Role of Notch signaling in colorectal cancer

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Notch signaling is an important molecular pathway involved in the determination of cell fate. In recent years, this signaling has been frequently reported to play a critical role in maintaining progenitor/stem cell population as well as a balance between cell proliferation, differentiation, and apoptosis. Thus, Notch signaling may be mechanistically involved in carcinogenesis. Indeed, many studies have shown that Notch signaling is overexpressed or constitutively activated in many cancers including colorectal cancer (CRC). Consequently, inactivation of Notch signaling may constitute a novel molecular therapy for cancer. CRC is one of the most common malignancies but the current therapeutic approaches for advanced CRC are less efficient. Thus, novel therapeutic approaches are badly needed. In this review article, the authors reviewed the current understanding and research findings of the role of Notch signaling in CRC and discussed the possible Notch-targeting approaches in CRC.

Introduction

Colorectal cancer (CRC) ranks the third and second among all commonly encountered malignancies in terms of incidence and mortality, respectively. The high mortality rate of advanced CRC is attributable to limited treatment options. In the search of better therapeutic options for CRC, Notch signaling has emerged as a potential target. Cleavage of Notch receptors by γ-secretase is an essential step for production of Notch intracellular domain (NICD), the active form of Notch. Translocation of NICD into nucleus and subsequent binding to transcriptional factors such as hairy-enhancer-of-split (Hes-1) and mastermind-like-1 (MAML-1) leads to the activation of Notch signaling. Current data indicate that activation of Notch signaling is critically involved in cell differentiation, proliferation, apoptosis, and angiogenesis. As Notch signaling is constitutively active in many human cancers including CRC, it is increasingly recognized as a therapeutic target for cancers.

In this article, the authors aim to review the relevant available literature on the role of Notch signaling in CRC and discuss the possible approaches and molecular mechanisms of Notch targeting in this malignancy.

Notch signaling—an overview

In human and mice, Notch-signaling pathway consists of Notch ligands (Jagged1, Jagged2, DLL1, DLL3 and DLL4), Notch receptors (Notch 1–4) and several downstream target genes such as p21, Hes-1 and Deltex (1,2). Notch receptors and their downstream target genes are widely expressed in mammalian tissues including embryonic tissues (3,4).

Activation of Notch signaling starts with binding of Notch ligands present on the neighboring cell (or signaling cell) to the Notch receptors on the bordering cell (receiving cell). Binding of Notch ligands to Notch receptors activates γ-secretase protein complex (5). Active γ-secretase can cleave the transmembranous Notch receptors, causing release of the NICD, which is the constitutively active domain of the Notch receptor. The released NICD then translocates to the nucleus where it binds to and forms a complex with one of the three transcriptional regulators CSL (a collective name of CBP or RBP-JK in vertebrates, Su (H) in Drosophila, and Lag-1 in Caenorhabditis elegans), MAML-1 and p300/CPB. The formation of these complex leads to a displacement of co-repressors previously bound to the transcription factors and recruitment of co-activators. The co-activators then induce expression of the target genes, such as the Hes and Hes-related proteins gene families, with Hes-1 being the most abundant one (6–10). Figure 1 briefly illustrates how Notch signaling is activated.

The biological role of Notch signaling as a regulator for cell differentiation was first identified in 1937, but its role in cancer was not recognized until 1991 when Notch1 was suspected to be causally related to the development of T-cell acute lymphocytic leukemia (T-ALL)/lymphoma (11). The discovery of the role of γ-secretase in the Notch signaling activation in 1999 had prompted intensive research on the potential application of γ-secretase inhibitors in the treatment of various cancers. It is now recognized that Notch signaling plays an important role in determining cell fate and maintaining progenitor cell population as well as the balance between cell proliferation, differentiation, and apoptosis (12).

Aberrantly activated Notch signaling has been observed during the carcinogenesis of many human cancers, such as pancreatic cancer (13–16), breast cancer (17,18), prostate cancer (19), liver cancer (20,21), cervical cancer (22–24), Ewing sarcoma (25), Kaposi sarcoma (26), lung cancer (27), ovarian cancer (28), lymphoma (29), renal cancer (30) and colon cancer (31,32). A direct introduction of activated Notch1 into mouse bone marrow produces changes typical of T-ALL (33).

In addition, overexpression of Notch signaling was found to be associated with poor prognosis or poor response to treatment of some solid tumors such as breast tumor (34,35) and prostate cancer (19). Thus, Notch signaling has been proposed as an important target for cancer therapy (36–38). In this review, we will focus on the role of Notch signaling in CRC. For Notch signaling in other gastrointestinal cancers, please refer to a recent review in ref. 39.

Notch signaling in normal colonic tissues

Notch signaling plays a critical role in the maintenance of the normal intestinal epithelia (40). Notch signaling is essential for regulating the differentiation of colonic goblet cells and stem cells/progenitor cells (41–44). Thus, Notch signaling is essential in maintaining the intestinal development and homeostasis. It is well known that colonic crypts are the principal niche for colonic stem cells. All Notch signaling component genes including all ligands, four receptors and several downstream target genes (Hes-1, 5, 6, 7 and Math1) are expressed in normal mouse intestinal crypts of various stages of differentiation and development (45–47). Notch receptors Notch1, Notch2 and Notch3 were highly expressed at the basal crypt of the human colon, and CSL and Notch ligand Jagged1 were highly expressed at the top of the crypts (48).

Such an expression pattern has some functional implications. Notch signaling is essential for regulating proliferation of crypt progenitor...
cells and the differentiation of colonic epithelial cells. Suppression of Notch signaling by depletion of Hes-1, the most abundant and direct downstream target gene of Notch signaling, was associated with significant increase in the secretory lineage of intestinal epithelial cells (49). On the other hand, activation of Notch signaling not only promote the proliferation of stem cells in the crypt but also redirect the gut progenitor cells to differentiate toward absorptive but not secretory lineage cells (41,50–52). Hes-1 regulates the expression of Math1 that is another important gene controlling intestinal differentiation (49,53). Mice deficient of RBP-Jκ or Hes-1 or those treated with γ-secretase inhibitor exhibited increased numbers of secretory epithelial cells (45,49). The role of Notch signaling in the control of gut crypt differentiation and proliferation was recently confirmed by a study in inducible gut-specific Notch-mutant mice, which showed that Notch signaling is involved in the regulation of cell cycle progression of crypt progenitor cells (43).

In addition, Notch appears to be necessary for the functional maintenance of Wnt signaling in the gut (54). A cooperation between the Notch signaling and Wnt signaling is required for the proliferation of intestinal precursor cells but not for the subsequent differentiation of the intestinal epithelial cells (54). Other studies have revealed that inhibition of Notch and/or Wnt pathways was able to increase the expressions of some colonic differentiation markers such as villin2, muc20 or TFF1 (55). These results indicate that under the physiological condition, activation of Notch signaling is probably involved in the maintenance of proliferative potential of intestinal epithelial cells.

**Notch signaling in colonic precancerous conditions**

Sporadic CRC usually develops from certain colonic precancerous conditions such as adenomatous polyps and inflammatory bowel diseases. The role of Notch signaling in these precancerous colonic lesions, however, has been scarcely studied. The expressions of Notch signaling genes including Hah1, Krüppel-like factor 4 (KLF4), Hes-1, Muc1, Muc2 and Muc4 were recently studied by real-time PCR, western blot and immunohistochemistry in patients with Crohn’s disease and ulcerative colitis (56). Upregulation of transcription factors that function downstream of Notch signaling, such as KLF4 and Hes-1, is probably responsible for the altered goblet cell differentiation and mucin formation in patients with inflammatory bowel diseases especially Crohn’s disease.

Increased Notch signaling may be linked to the increased susceptibility of colon cancer development in some precancerous conditions. For example, Jagged1 messenger RNA was found to be expressed at a significantly higher level in the normal colonic mucosa and adenomas of patients with familial adenomatous polyposis, compared with normal intestinal mucosal tissues of the healthy subjects (55). Increased colonic Notch1 and NICD were found in the dextran sodium sulfate-induced ulcerative colitis in mice (52), whereas treatment of the colitis by γ-secretase inhibitor LY411,575 strongly inhibits proliferation of the intestinal epithelial cells (52).

**Expression and activity of Notch signaling in CRC**

Currently, there are very little information indicating a cell-specific expression and function of Notch signaling in CRC compared with other solid tumors. Nevertheless, available data from many studies have demonstrated that CRC harbors aberrant activation of Notch signaling. Our recent studies found that the Notch ligand Jagged1 is expressed at a significantly higher level in CRC tissues than in their matched normal colonic mucosa. In addition, we observed that higher level of Jagged1, Jagged2, DLL1, DLL3, DLL4, Notch receptors 1–4

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**Fig. 1.** An illustration of Notch signaling and potential targeting approaches. Notch ligands on the ‘signaling cell’ bind to Notch receptor on the ‘receiving cell’. Five Notch ligands (Jagged1, Jagged2, DLL1, DLL3 and DLL4) and four Notch receptors (Notch1–4) have been identified. Notch receptors are transmembranous protein. The extracellular domains are for ligand binding that triggers cleavage of the intracellular portion of Notch receptor by γ-secretase and release of NICD. NICD then translocates into nucleus, binds to the transcription factors such CSL (RBP-Jκ) to form a transcription-activating complex. This complex can regulate the downstream target genes. Some endogenous regulators of Notch signaling include mammalian Numb (mNumb—a negative regulator) and musashi-1 (Msi-1—an inhibitor of mNumb). Potential therapeutic approaches include blocking Notch ligands, Notch receptors or major Notch downstream targets by siRNA technique, antagonizing antibodies or inhibitors of γ-secretase. Targeting endogenous Notch activator such as Msi-1 has also been attempted. Long T: inhibition; long arrow: activation.
and some downstream targets of Notch signaling (Hes-1, Deltex and NICTD) are present in >75% of CRC tissues compared with normal colonic tissues (L.Qiao and B.C.Y.Wong, unpublished data). Consistent with these findings, Notch signaling genes are not only highly expressed in CRC tissues but are also functionally active (57–59).

Activation of Notch signaling appears to be associated with the development of primary CRC rather than metastatic colon cancers (60), indicating that activation of Notch signaling may be an early event in CRC development. Activation of Notch signaling may also contribute to the treatment resistance of CRC. For example, resistance of CRC cells to Oxiaplatin, a platinum-derived chemotherapeutic drug, was closely correlated with a dose-dependent increase in Notch1 expression and NICD production (5), indicating that cancer cells may adaptively develop mechanisms to overcome therapy-induced cell killing via upregulating Notch signaling. The role of Notch-signaling activation in chemoresistance was further supported by the finding that Numb, a negative regulator of Notch signaling, is downregulated in advanced CRC (5). The mechanisms of constitutive activation of Notch signaling in CRC are not well understood, but like in any other cancers, genetic mutations at the Notch receptor loci may play some roles (61). However, significant mutations of Notch signaling components in CRC have not been reported.

It must be noted that not all Notch signaling components may be involved in the development of colon cancer. By using the Affymetrix U133A microarray analysis, it was found that the expression of Notch1 and Hes-1, but not Notch2, Jagged1 and Dll3, showed progressively increased expression from normal colonic mucosa to primary and metastatic colon cancer (5). In addition, we must note that Notch signaling does not always function as an oncogenic factor. In some cellular systems, Notch signaling may act as a tumor suppressor. This may be largely dependent on cell type, cellular context and the extent by which Notch is activated (62–64). Downregulation of Notch1 was found to be necessary in late stages of human papillomavirus-induced carcinogenesis (65).

In CRC, however, the vast majority of published references indicate that Notch signaling plays an oncogenic role. Thus, inhibition of Notch signaling may be of therapeutic benefit against CRC.

Implications of Notch signaling in colon carcinogenesis and CRC therapy

The role of Notch signaling in colon carcinogenesis may be best implicated by studies using APC-mutant mice, which are known to be highly susceptible for development of multiple colorectal tumors possibly due to activation of β-catenin. Studies in these mice have shown that Notch signaling is highly active in intestinal crypts and in the spontaneous adenomas in APC<sup>Min</sup> mice (45,55). Although Notch pathway inhibitors such as γ-secretase inhibitors were unable to ameliorate the intestinal neoplastic lesions (51), they have been shown to induce differentiation of colonic adenoma cells into goblet cells (55). Blocking Notch signaling by conditional removal of common Notch pathway transcription factor RBP-Jκ in mice or treatment with γ-secretase inhibitor dibenzazepine (DBZ) also caused a complete and rapid conversion of proliferative cells in the intestinal crypts and adenomas into post-mitotic goblet cells (45). Rodilla et al. (55) showed that deletion of a single Jagged1 allele reduced the size of intestinal tumors developed in the intestine of APC<sup>Mut<sup>−/−</sup></sup> mice, and APC<sup>Mut<sup>−/−</sup></sup>/Jag1<sup>+/−</sup> double-mutant mice are less vulnerable to develop intestinal tumors than APC<sup>Mut<sup>−/−</sup></sup>/Jag1<sup>−/−</sup> mice. All these suggested that under the physiological conditions, activation of Notch signaling conferred a growth advantage to colonic tumors in the background of APC mutations. These data have provided good foundation for experimentally testing the therapeutic efficacy of targeting Notch signaling in CRC. Several broad reviews articles have addressed the role of Notch signaling in the treatment of human diseases including cancers (61,66–68).

In targeting Notch signaling in CRC, chemical inhibitors of γ-secretase such as DBZ and Compound E significantly suppressed cell growth in colon cancer cell lines HT29 and HCT116 (44,69).

Intra-peritoneal injection of DBZ significantly inhibited the formation of intestinal adenoma in APC<sup>Mut<sup>−/−</sup></sup> mice (69). γ-Secretase inhibitors are able to sensitize colon cancer cells to chemotherapeutic agent-induced cell killing. For example, treatment of colon cancer cell lines SW480 and LDL-D1 by DAPT (N-[N-3(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine-]<t-butyl ester), Compound E and L-685,458 significantly enhanced taxane-induced mitotic arrest and apoptosis both in vitro and in vivo, although the inhibitors themselves did not have proapoptotic effect (32). Similarly, treatment of colon cancer HCT116 cells with another γ-secretase inhibitor GS934 significantly sensitized the cells to Oxiaplasin- and 5-fluorouracil-induced apoptosis and growth inhibition (5).

Other Notch-targeting approaches have also been attempted in colon cancer. Small interfering RNA (siRNA)-mediated knockdown of Notch receptors, Notch ligands, Notch downstream targets or endogenous Notch regulators has been shown in various in vitro and in vivo studies with various therapeutic effects. For example, siRNA-mediated knockdown of Notch1 was able to suppress the proliferation of HT29 cells (69). Downregulation of masashi-1, a positive regulator of Notch signaling that functions through its interaction and translational repression of mammalian Numb (an inhibitor of Notch signaling) (70) by its specific siRNA, was found not only to markedly inhibit the expressions of Notch1 and NICH but also significantly decrease proliferation, increase apoptosis and markedly retard the growth of xenograft colon tumor in mice (31). Similarly, siRNA-mediated knockdown of MAML-1, an important downstream transcription factor on the Notch pathway, by siRNA in CRC cell lines led to cell death (71).

Some published results, however, suggest that the effect of Notch signaling downregulation may be cell type or treatment specific. For example, downregulation of Notch1, Notch2 and Notch3 was not able to induce apoptosis or sensitize SW480 cells to paclitaxel-induced apoptosis and growth inhibition (32). Our own studies have showed that siRNA-mediated knockdown of Jagged1 was able to inhibit cell proliferation and migration in HCT116 and HT29 cells but were only mildly proapoptotic in these cells.

Targeting Notch has also been achieved by using antagonizing antibodies against Notch receptors or ligands. For example, antibody against DLL4 has been shown to suppress tumor angiogenesis (72,73). One particular aspect needs specific attention in Notch targeting-based experimental therapy for CRC is correct identification of patient populations. Patients whose cancer tissues harbor mutations of FBW7, a gene that encodes an ubiquitin ligase that is responsible for degradation of NICD, may not respond to γ-secretase inhibitors. It has been reported that T-ALL cell lines carrying FBW7 mutations were resistant to γ-secretase inhibitors (74). A pretreatment test of FBW7 mutation status would be helpful to identify the subgroup of patients suitable for γ-secretase inhibitor therapy.

Molecular mechanisms of Notch signaling targeting in CRC

Activation of Notch signaling can upregulate many signaling pathways that favor cell survival. PI3K/AKT signaling, whose activity is upregulated in ~40% of human CRC tissues, possibly as a result of inactivation of PTEN (75), is upregulated by Notch signaling activation (62,76,77). Other signaling pathways that are activated by Notch signaling include c-Myc and EGFR. For example, c-Myc is overexpressed in 70% of colon cancer (78), and a genome wide search showed that c-Myc is a direct target gene of Notch1 in breast cancer and lymphoma (79–82). In a recent study, it was revealed that MAML-1, a specific co-activator for the Notch pathway, can transcriptionally bind to the promoters of cyclin D1 and c-Myc in colon cancer cell lines (71). As cyclin D1 and c-Myc are closely related to cell cycle progression, the anticancer effect of Notch inhibition has been linked to its inhibitory effect on cell cycle progression (5,9,21,62,83).

Constitutive activation of EGFR has been convincingly demonstrated in many tumors and this pathway contributes heavily to uncontrollable cell growth, tumor cell survival as well as resistance to cytotoxic agents (84,85). Up to 80% of colon cancer expresses high
level of \textit{EGFR} (86,87) and 36\% of colon cancer overexpresses both \textit{EGF} and \textit{EGFR} (88). Recent studies have indicated that activation of \textit{Notch} signaling could induce cell proliferation through activation of \textit{EGFR} pathway in breast cancer and gliomas (34,89,90), possibly through a p53-dependnet pathway (90). Whether \textit{Notch} signaling interacts with \textit{EGFR} pathway in CRC remains further verification, but because both \textit{Notch} component genes and \textit{EGFR} are highly expressed in colonic mucosa and CRC tissues, it is possible that these two pathways might have interactions during colonic carcinogenesis.

Nuclear factor-kappaB (\textit{NF-kB}) is one of the most important transcription factors involved in the development of solid tumors including CRC (91–93). Activation of \textit{Notch} signaling has been shown to activate \textit{NF-kB} in several cell types (15,38,94–98). As activation of \textit{Notch} signaling and \textit{NF-kB} is frequently observed in CRC and constitutive activation of \textit{NF-kB} contributes to chemoresistance and treatment failure of cancers, it is possible that \textit{Notch}-mediated activation of \textit{NF-kB} is responsible for treatment failure in colon cancer. Furthermore, activation of \textit{Notch} signaling could upregulate the apoptosis-inhibiting genes including \textit{Bcl-2}, \textit{Bcl-XL} (77) and IAP family members such as \textit{survivin} (6,15).

Inhibition of \textit{KLF4}, a C2H2 zinc-finger containing transcription factor that is highly expressed in the gastrointestinal tract, can also be responsible for the anticancer effect of \textit{Notch} signaling. \textit{KLF4} is necessary to inhibit cell proliferation and maintain the terminal differentiation of goblet cells in the mouse intestine. \textit{APCMin/+} mice with \textit{KLF4} haploinsufficiency showed an increased susceptibility to developing colon cancer (99). Inhibition of proliferation of colon cancer cells by \textit{c-Myc} and \textit{KLF4} haploinsufficiency showed an increased susceptibility to developing colon cancer (99). Inhibition of proliferation of colon cancer cells by \textit{c-Myc} and \textit{KLF4} has been associated with markedly enhanced activity of \textit{KLF4} (44,69).

Blocking \textit{Notch} signaling by a dominant-negative \textit{RBP-Jκ}, an important transcription factor on the pathway of \textit{Notch} activation, led to increased \textit{KLF4} and decreased proliferation (44). Finally, activation of \textit{Notch} signaling suppressed the activity of \textit{transforming growth factor-β}, an important signaling that inhibits cell growth and a tumor suppressor (100–102).

Taken the above mechanisms together, inhibition of \textit{Notch} signaling may affect signaling pathways such as inhibition of \textit{PI3K/AKT} and \textit{EGFR}, inhibition of anti-apoptosis signals such as \textit{Bcl-2} and \textit{Bcl-XL} as well as suppression of transcription factors such as \textit{NF-κB}, \textit{c-Myc} and \textit{KLF4}. All these mechanisms may be separately or cooperatively involved in the tumor suppressive effects of \textit{Notch} inhibition. Figure 2 briefly depicts the \textit{Notch} signaling and its downstream targets or possible interacting genes in CRC.

**Angiogenesis and its regulation by Notch in CRC**

Angiogenesis is an important prerequisite for cancer development. As a solid tumor, the progressive development and subsequent metastasis of CRC are largely dependent on constant nutrient supply by neovascularization. The essential role of angiogenesis in CRC has been well recognized (103). The major drive force of angiogenesis comes from certain angiogenic growth factors derived from tumor tissues, among which vascular endothelial growth factor (\textit{VEGF}) is the most potent one (104,105). It is now known that overexpression of \textit{VEGF} occurs in the vast majority of human solid tumors including CRC (103), and a close correlation between high levels of \textit{VEGF} with angiogenesis, metastasis and poor prognosis has been demonstrated in patients with CRC (106,107). Thus, inhibiting angiogenesis through targeting \textit{VEGF} is now regarded as an important approach for the treatment of CRC. Indeed, the first generation of \textit{VEGF} inhibitor Bevacizumab (Avastin) was demonstrated as an effective anticancer agent in 2003 for patients with metastatic CRC (108) and was later approved by Food and Drug Administration (FDA) of the USA for clinical use in patients with advanced CRC. Other \textit{VEGF}-targeting agents such as Sorafenib (Nexavar) and Sunitinib malate (Sutent) have been subsequently developed and approved for clinical use in some solid tumors such as renal carcinoma and gastrointestinal cancers.

As antiangiogenesis therapy is a promising strategy for CRC, it is necessary to understand how angiogenesis is regulated in this
malignancy. Recent studies have showed that activation of Notch signaling affects multiple aspects of vascular development (73,109–112).

Notch signaling plays an important regulatory role both in the angiogenesis under physiological and pathological conditions. The important roles of Notch signaling in physiological angiogenesis are reflected by some of the previous research findings (i) many Notch signaling components such as Jagged1, Notch1, Notch4 and DLL4 are richly expressed in endothelial cells (113–117); (ii) DLL4 is a strong antiangiogenic factor (118) and mice carrying haploinsufficiency of DLL4 die embryonically due to vascular defects (118,119); (iii) Jagged1 is able to regulate the early cardiovascular development through regulating endothelial and vascular smooth muscle cells (116); (iv) systemic knockouts of Jagged1 and Notch1 or knockout of Notch receptors induce embryonic death with vascular defects (120–122) and (v) endothelium-specific systemic knockouts of Jagged1 also caused severe vascular defects and lead to embryonic lethality (121,122).

Some controversial results have also been published. For example, Notch4-transgenic mice (i.e. contain activated Notch4) exhibit vascular patterning defects (123) and loss of Notch signaling was found to be associated with an increase in endothelial VEGF receptor-2 expression (124). It has recently been reported that the antiangiogenic ligand DLL4 functions in an opposing manner to Jagged1 in the regulation of angiogenesis (117).

Among the Notch-signaling components, DLL4 appears to be the most potent factor in the regulation of new blood vessel formation in many solid tumors (125). DLL4 appears to be an endothelial cell-specific ligand for Notch signaling (116). There is a large body of evidence supporting the role of Notch signaling in tumor angiogenesis. For example, (i) expression of DLL4 is positively correlated with the level of VEGF in some solid tumors such as clear cell carcinoma of the kidney and bladder carcinoma (126,127); (ii) although inhibition of DLL4 caused overgrowth of the tumor vasculature, blockade of Notch signaling via inhibition of DLL4 was shown to inhibit tumor growth, possibly because DLL4 inhibition induced tumor blood vessels are functionally deficient (128) and (iii) Jagged1-expressing cancer cells were able to promote angiogenesis in solid tumors such as breast cancer (129). Jagged1-mediated tumor angiogenesis may be dependent on activation of the mitogen-activated protein kinase-signaling pathway (109,116). Recently, it was reported that TNF-α-induced angiogenesis was achieved through upregulation of Jagged1, further demonstrating a role of Jagged1-mediated Notch activation in regulating angiogenesis (130).

Overall, it is evident that Notch signaling plays a complicated and even controversial role in angiogenesis.

A possible regulatory role of Notch for colon cancer stem cells

In the last few years, a growing body of evidence indicates that tumors may derive from cancer stem cells (CSCs). These cells are not only responsible for tumor initiation, progression and relapse but also may well be responsible for resistance of cancers to conventional therapy. So far, CSCs have been identified in a number of human malignancies, including CRC. Normal colonic stem cells are found near the base of crypts (131) and are believed to have a relatively longer life span compared with the normal intestinal epithelial cell; thus, this special population is potentially exposed to and is able to harbor critical genetic alterations that are ultimately inductive to CRC. Typical human CRC and corresponding metastatic lesions contain heterogenous malignant tissues showing features of various differentiation stages even within an individual tumor, suggesting colon cancers are derived from a stem cell origin (132). In addition, common stem cell markers such as CD133, musashi-1, CD44, EpCAM and CD166 are widely expressed in colon cancer (133–135), further confirming the role of CSCs in CRC development. However, how colon CSCs are regulated is not clear.

Previous studies have suggested that Notch signaling components are richly expressed in the brain CSCs (136,137), indicating activation of Notch signaling may be involved in the regulation of brain CSCs. The role of Notch signaling in the regulation of colon CSCs is, however, not clearly defined. Nevertheless, the fact that Notch component genes are largely localized in the base of colonic crypts (45–48) provides a clue that Notch signaling may also be involved in the regulation of colonic stem cells. Indeed, the essential roles of Notch signaling in the maintenance and differentiation of the colonic mucosa progenitor cells have been suggested by several studies (41–43).

The possible clues that are indicative of an involvement of Notch signaling in regulating colon CSCs are available: (i) stem cell markers are expressed in the intestinal crypts where colonic stem cells reside; (ii) musashi-1 is an important positive regulator for Notch signaling (31,138); (iii) other important molecular pathways involved in the regulation of CSCs such as Wnt, Hedgehog, PI3K and BMP pathways may require cooperation with Notch pathway during stem cell proliferation (132,139) and (iv) activation of Notch signaling leads to activation of Bmi-1, a crucial stem cell regulator. These results are in support of a potential role of stem cells in CRC formation, and Notch is probably an important regulator for colonic stem cells. More studies are needed to clarify the regulatory role and molecular mechanisms of Notch signaling in colon CSCs.

A possible interaction between peroxisome proliferator-activated receptor gamma and Notch signaling in colon carcinogenesis and therapeutic implications

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor expressed in normal and malignant colonic tissues (140). Activation of PPARγ by its synthetic or natural ligands induces cell differentiation, cell cycle arrest, growth inhibition and apoptosis in colon cancer (140–146). In addition, activation of PPARγ coupled with other approaches such as downregulation of XIAP was found to have therapeutic effect against colon cancer (144–146). In murine preadipocytes, the expression of PPARγ requires the normal function of Notch1 (95,147). In 3T3 L1 preadipocytes, activation of Notch1 leads to activation of PPARγ (148). Similarly, activation of Notch signaling in keratinocytes by Jagged1 peptide led to a rapid increase in the expression of PPARγ (95), possibly through Jagged1-induced NF-κB activation. During adipogenesis, activation of Notch signaling was found to induce PPARγ expression via transcriptional activation of Hes-1 (149). Whether Notch signaling is interacting with PPARγ in colon cancer warrants further investigation.

Future directions

Like in many other cancers, there are many unsolved mysteries in the role of Notch signaling in CRC. Although Notch signaling is constitutively active in CRC, and an association between Notch activation and cancer development has been well documented, what is not clearly defined so far is whether Notch signaling activation is the cause or effect of colon carcinogenesis and whether it is just an essential factor for tumor growth once the malignant changes are initiated. More detailed studies are needed to unveil this mystery.

The literature on the potential therapeutic benefit of Notch targeting in cancers including CRC is rapidly expanding. The efficacy of Notch targeting varies with cancers. Even in the same cancer, various effects of targeting Notch signaling have been reported. Therefore, in addition to more extensive basic studies on the molecular mechanisms of how Notch signaling regulates the differentiation, growth and apoptosis of differentiated cancer cells and CSCs, investigations to improve the gene targeting or delivery efficacy are highly necessary. In terms of experimental therapy, future studies should not only be focused on targeting Notch signaling in differentiated cancer cells but should also be attempted in CSCs. Development and validation of reliable CSC markers are a necessary prerequisite in this aspect. Once more robust experimental data are available, it is pivotal to move into various stages of clinical trials to test the benefits and adverse effects of Notch-targeting agents in cancers.
In terms of targeting Notch signaling by chemical inhibition of γ-Secretase, the specificity of the current inhibitors on tumor cells is yet to be improved. Systemic use of the currently available six subgroups of γ-secretase inhibitor is associated with various adverse effects (150), possibly because of the fact that γ-secretase targets >30 physiologically important transmembrane proteins (151).

For example, systemic use of LY411,575 in mice was associated with a significant loss of immature cells in the thymus (52) and an impairment of the development of lymphoid cells (152), as well as a damaged regenerative ability of colonic epithelial cells (52). Other severe gastrointestinal tract toxicity such as massive diarrhea as a result of a marked increase in goblet cell differentiation has also been reported (150,153,154). These unwanted side effects may be the major obstacles preventing γ-secretase inhibitors from entering into clinical trials for CRC. A novel Notch inhibitor MK-0752 is currently under a phase I clinical trial for the treatment of T-ALL/lymphoma and advanced breast cancer (http://www.clinicaltrials.gov/ct2/show/NCT00106145?term=Notch&rank=1, ClinicalTrials Identifier ID: NCT00106145) but not yet for CRC (155). Hopefully, more intensive basic research on the role of Notch signaling in CRC and a more specific generation of γ-secretase inhibitors that, ideally, target cancer cells and CSCs will move the pace faster to enter into clinical trials of γ-secretase inhibitors in CRC.

Last but not least, detailed identification and functional analysis of the downstream target genes of Notch signaling are warranted to search for even more efficient target genes.

Summary and conclusions

Notch signaling components are constitutively active and overexpressed in CRC. Direct downregulation of Notch ligands, Notch receptors, NICD and Notch downstream targets or via chemical inhibition of γ-secretase showed therapeutic effects. Overall, inhibition of Notch signaling in CRC is able to suppress the cell growth and sensitize cancer cells to treatment-induced apoptosis. The mechanisms of Notch-signaling inhibition in cancer therapy are multiple and include but may not be limited to the following: (i) inhibition of cell proliferation associated genes, such as c-Myc, P13K/AKT, EGF/EGFR pathway genes and NF-κB pathway; (ii) inhibition of anti-apoptotic genes; (iii) inhibition of angiogenesis; (iv) inhibition of transforming growth factor-β pathway and (v) inhibition or redirection of colon CSCs. We can tentatively conclude that inhibition of Notch signaling is a potentially novel therapeutic target for CRC.

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