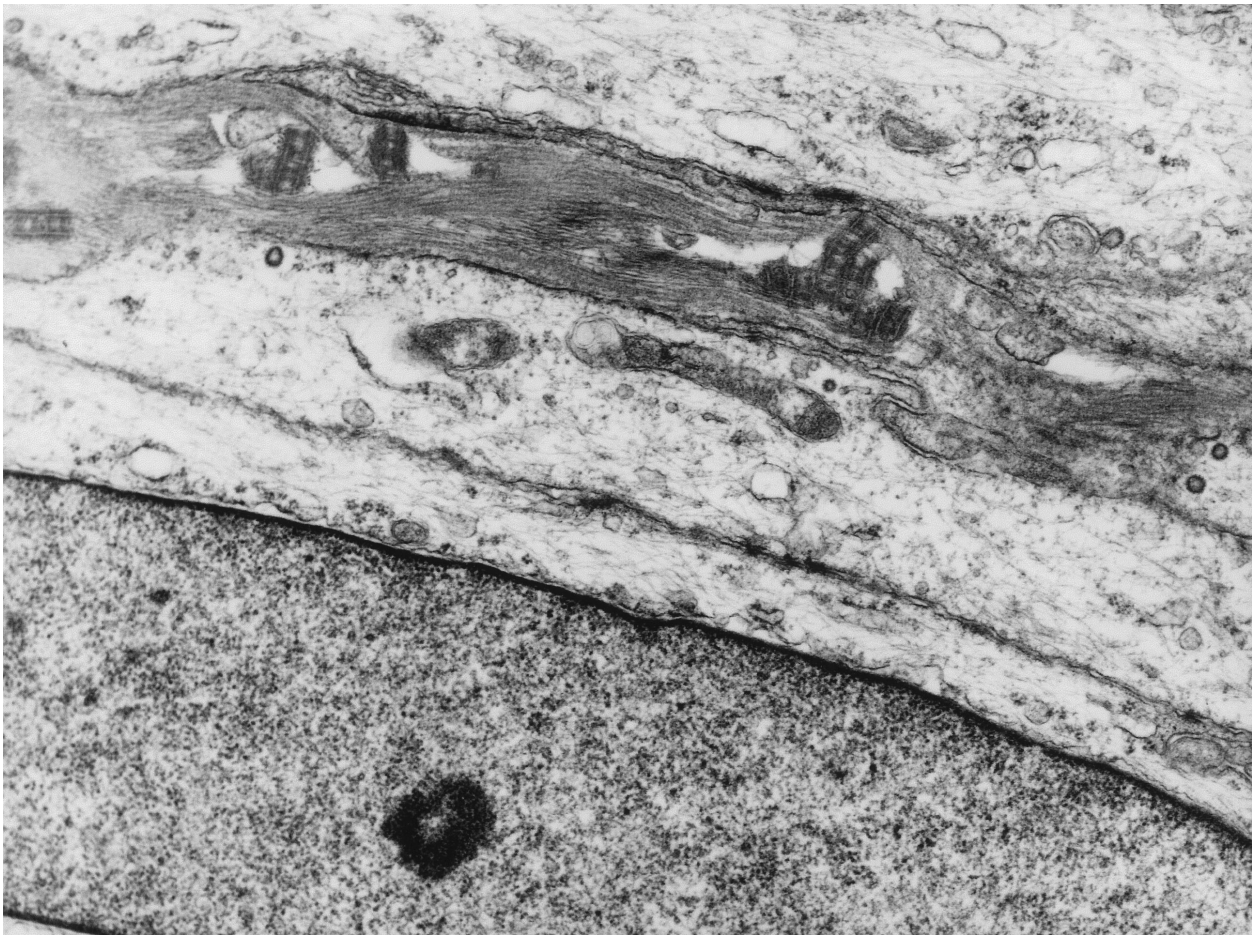

Fig. 10 A small intestinal GANT of 36 year old female with NF1 showed frequent precipitate with centriole-like configuration continuous to focal EL.

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