

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

editorial, we have briefly described the recent work on the evolution of cancer stem cells in gastro-intestinal tract with particular reference to the colon, and how they are involved in the development and progression of CRC, the incidence of which increases with advancing age.

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TEXT

The primary challenge in the study of aging is to gain an in-depth understanding of the intricate relationship between disease processes and aging. One of the most consistent pathological conditions with advancing age is a sharp rise in colorectal cancer (CRC), which typically occurs after the age of 50. It has been reported that a male in age group 70 years and above exhibits 54 times greater risk of developing CRC compared to younger male (birth to 39 years).

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^[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 epithelium 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^[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^[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^[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^[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.

Data generated from several investigations from our laboratory have revealed a progressive rise in CSCs in the colon with advancing age^[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^[9]. Both genetic and epigenetic alterations may affect stem cell function by altering transcriptome and lead to the processes of carcinogenesis (reviewed in^[10]). Thus the age-related rise in CRC could partly be due a rise in CSCs.

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^[11]. 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^[12]. Our earlier data suggested a pivotal role for Wnt/ β -catenin signaling for proliferation and maintenance of CSCs in the colon^[13]. 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^[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^[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^[7]. The number of colonic mucosal cells showing CD44⁺, CD166⁺ or EpCAM was markedly higher in the isolated mucosal cells in subjects over 55 years of age with polyps than 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^[15-19]. In addition, our data also revealed that CD166 and EGFR were co-localized in normal appearing mucosa of patients with adenomas. Interestingly, the co-expression of CD166 and EGFR was found to be markedly higher in individuals over 60 years

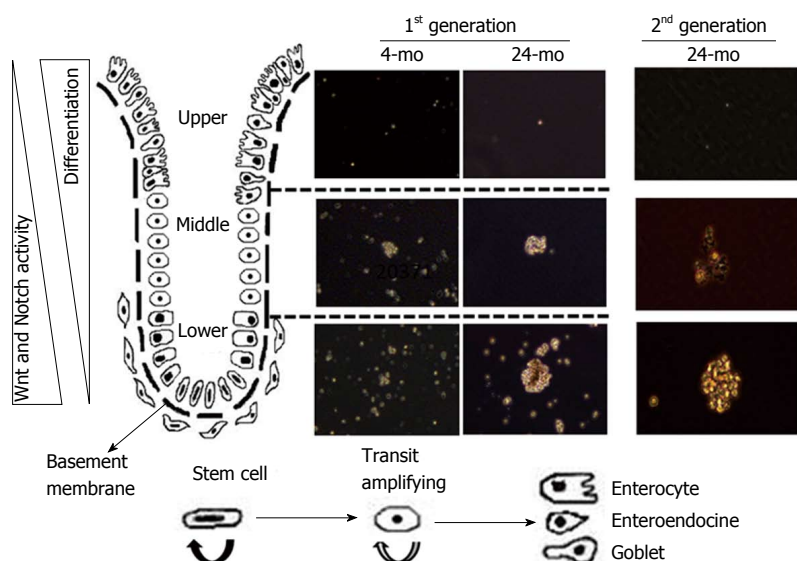


Figure 1 Changes in colonosphere forming potential of mucosal cells isolated from different regions of colonic crypt: Young (4-5 mo) and aged (22-24 mo) Fischer 344 rats were euthanatized by CO₂ asphyxiation following an overnight fast. The colon was removed, rinsed with cold PBS, everted, filled with 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 stem 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.

of age suggesting that with aging risk of developing CRC increases^[7]. Expression of CSC markers was also found to be higher in *Helicobacter pylori* gastritis^[20] and gastric cancers and also in normal appearing gastric mucosa from the aged^[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 1/3) of young (4-mo) and aged (24-mo) Fischer-344 rats and subjecting them to colonosphere formation and mutational analysis. Our results showed that the number of spheroids formed by the mucosal cells isolated from the middle and lower regions of the crypt from aged animals were higher than their younger counterparts. No such difference was observed in cells isolated from the upper region of the colonic crypt between the two age-groups. In addition, we also found cells from the lower and middle regions of colonic crypt of older animals to form spheroids for another generation. Although mucosal cells, isolated from bottom of the crypt of young rats did form a few colonospheres inconsistently, they were also smaller in size. In contrast, mucosal cells isolated from the mid and upper parts of colonic crypt of young rats did not form spheroids (Figure 1). The increased colonosphere formation by mucosal cells from older

animals was accompanied by a parallel rise in colonic 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, colonic 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.

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