Virchows Arch (2006) 449: 137–139
DOI 10.1007/s00428-006-0210-9

LETTER TO THE EDITOR

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An ultrastructural and immunohistochemical study of a combined submucosal granular cell tumor and lipoma of the colon showing a unique nodule-in-nodule structure: putative implication of CD34 or prominin-2-positive stromal cells in its histopathogenesis

Received: 14 October 2005 / Accepted: 3 April 2006 / Published online: 4 May 2006

Dear Editor

We recently had a patient with a unique combination of a granular cell tumor and a lipoma of the colon showing a nodule-in-nodule appearance by histopathological examination.

Our patient, a 55-year-old Japanese man, underwent colonoscopic examination as part of a routine health check. A whitish, polypoid lesion was found in the cecum (Fig. 1a, inset). Benign, submucosal tumor, such as a carcinoid tumor or a lipoma, was suspected and the lesion was resected endoscopically. The excised specimen measured 9×6 mm in size (Fig. 1a, inset).

Low-power light microscopic examination of the tumor revealed nodular proliferation of mature fat cells in the submucosal layer of the colon (Fig. 1a) without a definitive fibrous capsule. In the proliferative submucosal lipomatous nodule, a cluster of small fibrous granular cell tumors was sitting upon the lipoma nodule beneath the slightly thickened layer of the muscularis mucosa (Fig. 1a), forming a nodule-in-nodule (a granular cell tumor nodule in a lipoma nodule) structure (Fig. 1a). In the smaller granular cell tumor nodule, tumor cells with rich, finely granulated, clear cytoplasm formed cell nests in an alveolar or fasciculated pattern, sometimes in proximity to bundles of peripheral nerve fibers (Fig. 1b). Dense fibrous collagen, spindle-shaped or stellate stromal cells, and some small vasculature blood vessels were seen in the extracellular stromal tissue of the granular cell tumor (Fig. 1b). The boundary between the granular cell tumor and the lipoma tissue was not well defined, which consisted of mixed intervening fibrous stroma containing abundant spindle-shaped or stellate stromal cells (Fig. 1b). Most of the immunohistochemical characteristics were consistent with many previous literal descriptions of the immunohistochemical characteristics of granular cell tumors and lipomas. Briefly, negative controls were prepared by substituting the primary antibodies with nonimmunune immunoglobulin G. External organs served as positive controls. CD34 was expressed in endothelial cells, and prominin-2 was expressed in normal epithelial cells of the adult kidney and digestive tract. The granular cell tumor cells expressed various neurogenic markers of Schwann cells, including S-100, neuron-specific enolase, and CD56. Other neurogenic markers characteristic of neurons or neuroendocrine cells were also faintly positive, e.g., synaptophysin and chromogranins. Strong expression of markers of phagosomes or phagocytes (CD68) was also detected in the cells of the granular cell tumor component. Positive expression of CD34 was observed not only in the endothelial cells of the small vasculature, but also in the spindle-shaped or stellate stromal cells. Spindle-shaped or stellate stromal cells showing positive immunohistochemical staining for CD34 and/or prominin-2 were more conspicuous in the tumor nodules than other nontumorous areas of the colonic wall. (Fig. 1a). However, no expression of c-kit, myogenic cell markers (e.g. alpha smooth muscle actin, muscle actin of HHF35, desmin, and MEF-2), and CD10 was observed in the spindle-shaped or stellate stromal cells. A few spindle-shaped or stellate stromal cells among the cell nests of granular cell tumor were labeled by the antiprominin-2 antibody (Fig. 1c).

Ultrastructual examination of the granular cell tumor tissue specimens by transmission electron microscope revealed alveolar aggregations of granular cell tumor cells with rather clear cytoplasm demarcated by thin

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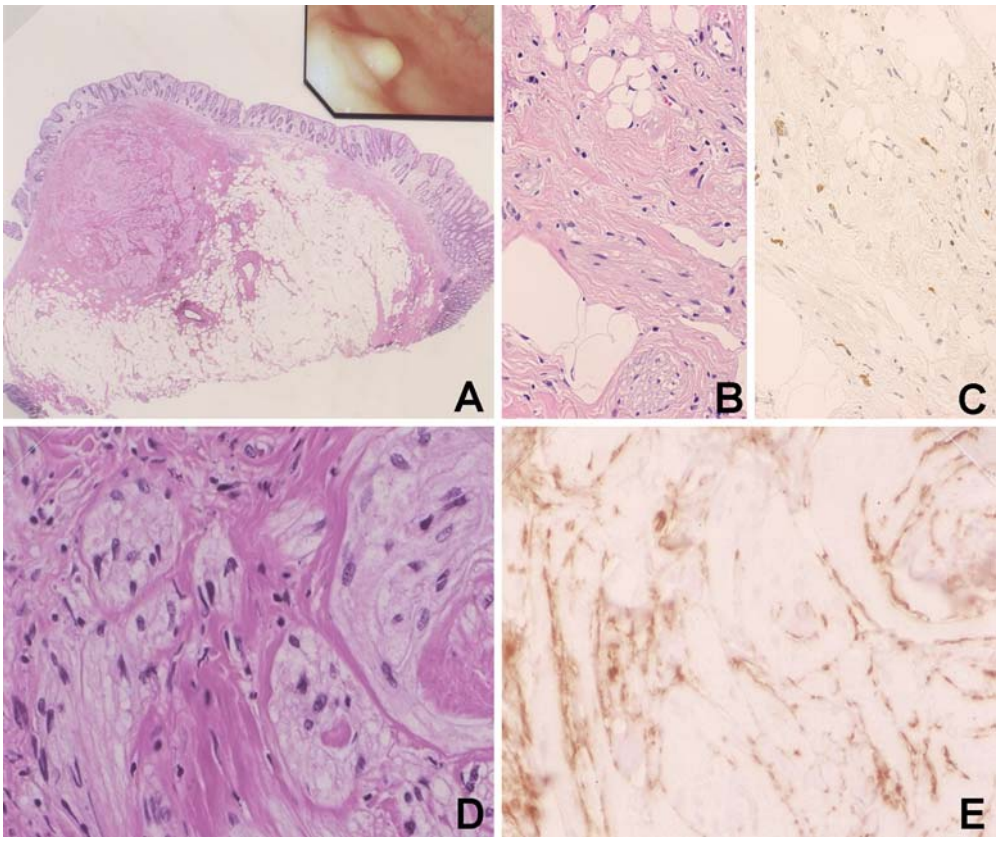

Fig. 1 Light microscopic and light microscopic immunohistochemical findings in the endoscopically resected polypoid lesion. **a** Low-power view of a section prepared from the resected polypoid lesion. Combined submucosal granular cell tumor (*upper left*) and lipoma (*lower right*) of the colon showing a nodule-in-nodule structure. Hematoxylin and eosin (H&E) stain, ×9. *Inset*: endoscopic view of the elevated *whitish* polypoid lesion. **b** Medium-power view of the granular cell tumor. H&E stain, ×190. **c** Immunostaining for prominin-2 in the granular cell tumor tissue. A few spindle-shaped stromal cells appeared in the stroma surrounding the granular cell tumor nests showing positive staining, ×190. **d** H&E stain in the granular cell tumor tissue. **e** Immunostaining for CD34 in the granular cell tumor tissue. Not only vascular endothelial cells, but also spindle-shaped stromal cells showing positive staining, ×190. A consequent section to subpanel **d** in the H&E section

lamina-like structures from the extracellular collagenous matrix. Either spindle-shaped, stellate stromal, or fibroblastic cells were embedded in the extracellular collagenous matrix, extending their thin cytoplasmic processes around the alveolar cell nests of the granular cell tumor (Fig. 2a,b).

Small electron-dense granular contents, possibly lipid-rich secondary lysosomes, and copious amount of vacuolated structure, which the contents were extracted during tissue processing for electron microscopy, were found in the vacuolated clear cytoplasm. The tumor cells had rather large nuclei with irregular nuclear membranes that were rich in euchromatin without conspicuous heterochromatin aggregation or large nucleoli. Their thin cytoplasm often extended far between their perikaryon and became embedded in the well-developed fibrous collagen-rich extracellular matrix in the tumor tissue. Occasional isolated cilia arising from the perikaryonic basal bodies were seen in both the spindle-shaped and stellate stromal or fibroblastic cells and granular cell tumor cells (Fig. 2b).

In the presented distinctive polypoid lesion of the colon, the positive immunohistochemical staining for cell proliferation markers and the detection of occasional isolated cilia on electron-microscopic examination [1] were consistent with the benign or slowly growing nature of this unique tumor. The tumor showed a unique nodule-in-nodule structure. The development of this peculiar tumor could be interpreted as a two-stage sequence, initiated by the formation of a lipoma component followed by the generation of a granular cell component.

Multipotent stromal stem cells in the tissue stroma may have a significant role in the histopathogenesis of this uncommon submucosal tumor characterized by the combination of a submucosal granular cell tumor and a lipoma of the colon demonstrating a nodule-in-nodule appearance. Previous reports describe clonal, plastic adherent stem cells from the bone marrow stroma to be capable of differentiating into osteoblasts, adipocytes, and chondrocytes. Recently, many investigators have demonstrated that multipotent stem cells can be recovered from a variety of other adult tissues and can differentiate into numerous tissue lineages, including myoblasts, cardiomyocytes, hepatocytes, and possibly even neural tissue. It is noteworthy that the primitive mesenchymal stem cells can be labeled by anti-CD133 (prominin, AC133), prominin-2 [2], or anti-low-affinity nerve growth factor receptor (p75, LNGFR) [4]. Prominin-2 is a pentaspan membrane glycoprotein predominantly expressed in neuroepithelial cells, hematopoietic stem cells, and epithelial cells of the adult kidney and the digestive tract. Tumor cells of PC3 prostate adenocarcinoma are also known to express prominin-2. CD34 showed diffusely positive staining in the cytoplasm of spindle-shaped stromal cells in the granular tumor cells. Gastrointestinal stromal tumors with neural differentiation are less likely to express CD34 compared to the neoplasms of the colon. CD34 molecule was once considered as one of the stem cell markers [1, 5]. CD34-positive stromal cells were shown to be distributed in the connective tissue surrounding mammary acini, salivary grand acini, thyroid follicles, hair follicles, sweat glands, and endocervical

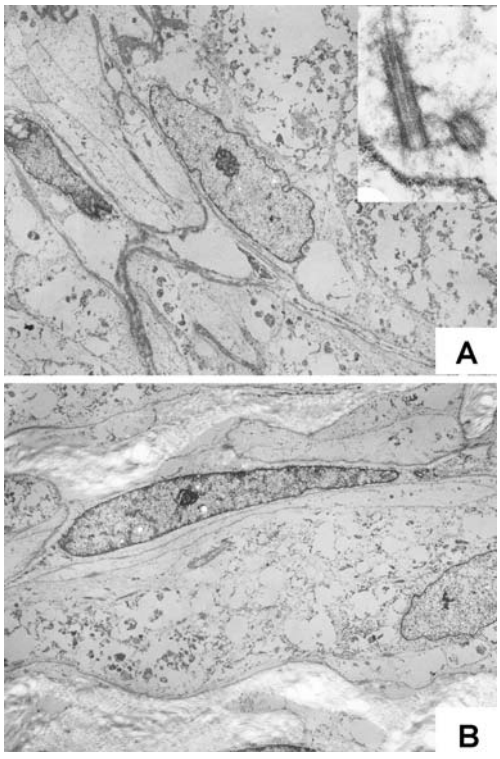

Fig. 2 Electron micrographs of the granular cell tumor in the resected polypoid lesion. **a** Higher-power view of the granular cell tumor cells with clear or vacuolated cytoplasm. Rather electron-dense and large aggregations of granular structures of possibly secondary lysosomes in the cytoplasm, ×7,000. *Inset*: isolated cilium arising from the perikaryonic basal bodies of the granular cell tumor cells, ×35,000. **b** High-power view of the granular cell tumor cells and spindle-shaped stromal or fibroblastic cells with elongated thin cytoplasmic processes, ×7,000

glands of the uterus, whereas no CD34-positive stromal cells were reported in the lamina propria of the colorectum or stomach [3]. In the colorectum and stomach, CD34-positive stromal cells were reported only in the submucosa, subserosa, muscularis propria, and muscularis mucosa [1]. It is also well known that tissue stromal cells might have the microenvironmental function of regulating the growth or differentiation of adjacent cell populations [1, 5].

Based on the immunoreactivity for CD34 and prominin-2 in spindle/stellate stromal cells, the authors speculate that these cells may have the potential to differentiate into neoplastic fat and granular cells.

In the present case, the strong predilection for spindle-shaped or stellate stromal cells to show positive staining for CD34 molecule in the tumor tissue is striking [5]. In addition to expression of CD34 in this exclusive population of spindle-shaped or stellate stromal cells, expression of prominin-2, a promising stem cell marker, was also observed in some of the cells [2]. We examined a few cases of spindle cell lipoma and granular cell tumors for expression of both markers (CD34/prominin-2). As a result, the spindle cell lipomas were positive for both markers and the granular cell tumors were positive in the spindle stromal cells for CD34. However, faintly positive staining for prominin-2 was detected around stromal cells.

As for markers of proliferating cells, topoisomerase II, proliferating cell nuclear antigen (PCNA), and Ki-67 showed various staining patterns among the three major cellular components of this rare tumor, namely, (1) tumor cells of granular cell tumor, (2) spindle-shaped or stellate stromal cells, and (3) lipoma cells. P53 expression was not conspicuous in any of these three cell components. MEF2 is a transcription factor considered as a specific marker for myogenic or cardiomyocytic lineages. Although its precise mechanism is yet to be defined, some mesenchymal stem cell and intrathoracic sarcoma exhibit positive staining for MEF2. PCNA and Ki-67 are well known as proliferation markers. PCNA and Ki-67 were expressed in the granular cell tumor cells more frequently compared with spindle-shaped/stellate stromal cells and lipoma cells. However, MEF2 were not expressed in granular/spindle/adipocyte cells of the presented tumor.

It is interesting that these cells showed no expression of c-kit and some myogenic markers (alpha smooth muscle actin, muscle actin of HHF35, desmin, and MEF-2) and CD10 in the present study [1, 5]. Our ultrastructural analysis also revealed that well-developed thin cytoplasmic processes of the spindle-shaped or stellate stromal cells could generate intimate cell-to-cell or cell-to-matrix interaction through the wide surface area of the cell membrane [5]. This cell-to-cell or cell-to-matrix interaction may be a fundamental process in tissue microenvironmental functions [5]. Our results suggest that CD34-positive stromal cells are essential for the integrity of gastrointestinal mesenchymal elements, including smooth muscles, neural elements, and fat tissue [5]. CD34-positive stromal cells or a few similar cells labeled by prominin-2 [2] may have the potential to differentiate into neoplastic fat cells in the lipoma and tumor cells in the granular cell tumor. These cells may play some role in regulating the growth or the differentiation of both types of tumor cells in this unique submucosal tumor displaying a nodule-in-nodule appearance as a combination of a granular cell tumor and a lipoma.

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