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Differential staining of γ synuclein in poorly differentiated compared to highly differentiated colon cancer cells

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Abstract. Synuclein α, β and γ are proteins usually found in neurodegenerative diseases. However, interestingly synucleins are expressed in cancer cells of several organs including ovary, mammary gland and colon. By immunocytochemistry using specific antibodies to γ synuclein (SNCG), we examined the distribution of this protein in poorly differentiated, compared to highly differentiated colon cancer cells. In poorly differentiated cancer cells tumors were very frequently stained intensely with antibodies to SNGG, suggesting high expression of this protein. In contrast, in highly differentiated cells, there was no labeling. Labeled cells could be found only at the edges or in between the lobules of the differentiated tumor cells. However, in moderately differentiated tumors, a weak cytoplasmic staining of SNCG was evident. Interestingly in cancer patients (stage II-IV) both poorly and highly differentiated tumor cells were often present in the same patient. Labeled cancer cells with SNCG were evident also in lymph nodes, around the wall of blood vessels and in fat tissue, where only poorly differentiated cancer cells were exclusively present. Since cancer cells with poor differentiation are believed to be aggressive with metastases formation it is suggested that SNCG can serve as a marker for the potential of the tumor cell for the rapid spreading and metastazing of the non-differentiated tumors.

Introduction

The synucleins are small, soluble highly conserved group of neuronal proteins that have been implicated in both neurodegenerative diseases and cancer. The synuclein family consists of α, β and γ synuclein. They are naturally unfolded group of proteins that share sequence homologies and structural properties (1-3). So far, the biological functions of the synucleins are still unclear, but their involvement in neurodegenerative diseases and cancer may provide insights into the pathological processes that result from these two groups of debilitating diseases and present the possibility to use them as potential targets for early diagnosis and treatment (2-4).

Recently, elevated levels of SNCG protein have been detected in various cancers, especially in advanced stages of the disease. Moreover, it was recently reported that all three synucleins: α, β and γ are expressed in colorectal cancer (2).

Furthermore, studies to date indicate that overexpression of SNCG compromises normal mitotic checkpoint controls, resulting in multinucleation as well as faster cell growth (5,6). SNCG has also been shown to promote invasion and metastasis in *in vitro* assays as well as in animal models (reviewed in ref. 1). Overexpression in SNCG also interferes with drug-induced apoptotic response (4,7). Interestingly this feature is probably due to enhanced cancer cell survival and inhibition of drug-induced apoptosis by modulating MAPK pathways (4,7). These observations raise questions about the involvement of SNCG in the process of tumorigenesis and metastasis, and efforts have already been made to use SNCG as a marker for assessing breast cancer progression (1,2). However, such an attempt has not yet been applied to colon cancer. It should be noted that overexpression of synucleins occurred by hypomethylation of the genes coding for these proteins (9,10). However, the mechanism which trigger this process is not completely clear.

In the present study we found that SNCG can be used as an early marker for the development of colon cancer. Moreover, immunocytochemistry can distinguish between highly differentiated and poorly differentiated colon cancer cells.

Materials and methods

Tissues of colon cancer fixed with formalin and embedded in paraffin from 20 patients were collected. The tissues contained also areas at the edge of the tumor that were classified according to pathological inspection as normal. Section of 4 μm in thickness were stained with primary antibodies to SNCG (anti-SNCG clone EP1539Y Millipore) followed by second antibodies conjugated to peroxidase, followed by staining with hematoxylin for 90 sec (14-16). Stained sections

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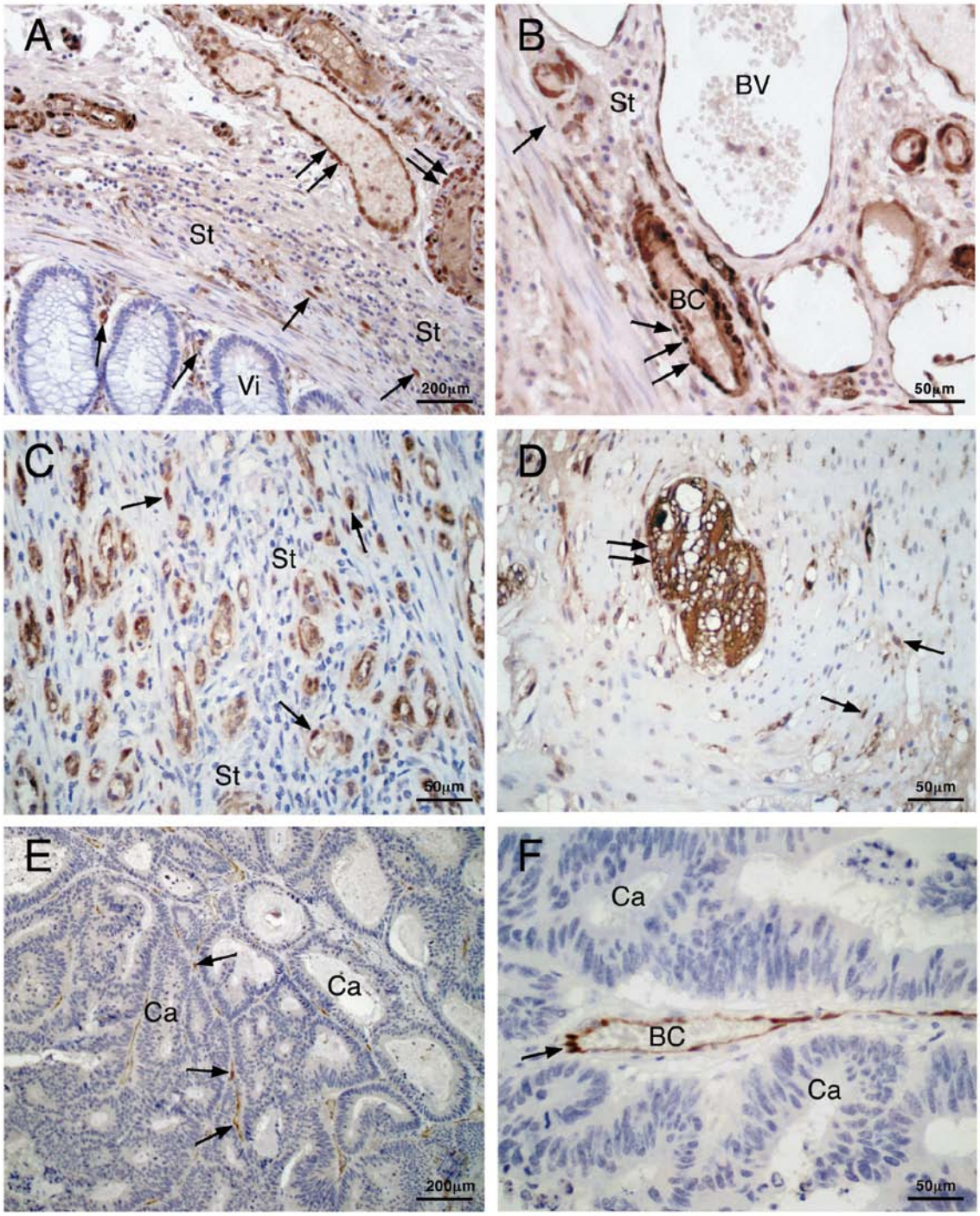

Figure 1. Labeling of colon cancer cells with SNCG. (A) A small magnification showing colon villi unstained (Vi). Some small tumors embedded in the stroma can be seen in between the villi (arrows). Larger tumors are seen deeper in the stroma (double arrows) and at the circumference of a blood vessel (BV). (B) Higher magnification showing stained cells around blood capillary (BC and double arrows). Tiny tumors are also visible (arrows). Original magnification, x400. (C) Small foci of tumor development embedded in the stroma are stained for SNCG (arrows); St, stroma. Original magnification, x400. (D) Large tumor stained with SNCG (double arrows) embedded in the stroma (St). Small tumors are also visible (arrows). (E) Low magnification of differentiated tumor. Tiny labeling is seen in between the tumor lobules (arrows); Car, carcinoma. Original magnification, x100. (F) High magnification of differentiated tumor. Tumor lobules are unstained. (Car, carcinoma). Stained cells with anti-SNCG antibodies is evident around tiny blood vessel (BV). Original magnification, x400.

were visualized and photographed at a magnifications of x100 or x400 using the Nikon microscope. The unstained areas of sections served as a control for the specificity of the staining. Permission for the research from the Helsinki Committee was obtained from Kaplan-The Hebrew University Medical Center and from Wolfson Hospital.

Results

In order to examine the distribution of SNCG in colon cancer, we visualized by indirect immunocytochemistry 4 μm sections of formaldehyde-fixed and paraffin-embedded tissue, collected from 20 patients. In control tissues taken from the

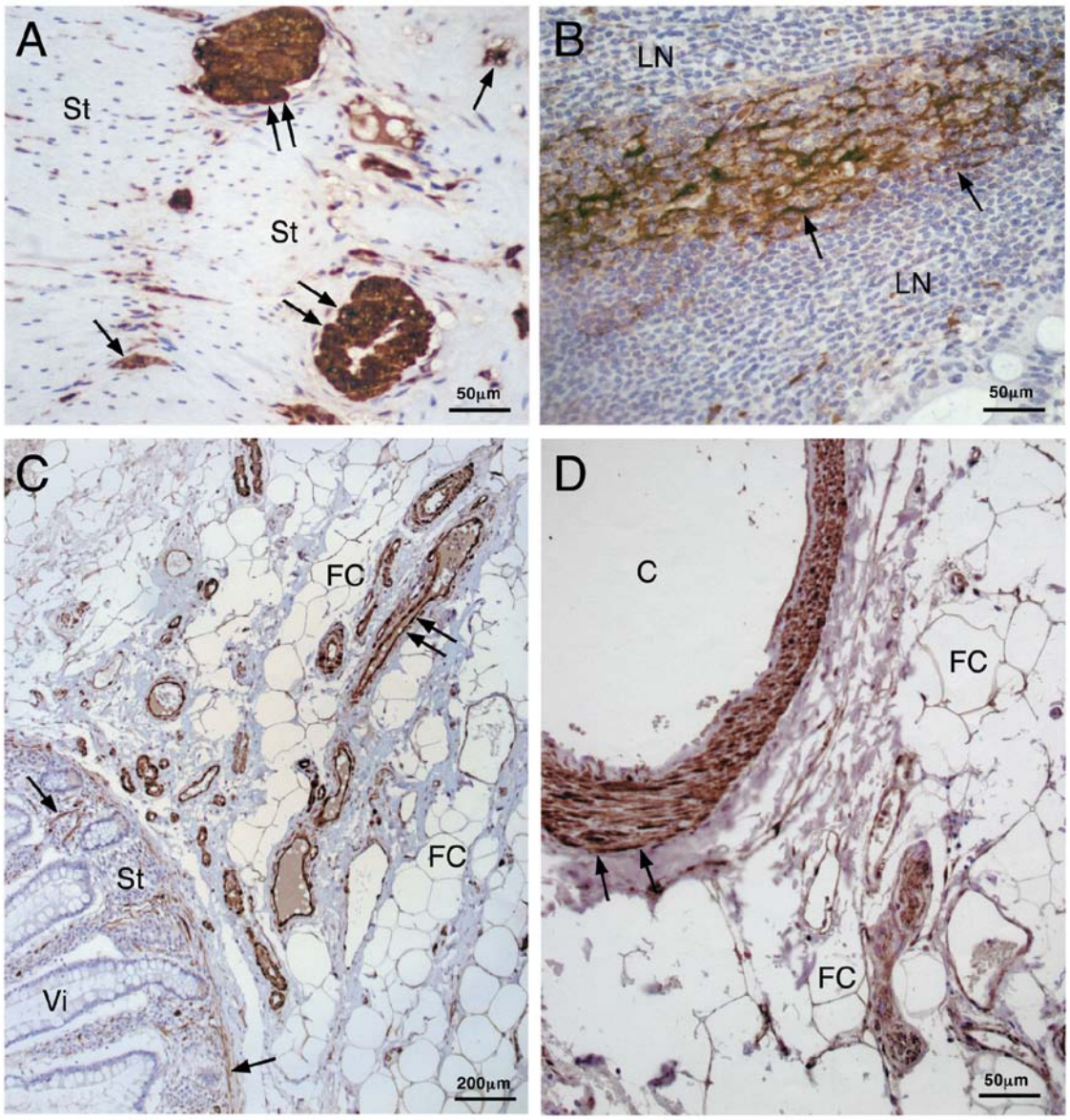

Figure 2. Labeling of colon cancer cells at the late stage of tumor development (stage II-IV). (A) High magnification demonstrating two medium size tumors that are heavily labeled, embedded in the stroma (double arrows). Smaller tumors are also visible (arrows). Original magnification, x400. (B) A part of a lymph node (LN). The center is loaded with cancer cell containing SNCG (arrows). Original magnification, x400. (C) Small size to medium size tumors labeled with antibodies to SNCG (arrows) are embedded in the fat tissue fat cells (FC). Some labeling is also evident embedded in the stroma close to the villi (Vi, single arrows) and some labeling is also found around small blood vessels. Original magnification, x100. (D) Part of large tumor (arrows) with empty cavity (EC) stained with anti-SNCG and showing moderate degree of organization. The tumor is embedded in fat tissue (FC, fat cells). C, an empty cavity. Original magnification, x400.

edge of the tumors about 50% showed already initial staining with antibodies to SCNG and the rest showed no label either in the stroma or in the villi area of the colon (data not shown). At stage I we found clear labeling of SNCG in tiny undifferentiated tumors in the stroma, in between the villi and around blood vessels (Fig. 1A and B). The tiny tumors consist very often only of a couple of cells (Fig. 1C). In a more advanced stage of the cancer (stage II-IV) larger undifferentiated tumor cells embedded in the stroma were heavily stained SNCG antibodies (Fig. 1D). In contrast, in differentiated tumors with typical appearance of carcinomas no labeling of SNCG was evident. However, some labeling appeared between the lobules of the tumors (Fig. 1E). High magnification revealed intensely stained cells with SNCG associated with blood capillaries between the carcinoma lobules (Fig. 1F). In moderately differentiated tumors a weak labeling of SNCG was evident (data not shown).

In advanced stages of the cancer, the staining of the undifferentiated tumors with SNCG became much more intensive (Fig. 2A). In addition, intensive labeling was found in the center of the lymph nodes (Fig. 2B). Undifferentiated tumor cells stained with antibodies to SNCG clearly penetrated into fat tissue (Fig. 2C). Occasionally some degree of organization was evident in the large tumors with empty cavity, in the center, upon staining with antibodies to SNCG (Fig. 2D).

Discussion

In the present study we, for the first time, demonstrated that colon cancer originate from the colon stroma preferentially

synthesized SNCG, in contrast to highly differentiated cancer cells, which originate from the colon epithelial cells. SNCG only rarely was found between the lobules of the differentiated tumor cells, which appeared as typical carcinoma.

The non-differentiated tumors are probably more aggressive than the differentiated ones for the following reasons: i) we demonstrated that SNCG producing cells penetrated to the fat tissue. In contrast, there was no invasion to fat tissue of highly differentiated tumors. ii) In the present study we found that SNCG producing cells accumulated around blood vessels and probably upon invasion to the blood vessels the spreading of the tumor cells could be a much more rapid process. This is in line with a recent report that patients suffering from colon cancer, with poorly differentiated tumors, showed clearly and significantly higher mortality than those patients with highly differentiated tumors (14).

We, for the first time, suggest that SNCG producing cells could serve as a marker for early development of colon cancer, since we found that patients of phase I contained already tiny tumors of cells producing SNCG as small as containing a couple of cells, which probably resemble initiation of foci of colon cancer development. Therefore, we cannot exclude the possibility that the cancer stem cells are in close vicinity to these tiny tumors (15,16). Double staining of the sections with antibodies to SNCG on one hand, and specific antibodies to one of the cancer stem cell-specific antigen (17) on the other hand, may resolve this issue.

Recently it was reported that follicular dendritic cell sarcoma and benign and malignant vascular tumors could also produce SNCG (18). However, these findings cannot blur the view that early detection of colon cancer exemplified by staining of SNCG can assist in prognosis and choosing the appropriate therapy for colon cancer. Moreover, SNCG expression can assist in distinguishing between non-invasive and invasive colon cancer.

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References

1. Bruening W, Giasson BI, Klein-Szanto AJ, Lee VM, Trojanowski JQ and Godwin AK: Synucleins are expressed in the majority of breast and ovarian carcinomas and in preneoplastic lesions of the ovary. Cancer 88: 2154-2163, 2000.
2. Ye Q, Wang TF, Peng YF, Xie J, Feng B, Qiu MY, Li LH, Lu AG, Liu BY and Zheng MH: Expression of α, β and γ-synuclein in colorectal cancer, and potential clinical significance in progression of the disease. Oncol Rep 23: 429-436, 2010.
3. Lavedan C, Leroy E, Dehejia A, Buchholtz S, Dutra A, Nussbaum RL and Polymeropoulos MH: Identification, localization and characterization of the human γ-synuclein gene. Hum Genet 103: 106-112, 1998.
4. Singh VK and Jia Z: Targeting synuclein-γ to counteract drug resistance in cancer. Expert Opin Ther Targets 12: 59-68, 2008.
5. Ahmad M, Attoub S, Singh MN, Martin FL and El-Agnaf OM: γ-synuclein and the progression of cancer. FASEB J 21: 3419-3430, 2007.
6. Hu H, Sun L, Guo C, Liu Q, Zhou Z, Peng L, Pan J, Yu L, Lou J, Yang Z, Zhao P and Ran Y: Tumor cell-microenvironment interaction models coupled with clinical validation reveal CCL2 and SNCG as two predictors of colorectal cancer hepatic metastasis. Clin Cancer Res 15: 5485-5493, 2009.
7. Pan ZZ, Bruening W, Giasson BI, Lee VM and Godwin AK: γ-synuclein promotes cancer cell survival and inhibits stress- and chemotherapy drug-induced apoptosis by modulating MAPK pathways. J Biol Chem 277: 35050-35060, 2002.
8. Shimoda T, Ikegami M, Fujisaki J, Matsui T, Aizawa S and Ishikawa E: Early colorectal carcinoma with special reference to its development de novo. Cancer 64: 1138-1146, 1989.
9. Czekierdowski A, Czekierdowska S, Wielgos M, Smolen A, Kaminski P and Kotarski J: The role of CpG islands hypomethylation and abnormal expression of neuronal protein synuclein-gamma (SNCG) in ovarian cancer. Neuro Endocrinol Lett 27: 381-386, 2006.
10. Liu H, Liu W, Wu Y, Zhou Y, Xue R, Luo C, Wang L, Zhao W, Jiang JD and Liu J: Loss of epigenetic control of synuclein-γ gene as a molecular indicator of metastasis in a wide range of human cancers. Cancer Res 65: 7635-7643, 2005.
11. Zeren T, Inan S, Seda Vatansever H, Ekerbicer N and Sayhan S: Significance of tyrosine kinase activity on malign transformation of ovarian tumors: a comparison between EGF-R and TGF alpha. Acta Histochem 110: 256-263, 2008.
12. Ginath S, Menczer J, Friedmann Y, Aingorn H, Aviv A, Tajima K, Dantes A, Glezerman M, Vlodavsky I and Amsterdam A: Expression of heparanase, Mdm2, and erbB2 in ovarian cancer. Int J Oncol 18: 1133-1144, 2001.
13. Singer G, Stöhr R, Cope L, *et al*: Patterns of p53 mutations separate ovarian serous borderline tumors and low- and high-grade carcinomas and provide support for a new model of ovarian carcinogenesis: a mutational analysis with immunohistochemical correlation. Am J Surg Pathol 29: 218-224, 2005.
14. Shaikh AJ, Raza S, Shaikh AA, Idress R, Kumar S, Rasheed YA, Lal A and Masood N: Demographics, pathologic patterns and long-term survival in operable colon cancers: local experience in Pakistan. Asian Pac J Cancer Prev 10: 361-364, 2009.
15. Vogelstein B, Fearon ER, Hamilton SR, *et al*: Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525-532, 1988.
16. Medema JP and Vermeulen L: Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. Nature 474: 318-326, 2011.
17. Todaro M, Francipane MG, Medema JP and Stassi G: Colon cancer stem cells: promise of targeted therapy. Gastroenterology 138: 2151-2162, 2010.
18. Zhang H, Maitta RW, Bhattacharyya PK, Florea AD, Sen F, Wang Q and Ratech H: γ-synuclein is a promising new marker for staining reactive follicular dendritic cells, follicular dendritic cell sarcoma, Kaposi sarcoma, and benign and malignant vascular tumors. Am J Surg Pathol 35: 1857-1865, 2011.