Crosstalk between tumor cells and microenvironment via Wnt pathway in colorectal cancer dissemination

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Abstract

Invasion and metastasis are the deadly face of malignant tumors. Considering the high rate of incidence and mortality of colorectal cancer, it is critical to determine the mechanisms of its dissemination. In the parallel investigation of the invasive front and tumor center area of colorectal cancer (CRC), observation of heterogeneous β-catenin distribution and epithelial-mesenchymal transition (EMT) at the invasive front suggested that there might be a crosstalk between tumor cells and the tumor microenvironment. Wnt signaling pathway is also involved in the cancer progression due to its key role in CRC tumorigenesis. Moreover, in recent years, there is increasing evidence that the regulators of microenvironment, including extracellular matrix, growth factors and inflammatory factors, are associated with the activation of Wnt pathway and the mobility of tumor cells. In this review, we will try to explain how these molecules trigger metastasis via the Wnt pathway.

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Key words: Invasion; Microenvironment; Colorectal cancer; Epithelial-mesenchymal transition; Wnt; β-catenin

INTRODUCTION

Colorectal cancer (CRC) is one of the major malignancies worldwide and the second leading cause of cancer death in the United States[1]. In the past decades, many researches in tumorigenesis and progression of CRC have focused on genes and epigenetic changes. Recently, increasing attention has been paid to cellular signal transduction in CRC, especially Wnt pathway which regulates cell growth, differentiation and death in embryogenesis and tumor development, attributing to the presence of an activating mutation of the canonical Wnt signaling pathway in about 90% of all CRCs[2-6]. Activation of the Wnt signaling pathway is characterized by the accumulation of β-catenin in nuclei[7]. It was reported that nuclear β-catenin is detectable in colorectal tumors and its amount is increased from early adenomas to adenocarcinomas[8]. However, the distribution of β-catenin within an individual tumor is very heterogeneous. Immunohistochemical analysis of moderately- and well-differentiated colon adenocarcinomas reveals that accumulation of nuclear β-catenin is observed in dedifferentiated tumor cells at the invasive front and scattered in the adjacent stromal compartment. Contrarily, in central differentiated area, it is detectable on the membrane and its translocation is not found[9,10]. Consequently, there is considerable interest in finding the means to explain such dynamic changes. Recent researches highlight the role of tumor microenvironment in cancer dissemination where cells located at the invasive front are exposed to cytokines, such as growth factors, chemokines, inflammatory factors, and extracellular matrix, which may interact with the Wnt signaling pathway resulting in the heterogeneous intracellular distribution of β-catenin[11-13]. Therefore, this review will concentrate on the relationship between microenvironment and Wnt pathway in invasion and metastasis of CRC.

WNT PATHWAY IN CRC

The Wnt signaling pathway is involved in various differentiation events both in embryogenesis and in tumor formation when aberrantly activated. Molecular studies demonstrated that constitutive activation of Wnt/β-catenin signaling occurs in nearly all colorectal tumors due to mutations either in APC gene or in less frequently β-catenin[14,15]. Therefore, understanding the role of this pathway in CRC carcinogenesis is important.

In the absence of Wnt signaling, intracellular β-catenin levels are regulated by multiprotein complex encompass-
The capability of invasion and metastasis is the hallmark of malignant tumors. The progression of tumor cellular dissemination leading to invasive growth includes the detachment from primary cancer, migration, access to blood or lymphatic vessels and development of secondary tumors. Cellular dissemination is characterized by disordered cell-cell interactions and cell adhesion. Disintegration of cell adhesion molecules, especially β-catenin, has been implicated in this process. However, only β-catenin in the membranes, a stable subcellular localization, forms an adherent complex with α-catenin and E-cadherin which is regulated by tyrosine phosphorylation. Phosphorylated β-catenin is dissociated from the adherent complex and transferred to the cytoplasm, where β-catenin can be degraded or translocated into nuclei, triggering dysregulation of Wnt pathway. Importantly, cooperative effects on tumor development of defects in E-cadherin-mediated cell adhesion and activation of β-catenin-mediated signal transduction are observed in human CRC[28]. Moreover, a tissue microarray-based analysis of a large number of cases, performed by Lugli et al[20] demonstrated that increased nuclear β-catenin expression and loss of membranous E-cadherin are two independent, adverse prognostic factors in sporadic CRC, suggesting that the role of β-catenin in tumor invasion and metastasis is not just attributed to interaction with E-cadherin, therefore other mechanisms may be involved, such as Wnt/β-catenin signaling pathway.

Furthermore, as the downstream effector of canonical Wnt pathway, nuclear β-catenin cooperating with TCF/LEF initiates expression of target genes (Table 1), some of which can improve tumor progression. MMP-7, a target of β-catenin/TCF signaling, is expressed in up to 90% of CRCs and its expression in the invasive front as well as in urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR) is related to unfavorable outcome in CRC[22,23,24]. Fascin, a novel target of β-catenin/TCF signaling, is expressed at the invasive front of human colon cancer, suggesting that it plays a potential role in the development of colon cancer metastasis[28]. It was reported that intratumorous heterogeneity in CRC correlates with differential expression of 510 genes between the central tumor region and the invasive front, isolated by laser-microdissection in the same tumor samples[29]. This in vivo analysis shows over-expression of known Wnt/β-catenin target genes either in the entire

| Table 1 | β-catenin target genes related to cancer |
|---------|----------------------------------------|
| Function | Target gene |
| Cell proliferation | C-myc; Cyclin D1 |
| Inhibition of apoptosis | MDR1/PGP; COX-2; PPAR |
| Tumor progression | MMPs; uPAR; uPA; CD44; Laminin γ2; Nt-CAM |
| Growth factors | c-met; VEGF; WISP-1; BMP-4 |
| Transcription factors | c-jun, fra-1; ITF-2; Id2; AF17 |
| Negative feedback targets | Conductin; Tcf-1; Nkd |

β-CATENIN IN CRC PROGRESSION

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tumor tissue or specifically at the invasive front. Whether these target genes expressed at the front are involved in the tumor invasive process still needs to be further studied. Furthermore, the concomitant high expression in 2 groups of Wnt/β-catenin target genes, inflammation- and tissue repair-related genes, at the invasive front supports the hypothesis that inflammation-activated microenvironment may trigger selective Wnt/β-catenin target gene expression and contribute to the progression of CRC. Accordingly, similar in tumor initiation, Wnt pathway activation (detectable by nuclear accumulation of β-catenin and expression of some target genes) might be functionally associated with cancer dissemination.

In modestly- and well-differentiated tumor, membranous expression of β-catenin in tumor center retains whereas nuclear β-catenin is observed in dedifferentiated tumor cells localized in the invasive area. Since tumor cells in an individual tumor harbor APC mutations, this alteration alone cannot lead to the heterogeneous distribution of β-catenin, but its translocation has to be explained by additional events. Whether nuclear β-catenin accumulation is the sign of motility enhancement of tumor cells and what initiates β-catenin heterogeneous distribution, are two questions arising from these observations.

**EPITHELIAL-MESENCHYMAL TRANSITION IN CRC DEVELOPMENT**

In the majority of sporadic CRCs, well-, modestly-, and well-differentiated adenocarcinomas, tumor cells at the invasive front lose their epithelial characteristics and take on the properties that are typical of mesenchymal cells, which require complex changes in cell architecture and behavior. Such transition from epithelial- to mesenchymal- cells, dubbed as epithelial-mesenchymal transition (EMT), is considered a fundamental event in the metastatic cascade. The essential features of it are the disruption of intercellular contacts and the enhancement of cell motility, thereby leading to release of cells from the parent epithelial tissue. The resulting phenotype is suitable for migration and, thus, for tumor invasion and dissemination, allowing metastasis progression to proceed. Although the molecular bases of EMT have not been completely elucidated, several interconnected transduction pathways and a number of potentially involved signaling molecules, including β-catenin, have been identified.

Activated β-catenin is directly linked to EMT. The activation of Wnt signal pathway results in the activation of β-catenin/TCF transcriptional regulators such as snail and slug, which regulate the changes in gene-expression patterns underlying EMT. Similarly, in the study of breast cancer cells, Yook et al demonstrated that canonical Wnt pathway engages tumor cell dedifferentiation and tissue-invasive ability through an axin-2-dependent pathway to identify a new mechanistic β-catenin-TCF-regulated axin2-GSK3β-Snail1 axis, thus gaining insight into cancer-associated EMT program. It was reported that Wnt/β-catenin signaling pathway plays a pivotal role either in gastric cancer formation or in tumor invasion and dissemination. In cell culture experiments, cells with β-catenin activation lose their polarity and disrupt cell-cell contacts and EMT morphologically. Moreover, immunohistochemical stains demonstrate alternations of the actin cytoskeleton in these cells, indicating that nuclear β-catenin accumulation is functionally related to EMT in budding tumor cells at the tumor-host interface.

**MICROENVIRONMENTAL REGULATION IN β-CATENIN TRANSLOCATION AND EMT INDUCTION**

The dynamic changes in the above non-random distribution of β-catenin and EMT of tumor cells at the invasive front of CRC, can be at least partially explained by interactions with the tumor environment. A micro-ecosystem exists at the invasive front of tumor where the stromal cells interact with parenchymal cells by producing extracellular matrix and secreting cytokines that directly or indirectly promote cell invasion. Moreover, it also appears that inflammatory cells are involved in the formation of tumor metastasis.

Epithelial-mesenchymal interactions are essential for intestinal development. Thus, more investigations should be focused on mesenchymal factors, particularly the components of extracellular matrix, because they have a potent regulatory effect on tumor cells. Recent studies demonstrated that Wnt ligands are expressed in both mesenchymal and epithelial cells of the colon. It was also reported that local regulation by Wnt signals of diverse cell signaling pathways in fibroblasts could have multifaceted consequences for tissue microenvironments in vivo, including the balance between cell differentiation and proliferation, as well as between cell migration and adhesion. Mesenchymal forkhead transcription factors, Foxf1 and Foxf2, can limit paracrine Wnt signaling and promote extracellular matrix production in gut, and deletion of Foxf1 and Foxf2 is accompanied with increased mesenchymal expression of Wnt5a and β-catenin nuclear accumulation in epithelial cells, indicating that there is a crosstalk between stromal cells and parenchymal cells involving Wnt signaling. There are extensive data to support the relation between extracellular matrix and signal pathway in tumorigenesis. Tsuboi K et al investigated the relationship of galectin-3 expression, a component of extracellular matrix, to the clinicopathological factors, and found that reduced galectin-3 expression is related to invasion and metastasis of CRC. In contrast to β-catenin, the expression of galectin-3 is lower at the invasive front of a tumor. Whether β-catenin regulates galectin-3 expression or other signaling pathways are involved in the process is still controversial.

In addition, cell culture experiments have also revealed a role of cytokines, such as growth factors, in the intracellular β-catenin distribution, as well as in the induction of EMT. One of the related growth factors is the hepatocyte growth factor (HGF), which is found in CRC. It was reported that HGF and β-catenin physically interact in a complex, which is disassembled after HGF treatment. Moreover, HGF treatment promotes β-catenin/TCF transcriptional activity in CRC cells. HGF also stimulates...
cells leading to cell scattering. Therefore, a self-amplifying positive feedback loop between HGF and β-catenin in CRC promotes tumor growth and invasion. Like HGF, Platelet-derived growth factor (PDGF) also can activate EMT in CRC cells by enhancing Wnt signaling. A recent study has shown a novel Wnt-independent pathway that enhances β-catenin signaling to nuclear β-catenin. PDGF promotes tyrosine phosphorylation of p68, which binds to β-catenin and inhibits its phosphorylation by GSK3β. A new EMT pathway from PDGF and another route to nuclear β-catenin signaling have been identified. Similarly, the epidermal growth factor (EGF) and transforming growth factor β (TGFβ) can also enhance Wnt/β-catenin signaling by phosphorylating p68.

It has been widely accepted that inflammatory cells in colorectal tumors are associated with the progression to malignancy. Brown et al. reported that non-steroidal anti-inflammatory drugs (NSAIDs) can decrease the number and size of intestinal polyps in Apc-mutation mice by inhibiting cyclooxygenase-2 (COX-2), one of the main enzymes in prostaglandin biosynthesis. To investigate the mechanism, a recent study by Castellone and collaborators indicate that COX-2 and its proinflammatory mediator prostaglandin E2 (PGE2) enhance colon cancer progression via a heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptor, EP2. This signaling route involves the activation of PI3K and protein kinase A by free G protein and is directly associated with the G protein signaling (RGS) domain of axin, thus leading to GSK3β inactivation, relief of inhibitory phosphorylation of β-catenin and activation of Wnt signaling pathway. Therefore, these findings suggest that COX-2 and inflammation can promote the progression of colon cancer. It was recently reported that co-culture of tumor cells and macrophages leads to up-regulation of Wnt5a in the latter and that non-canonical signaling via Wnt5a in cancer cells is critical for invasion. However, whether a similar interaction between cancer cells and tumor-associated macrophages occurs in CRC is still unknown.

SUMMARY

Since tumor cells at the invasive front display nuclear accumulation of β-catenin and EMT features associated with local activation of Wnt signaling pathway, dissemination of cancer cells is not due to gene mutation alone. The importance of tumor microenvironment where extracellular matrix, growth factors and inflammatory factors play a key role in tumor invasion cannot be overlooked. A complex network, which is orchestrated by Wnt pathway and other signaling pathways, may be involved in the regulation of tumor-microenvironment crosstalk. Further study is needed to investigate the specific role of tumor cells and the microenvironment of tumor in invasiveness. Although recent researches have illuminated the involvement of Wnt pathway in cancer development, a more comprehensive view of how cancer spreads will likely emerge in the future, allowing us to provide new potential therapeutic targets for the treatment of aggressive and recurrent CRC in clinical practice.

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