Role of cancer stem cells in age-related rise in colorectal cancer

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Abstract

Colorectal cancer (CRC) that comprises about 50% of estimated gastrointestinal cancers remains a high mortality malignancy. It is estimated that CRC will result in 9% of all cancer related deaths. CRC is the third leading malignancy affecting both males and females equally; with 9% of the estimated new cancer cases and 9% cancer related deaths. Sporadic CRC, whose incidence increases markedly with advancing age, occurs in 80%-85% patients diagnosed with CRC. Little is known about the precise biochemical mechanisms responsible for the rise in CRC with aging. However, many probable reasons for this increase have been suggested; among others they include altered carcinogen metabolism and the cumulative effects of long-term exposure to cancer-causing agents. Herein, we propose a role for self-renewing, cancer stem cells (CSCs) in regulating these cellular events. In this editorial, we have briefly described the recent work on the evolution of CSCs in gastro-intestinal track especially in the colon, and how they are involved in the age-related rise in CRC. Focus of this editorial is to provide a description of (1) CSC; (2) epigenetic and genetic mechanisms giving rise to CSCs; (3) markers of CSC; (4) characteristics; and (5) age-related increase in CSC in the colonic crypt.

Key words: Cancer stem cells; Aging; Colorectal cancer; Colonospheres; Colonic crypt

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Core tip: Sporadic colorectal cancer (CRC), an age-related disease, occurs in 80%-85% of patients with CRC. The changes that occur at the cellular and molecular levels during ageing leading to a rise in CRC are poorly understood. We have postulated a role for cancer stem/stem-like cells that are shown to possess self-renewing, pluripotent properties. These cells, which reside at the bottom of the colonic crypt, are thought to regulate the processes of carcinogenesis. In this
Like cells may play a pivotal role in initiation, development and progression of colorectal carcinoma.

Data generated from several investigations from our laboratory have revealed a progressive rise in CSCs in the colon with advancing age\textsuperscript{[7-8]}. Stem cells, present in all vertebrates, are constantly replenishing dying cells or regenerating damaged or injured tissue. With aging, DNA repair system has been shown to be impaired that results in an increased DNA damage. DNA damage leading to reduction in some stem cells through apoptosis can result in genetic and epigenetic changes in stem cells that have survived the DNA repair mechanisms\textsuperscript{[9]}. Both genetic and epigenetic alterations may affect stem cell function by altering transcriptome and lead to the processes of carcinogenesis (reviewed in\textsuperscript{[10]})

CSCs can be identified by surface epitopes or their functional characteristics. Colon CSCs are characterized by the expression of several markers that represent the surface epitopes which among others include CD44, CD166, CD133 and EpCAM\textsuperscript{[11-12]}. In addition, colonosphere formation is considered to be another functional assay for identification of CSCs.

Another characteristic of CSCs is the acquisition of epithelial to mesenchymal transition (EMT), which provides the cells ability to migrate, invade and metastasize. EMT can be determined by E-cadherin and vimentin expression, which are downstream targets of Wnt/β-catenin and notch signaling\textsuperscript{[12]}. Over-expression and/or induction of epidermal growth factor receptor (EGFR) signaling and/or other members of receptor tyrosine kinase family, especially ErbB-2 has also been shown to occur in many cancers including the colon and is considered to be an indicator of poor prognosis. We have postulated that activation of EGFR in the gastrointestinal tract may lead to stem cell proliferation and maintenance as inhibition of EGFR by cetuximab reduced CSCs in the colon\textsuperscript{[14]}

In view of the recent evidence indicating the appearance of CSCs is one of the initial events in carcinogenesis, we have investigated and confirmed that age-related increases in adenomatous polyps are associated with increases in mucosal CSCs\textsuperscript{[7]}. We demonstrated that with advancing age there is a progressive rise in CSCs in the colon not only in adenomas, but also in normal appearing mucosa. This observation indicates that aging increases the risk of CRC\textsuperscript{[7]}. The number of colonic mucosal cells showing CD44\textsuperscript{+}, CD166\textsuperscript{+} or EpCAM was markedly higher in the isolated mucosal cells in subjects over 55 years of age with polyps than in the younger ones.

We also reported an age-related rise in expression and activation of all members of EGFRs with the exception of EGFR-4, which was not studied\textsuperscript{[15-16]}. In addition, our data also revealed that CD166 and EGFR were co-localized in subjects over 55 years of age with polyps than the normal controls. We also demonstrated that CD166 and EGFR were co-localized in adenomas and it is suggested that these self-renewing, pluripotent cancer stem/stem-like cells may play a pivotal role in initiation, development and progression of colorectal carcinoma.

According to a well accepted model of CRC progression by Vogelstein, this malignancy arises as a result of accumulation of mutations in tumor suppressor genes and oncogenes\textsuperscript{[1,2]}. For a malignant tumor to be initiated mutations in at least 4-5 genes are required and it is the total number of mutations rather than their sequence that is important for malignant transformation to occur. Transformation from the initial events to an invasive carcinoma takes about 8-12 years. As colonic mucosa is a highly dynamic tissue and the mucosal surface epithelial cells are constantly replaced with cells derived from crypt stem cells; it is reasonable to assume that only the long-lived cells (stem cells) may serve as reservoirs for accumulation of such precancerous mutations. In a normal colon, these cells are typically present at the bottom of the colonic crypts\textsuperscript{[3]}. Cancer stem cells (CSCs), that possess remarkable similarity with normal stem cells, are thought to be the result of accumulated mutations, specifically in tumor suppressor genes and/or oncogenes\textsuperscript{[4]}. Like normal stem cells, CSCs are also able to proliferate indefinitely and also possess the property of pluripotency indicating their capability to differentiate into more than one cell lineages. Recent evidence show that CSCs are present in many malignancies, including CRC\textsuperscript{[4-6]}. Self-renewing properties of CSCs allow these cells to form tumors representing the original tumor in immuno-compromised mice. Within the epithelial malignancies, CSCs were first identified in breast cancer and characterized by specific cell surface markers\textsuperscript{[4,6]}. Since then, they have been reported in a multitude other human malignancies. It is suggested that these self-renewing, pluripotent cancer stem/stem-like cells may play a pivotal role in initiation, development and progression of colorectal carcinoma.
of age suggesting that with aging risk of developing CRC increases\cite{1,3}. Expression of CSC markers was also found to be higher in *Helicobacter pylori* gastritis\cite{20} and gastric cancers and also in normal appearing gastric mucosa from the aged\cite{21}. The precise underlying mechanisms for the age-associated increase in gastrointestinal malignancies, specifically CRC remain to be elucidated. We have hypothesized that CSCs, which are thought to arise from mutations of normal stem cells residing at the bottom of the crypt, will proliferate and migrate with time to occupy the entire crypt. This will eventually lead to the age-related rise in colon cancer. We tested this hypothesis by isolating mucosal cells from three different regions along the colonic crypt (upper, middle and lower) of young (4-5 mo) and aged (22-24 mo) Fischer 344 rats were euthanatized by CO₂ asphyxiation following an overnight fast. The colon was removed, rinsed with cold PBS, everted, filled with a 5-10 mL protease solution [1 mg/mL collagenase 1 and 20 µg/mL hyaluronidase 1 in 0.05% Trypsin-EDTA (1X) with 2% BSA] and ligated at both ends. The colon was placed in 0.05% Trypsin-EDTA (1X) and incubated for 30 min at room temperature. To obtain the cells from the upper part of the colonic crypt, the colonic bag was transferred into 50 mL DMEM/F12 and incubated for 60 min at room temperature. For cells from the middle region of the crypt, the colonic bag was transferred into fresh 50 mL DMEM/F-12 and incubated at room temperature for another 45 min. Finally, the colonic bag was incubated further for 45 min at room temperature to obtain the cells from the lower part of the crypt. The dispersed mucosal cells were collected by centrifugation at 500 g for 5 min, washed with DMEM/F12, immediately suspended and cultured in serum-free cell medium containing DMEM/F12 (1:1) supplemented with B27, 20 ng/mL epidermal growth factor, 10 ng/mL fibroblast growth factor, 50 µg/mL gentamicin and antibiotic-anti-mycotic. First generation colonospheres were observed after 7 and 14 d. The colonospheres were collected, trypsinized and re-suspended in stem cell medium for formation of second generation colonospheres. PBS: Phosphate buffer saline; BSA: Bovine serum albumin.

animals was accompanied by a parallel rise in clonal CSC marker CD44 and also β-catenin, which is known to be dysregulated in colon cancer. On the other hand, the levels of the differentiation marker CK-20 in the middle and upper part of the crypts of older animals were markedly higher than the levels noted in the lower region. Likewise, colon mucosal cells from the lower region of aged rats exhibited an increased frequency of mutations of the colonic crypt of than their younger counterparts.

In conclusion, our data demonstrate a gradual increase in CSCs in the colonic crypt with advancing age, which could partly contribute to the age-related rise in CRC. Although the underlying reasons for the rise in CSCs in the colon with advancing age remain to be fully explored, one possibility could be that aging renders the gastrointestinal mucosa more susceptible to ever-increasing environmental or other toxicants.

**REFERENCES**

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorgenesis. *Cell* 1990; 61: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90156-I].

2. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; 319: 525-532 [PMID: 2841597 DOI: 10.1056/NEJM198809013190901].

3. Potten CS. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. *Philos Trans R Soc Lond B Biol Sci* 1998; 353: 821-830 [PMID: 9684279 DOI: 10.1098/rstb.1998.0246].

4. Jordan CT, Guzman ML, Noble M. Cancer stem cells. *N Engl J Med* 2006; 355: 1253-1261 [PMID: 16990388 DOI: 10.1056/...
5 Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, Tripodo C, Russo A, Gulotta G, Medema JP, Stassi G. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell 2007; 1: 389-402 [PMID: 18371377 DOI: 10.1016/j.stem.2007.08.001]

6 Dick JE. Stem cell concepts renew cancer research. Blood 2008; 112: 4793-4807 [PMID: 19064739 DOI: 10.1182/blood-2008-08-077941]

7 Patel BB, Yu Y, Du J, Levi E, Phillip PA, Majumdar AP. Age-related increase in colorectal cancer stem cells in macroscopically normal mucosa of patients with adenomas: a risk factor for colon cancer. Biochem Biophys Res Commun 2009; 378: 344-347 [PMID: 19010307 DOI: 10.1016/j.bbrc.2008.10.179]

8 Patel BB, Yu Y, Du J, Rishi AK, Sarkar FH, Tarca AL, Wali A, Majumdar AP. Schlafen 3, a novel gene, regulates colonic mucosal growth during aging. Am J Physiol Gastrointest Liver Physiol 2009; 296: G955-G962 [PMID: 19228883 DOI: 10.1152/ajpgi.90726.2008]

9 Finkel T, Serrano M, Blasco MA. The common biology of cancer and ageing. Nature 2007; 448: 767-774 [PMID: 17700693 DOI: 10.1038/nature05985]

10 Roy S, Majumdar AP. Cancer Stem Cells in Colorectal Cancer: Genetic and Epigenetic Changes. J Stem Cell Res Ther 2012; Suppl 7: pii: 10342 [PMID: 23565347]

11 Sanders MA, Majumdar AP. Colon cancer stem cells: implications in carcinogenesis. Front Biosci (Landmark Ed) 2011; 16: 1651-1662 [PMID: 21196254 DOI: 10.2741/3811]

12 Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997; 275: 1787-1790 [PMID: 9065402 DOI: 10.1126/science.275.5307.1787]

13 Kanwar SS, Yu Y, Nautiyal J, Patel BB, Majumdar AP. The Wnt/beta-catenin pathway regulates growth and maintenance of colonospheres. Mol Cancer 2010; 9: 212 [PMID: 20691072 DOI: 10.1186/1476-4598-9-212]

14 Nautiyal J, Du J, Yu Y, Kanwar SS, Levi E, Majumdar AP. EGFR regulation of colon cancer stem-like cells during aging and in response to the colonic carcinogen dimethylhydrazine. Am J Physiol Gastrointest Liver Physiol 2012; 302: G655-G663 [PMID: 22281474 DOI: 10.1152/ajpgi.00323.2011]

15 Majumdar AP. Regulation of gastrointestinal mucosal growth during aging. J Physiol Pharmacol 2003; 54 Suppl 4: 143-154 [PMID: 15075456]

16 Majumdar AP, Du J. Phosphatidylinositol 3-kinase/Akt signaling stimulates colonic mucosal cell survival during aging. Am J Physiol Gastrointest Liver Physiol 2006; 290: G49-G55 [PMID: 16123202 DOI: 10.1152/ajpgi.00106.2005]

17 Majumdar AP, Du J, Yu Y, Xu H, Levi E, Patel BB, Rishi AK. Cell cycle and apoptosis regulatory protein-1: a novel regulator of apoptosis in the colonic mucosa during aging. Am J Physiol Gastrointest Liver Physiol 2007; 293: G1215-G1222 [PMID: 17932228]

18 Nautiyal J, Rishi AK, Majumdar AP. Emerging therapies in gastrointestinal cancers. World J Gastroenterol 2006; 12: 7440-7450 [PMID: 17167831]

19 Patel BB, Majumdar APN. HER family of receptors as treatment targets. In: Pancreatic cancer. Anderson Solid Tumor Oncology Series. 1st ed. Lowy AM, Leach SD, Phillip PA, editors. New York: Springer, 2008: 609-634

20 Bertaux-Skeirik N, Feng R, Schumacher MA, Li J, Mahe MM, Engevik AC, Javier JE, Peek RM, Ottemann K, Orian-Rousseau V, Boivin GP, Helmrath MA, Zavros Y, CD44 plays a functional role in Helicobacter pylori-induced epithelial cell proliferation. PLoS Pathog 2015; 11: e1004663 [PMID: 25658601 DOI: 10.1371/journal.ppat.1004663]

21 Levi E, Sochacki P, Khoury N, Patel BB, Majumdar AP. Cancer stem cells in Helicobacter pylori infection and aging: Implications for gastric carcinogenesis. World J Gastrointest Pathophysiol 2014; 5: 366-372 [PMID: 25133037]

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