Cancer Stem Cells and Signaling Pathways in Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC) is the third most common cancer in males, the second in females and is the second leading cause of cancer related death worldwide. Despite recent advances in chemotherapy, and targeted therapy for CRC, the prognosis for patients with advanced cancer has remained poor, due to drug resistance, metastasis and recurrence. A small fraction of cells possess tumor propagation abilities. These are termed "cancer stem cells (CSCs). A subset of colorectal cancer stem cells, may hold a key to controlling cancer. The cancer stem cell (CSC) model suggests that tumors are hierarchically organized, only CSCs possess cancer-promoting potential. The killing of CSCs is thought to be a critical component of effective antitumor therapies. A number of signaling pathways, most notably the Wingless related (Wnt), transforming growth factor-beta (TGF-β), Notch and Hedgehog signaling and other mechanisms have been found to be associated with CSCs in CRC. They play important roles in maintaining the growth and functional integrity of CSC. Many new molecules are now being studied to block theses pathways. Some of the molecules block the self-renewal and induction of apoptosis in CSCs. The design of CSC-targeted interventions is a rational target, and reduce local recurrence and metastasis. This review aims to summarize the issue on CSCs and signaling pathway relevant for CRC, which may lead to more effective therapeutic strategies for CRC.

Keywords: *cancer stem cell, colorectal cancer, signaling pathway*

ABSTRAK

Kanker kolorektal (KKR) merupakan kanker urutan ketiga terbanyak pada pria, dan kedua pada wanita dan penyebab kematian kedua akibat kanker di dunia. Meskipun dengan kemajuan terkini pengobatan kemoterapi dan target terapi, prognosis pasien KKR stadium lanjut masih tetap jelek, karena resistensi obat, metastasis dan kambuhnya kanker. Suatu fraksi kecil sel mempunyai kemampuan untuk berkembangnya kanker yang disebut dengan cancer stem cells (CSCs). Subset cancer stem cells tersebut mengontrol sel kanker kolorektal. Menurut model CSC, tumor diatur secara hirarki dan hanya CSCs yang memiliki potensi menjadikan kanker. Mematikan CSCs merupakan komponen penting dalam terapi anti tumor yang efektif. Sejumlah pathway terutama Wingless (Wnt), transforming growth factor-beta (TGF-β), Notch dan Hedgehog signaling serta mekanisme lainnya telah ditemukan berhubungan dengan CSCs pada KKR. Pathways tersebut memegang peranan penting dalam mempertahankan pertumbuhan dan integritas fungsional dari CSCs. Banyak bahan molekul baru sedang diteliti untuk memblok pathway tersebut. Sebagian dari molekul memblok self-renewal dan menginduksi apoptosis CSCs . Rancangan target intervensi terhadap CSC adalah suatu target yang rasional, dan dapat menurunkan rekurensi lokal dan metastasis. Tinjauan ini mengemukakan masalah terkait CSCs dan signaling pathway terkait KKR, yang bisa memberi arah strategi terapi KKR yang lebih efektif.

Kata kunci: *Cancer stem cell, kanker kolorektal, signaling pathway*

INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed and lethal cancers worldwide.[1,2] It is the third most frequent cancer in males, and the second in females, and is the second leading cause of cancer related death worldwide. Indonesia with its 250 million population, has age standardized incidence rates for colorectal cancer per 100,000 population were 15.2 for males and 10.2 for females, and has estimated 63,500 cases per year, and an almost similar burden to other countries with increasing populations.[2,3,4] Most of CRC were located in the rectum (74.6%) than in colon (25.4%).[5]

Colorectal carcinogenesis results from a series of genetic/epigenetic alterations and interactions with microenvironmental and germ-line factors that transform the normal colonic mucosa into an aberrant phenotype.[1,6,7] There are two models of carcinogenesis: the stochastic and the cancer stem cell (CSC) model. The CSC model suggests that tumors are hierarchically organized and only CSCs possess cancer-promoting potential. Besides all the advances in target therapy, many patients still fail to survive because they develop primary and acquired resistance.[8] The cause not only on tumor heterogeneity, but also that the tumor grows up in a complex ecosystem, that can influence the tumor main driver pathway to survival.[8,9,10] Genetic diversity, tumor micro-environment and epigenetics are coming together and influence the concept of maintenance of stem cell state. This revolutionary idea changed the historical concept that tumor cells may harbour stem cells, and with these active properties they may influence carcinogenesis and patient's outcome as never seen before.[8]

Carcinogenesis of Colorectal Cancer (CRC)

According to the stochastic model of tumorigenesis suggest that any kind of cell is capable of initiating and promoting cancer development. The CSC model suggest that tumors are hierarchically organized and only a small fraction of cells (CSCs) possess tumor propagation abilities or cancer-promoting potential, and various molecular pathways, such as Wingless/Int (Wnt), Notch and Hedgehog, as well as the complex crosstalk network between microenvironment and CSCs, are involved in CRC.[1,11] The CSC model modifies the classic Fearon and Vogelstein model, which is characterized by step-by-step genetic modifications of the adenoma to carcinoma sequence, by placing the normal stem cell (SC) as the primary candidate for being the cell of origin, by underlying the crucial importance of microenvironmental signals, and by explaining tumor heterogeneity within the context of a clonally evolved CSC model.[1, 12]

Large Intestine and Stem Cell

The inner luminal lining of the large intestine is a single layer of epithelial columnar cells folded into finger-like invaginations, which are embedded in the submucosal connective tissue to form the functional unit of the intestine, called the crypt of Lieberkuhn. Normal human colon consists of millions of crypts, each containing about 2000 cells.[12-16] The crypt base columnar cells (CBCCs) are regarded as stem cells in normal colon crypt. It is now recognized that colorectal cancer stem cells (CCSCs) derive from normal colonic crypt stem cells, located at the bottom area of the normal crypt, and differentiate into a variety of crypt cells under normal circumstances. Current evidence suggest that CCSCs are a special subgroup of cells in colorectal cancer with the ability to initiate differentiation towards malignant cells and exhibit self-renewal and metastasis potential. These cells differentiate into mature cells, and recruite cancer cells in the mature cancer tissue during CRC carcinogenesis.[12-15] Overall, 3 main epithelial cell lineages comprises a crypt: the columnar cells or colonocytes, the mucin-secreting cells or goblet cells, and the endocrine cells. Turnover of these cell lineages is a constant process, occurring every 2-7 days under normal circumstances and increasing following tissue damage.[12,17] This complex process is regulated by adult stem cells (ASCs) located within the crypt unit.

The Cancer Stem Cell Model in Colorectal Cancer (CRC)

The silencing of key genes can foster increased CRC-related pathway signaling (such as Wnt pathway), resulting in genomic instability and mutations in the downstream pathway genes, such as APC or β-catenin, and further activate these signaling pathways to foster colon tumorigenesis.[18-19] In the CSC model, precursor cells are a type of partially differentiated stem cell which has the capacity to differentiate into one cell type, and therefore are also called unipotent stem cells. Epigenetic changes, such as aberrant methylation, may result in silencing of genes p16, SFRPs, GATA-4/-5 and APC in stem/precursor cells of adult cell-renewal systems and may lock these cells into stem-like states that foster abnormal cell clonal expansion, and the

stem/precursor cells are transformed into preinvasive cancer stem cells.[20] At this stage, preinvasive cancer stem cells turn into cancer stem cells that will ultimately become cancer cells. Epigenetic and genetic alterations play crucial roles in CRC carcinogenesis, while epigenetic alterations may be a predominant factor during early malignant transformation of colonic stem cells in the stem cell model.

The current chemotherapies generally aim at mature cancer cells, not the CRC CSCs. Although these treatments can reduce the size of cancer tissue, they cannot completely kill CSCs. CSCs have higher proliferative potential and stronger resistance to chemotherapy and radiotherapy and differentiate into mature cancer cells when therapy is withdrawn, resulting in cancer recurrence and metastasis. Therefore, development of therapy targeting CSCs has a therapeutic potential to achieve better treatment to radically suppress cancer growth and metastasis.[21]

CSCs are characterized by self-renewal, multipotency, limitless proliferation potential, angiogenic, and immune evasion features. These cells are considered highly malignant, fundamental for the growth of neoplasia, for recurrence, and for metastasis. Also they are considered resistant to chemotherapy, radiotherapy and target therapeutics. CSCs in the colorectal cancer, becoming a potential target for the treatment of the disease.[8, 22-26] CSCs have been isolated from many solid tumors in humans using the combination of cell surface markers, including CD44, CD24, ESA 18 among others. Several biomarkers of colorectal CSCs are as follows: CD133+/ CD44+/ ALDH1+, EpCAM+/ CD44+/CD24+, Lgr5+/ GPR49+, and CD133+/CD26+.[8,22,27] These CSC play a predominant role in the initial phase of tumorigenesis. These facts suggest that inhibition of CSCs may be a therapeutic target for cancer.[8] The killing of cancer stem cells is thought to be a critical component of effective antitumor therapies.

Signaling Pathways

Some pathways, including the wingless related (Wnt), transforming growth factor-beta (TGF-β), Notch and Hedgehog signaling pathways and other mechanisms have been found to be associated with CSCs in many cancer.[27, 28, 29] The alterations in microenvironment may also be responsible for the tumor formation by dominance in growth promoting signals over the growth inhibiting signals. Therapeutic options follow the basic characteristic features or related features of the CSCs; however need to elucidate further for careful clinical applications.[30]

The Wnt pathway plays an essential role in the growth and maintenance of CSCs. This pathway is regulated at the level of β-catenin, which is degraded by adenomatous polyposis coli (APC). Mutations in the APC gene are found in most colorectal tumors. As a result, β-catenin is accumulated in the nucleus, where it activates target genes with important functions in colorectal cancer development.[31-33] Wnt signaling pathway plays a pivotal role in the regulation of epithelial stem cell self renewal. In contrast, dysregulation of this signaling has been implicated in many epithelial cancers, including colon carcinogenesis.[34,35,36]

TGF-β signaling pathway is one of the most commonly altered pathways in human cancers. This pathway regulates cell proliferation, differentiation, migration, apoptosis, stem cell maintenance and function TGFβ superfamily ligands bind to a type II serine/threonine kinase receptor, which recruits and phosphorylates type I receptor.[31,37,38] The TGF-β pathway acts as a tumor suppressor pathway in healthy tissues but as a promoter in colorectal cancers.[39] Notch signaling is active in colon cancer initiating cells (CC-ICs) and is essential for the intrinsic maintenance of CC-ICs self-renewal and the repression of secretory cell lineage differentiation gene.[40] Notch signaling is an evolutionarily conserved pathway in multicellular organisms, regulates cell-fate determination during development and in stem cells. It mediates juxtacrine signaling among adjacent cells. Interaction between Notch and its ligands initiates a signaling cascade that regulates differentiation, proliferation, and apoptosis.[41]

Hedgehog signaling, which is active in both colon cancer epithelial cells and, strikingly, CD133(+) cancer stem cells, promotes colon cancer growth, stem cell self renewal and metastatic behavior in advanced cancers.[42] The hedgehog signaling is named after the polypeptide ligand, an intercellular signaling molecule called Hedgehog (Hh) found in Drosophila.[43] The proliferation, migration, and differentiation of target cells are regulated by Hh signaling in a spatial, temporal, and concentration dependent manner. In mammals, three Hedgehog homologues are present, of which Sonic hedgehog (Shh) is the best studied.[43] Several CSC target and agents targeting dysregulated signaling pathways in CSC, are as follows: (1) STAT3 (Napabucasin); (2) LRP/ FZD (Vantictmab); (3) WNT(Ipafricept); (4) Anti-DLL4 (Demcizumab); (5) Notch (Tarex).

Many new molecules are now being developed and tested in clinical trials, to block these pathways. Some of these new small molecules block the self-renewal

and induction of apoptosis in CSCs. They act inhibiting the Wnt/β-catenin pathway, the Notch pathway and the hedgehog pathway. Inhibition of the STAT3 pathway inhibits cell proliferation *in vitro* and reduces tumor growth *in vivo*. This pathway is critical for the self-regeneration and survival of CSCs in various neoplasms. The STAT3 pathway is connected to/β-catenin pathway activity, which is also very important in the early stage of carcinogenesis and progression of disease in many cancers.[8,28,29]

The Wnt/β-catenin pathway is mostly dysregulated in colorectal cancer and epidermal cancer; the hedgehog pathway is dysregulated in colorectal cancer, gastric cancer, pancreatic cancer, basal cell carcinoma and medulloblastoma; the Notch pathway dysregulated in colorectal cancer, pancreatic cancer, breast cancer and leukemia, and the JAK/STAT3 pathway in colorectal cancer, gastric cancer, breast cancer, and glioblastoma.[8, 22, 27]

THERAPEUTIC OPTIONS

The CSC-targeted interventions is a rational target, which will enhance responsiveness to traditional therapeutic strategies and reduce local recurrence and metastasis. The problem is how to identify these subclones which express dysregulation of these crucial pathways? Science has advanced and identified sub-populations, which are eventually responsive to the blockage of these new molecules.[1,45,46,47]

It has become clearer that a tumor does not have a single genome, but multiple genomes, which belong to different sub-clones. These different sub-clones will contribute to intra-tumoral heterogeneity. Nevertheless, these different sub-clones don't all behave in the same way: some are active and maintain their capacity of auto-renewal and are pluripotent, others remain dormant in a quiescent form and others are in a post-mitotic condition and run into apoptosis.[8] The new concept that one or more of these clones may harbour CSC, redefines the driver clone "the harmful cancer clone" that attributes the growth and survival potential. These cells maintain the embryological potential to maintain its primary capacity to stimulate their own oncogenes and inhibit the tumor suppressor genes, favouring carcinogenesis. These clones are the hierarchy of tumor survival, and it should be the main aim in personalized medicine in the near future. The future of treatment of CRC lies in research on CSCs, signaling pathways. If these CSCs and signaling pathways better understood, CSC targeting via markers and targeting these aberrant signaling pathways are important offers a new strategy for cancer therapy.[8,45,46,47]

CONCLUSION

Stem cells may become cancer stem cells under a series of epigenetic and genetic alterations. CSCs possess cancer promoting potential, and various molecular signaling pathways as well as the complex crosstalk network between CSCs and microenvironment are involved in CRC. The design of CSC-targeted interventions and agents targeting dysregulated signaling pathways in CSC, will enhance responsiveness to therapeutic strategies and reduce local recurrence and metastasis.

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