Notch signaling in gastrointestinal tract (Review)

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Abstract. Notch signaling is one of key pathways constituting the stem cell signaling network. DLL1, DLL3, DLL4, JAG1 and JAG2 with DSL domain are typical Notch ligands, while DNER, F3/Contactin and NB-3 without DSL domain are atypical Notch ligands. Notch-ligand binding to NOTCH1, NOTCH2, NOTCH3 or NOTCH4 receptor induces the receptor proteolysis by metalloprotease and γ-secretase to release Notch intracellular domain (NICD). Typical Notch ligands transduce signals to the CSL-NICD-Mastermind complex for the maintenance of stem or progenitor (transit-amplifying) cells through transcriptional activation of *HES1, HES5, HES7, HEY1, HEY2* and *HEYL* genes, and also to the NF-κB-NICD complex for the augmentation of NF-κB signaling. Atypical Notch ligands transduce signals to the CSL-NICD-Deltex complex for the differentiation of progenitor cells through *MAG* transcriptional activation. Notch signals are transduced to the canonical pathway (CSL-NICD-Mastermind signaling cascade) or the non-canonical pathway (NF-κB-NICD and CSL-NICD-Deltex signaling cascades) based on the expression profile of Notch ligands, Notch receptors, and Notch signaling modifiers. Canonical Notch signaling is activated in the stem or progenitor domain of gastrointestinal epithelium, such as basal layer in esophagus and lower part of the crypt in colon. Notch signaling to inhibit secretory cell differentiation is oncogenic in gastric cancer and colorectal cancer, while Notch signaling to promote keratinocyte differentiation is anti-oncogenic in esophageal squamous cell carcinoma (SCC). Single nucleotide polymorphism (SNP), epigenetic change, and genetic alteration of genes encoding Notch signaling-associated molecules will be utilized as biomarkers for gastrointestinal cancer. γ-Secretase inhibitors, functioning as Notch signaling inhibitors, will be applied as anti-cancer drugs for gastric cancer and colorectal cancer.

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1. Overview of Notch signaling landscape

Overview. Notch signaling pathway is implicated in self-renewal of stem cells, cell-fate determination of progenitor cells, and terminal differentiation of proliferating cells (1-6). Notch-ligand binding induces the cleavage of Notch receptor by metalloprotease and γ-secretase to release Notch intra-cellular domain (NICD). Canonical Notch signaling to CSL-NICD-Mastermind complex inhibits the differentiation of stem cells or progenitor (transit-amplifying) cells, while non-canonical Notch signaling to CSL-NICD-Deltex complex promotes the differentiation of progenitor cells.

Notch ligand. Delta homologs, DLL1, DLL3 and DLL4, are type I transmembrane proteins with extracellular N-terminal DSL domain and EGF-like repeats (6,7). Serrate homologs, JAG1 and JAG2, are type I transmembrane proteins with extra-cellular N-terminal DSL domain, EGF-like repeats, cysteine-rich domain and von Willebrand factor type C domain (8-10). DNER is a type I transmembrane protein with extracellular EGF-like repeats (11). F3/Contactin and NB-3 are glycosyl phosphatidylinositol (GPI)-anchored proteins with immuno-globulin-like domains and EGF-like repeats (12,13). DLL1, DLL3, DLL4, JAG1 and JAG2 with DSL domain are typical Notch ligands with higher affinity, while DNER, F3/Contactin and NB-3 without DSL domain are atypical Notch ligands with lower affinity.

Notch receptor. Notch family receptors, NOTCH1, NOTCH2, NOTCH3 and NOTCH4 are type I transmembrane proteins with extracellular EGF-like repeats, Lin12/Notch repeat region, cytolasmic RAM23 domain, Ankyrin repeats and PEST domain (14,15). Notch is cleaved at the S1 site by a Furin-like convertase to generate the mature heterodimeric receptor. On ligand-binding, Notch receptor is further cleaved at the S2 site by metalloprotease tumor necrosis factor-α converting enzyme, and at the S3 site by γ-secretase complex to give rise to NICD. Notch family receptors
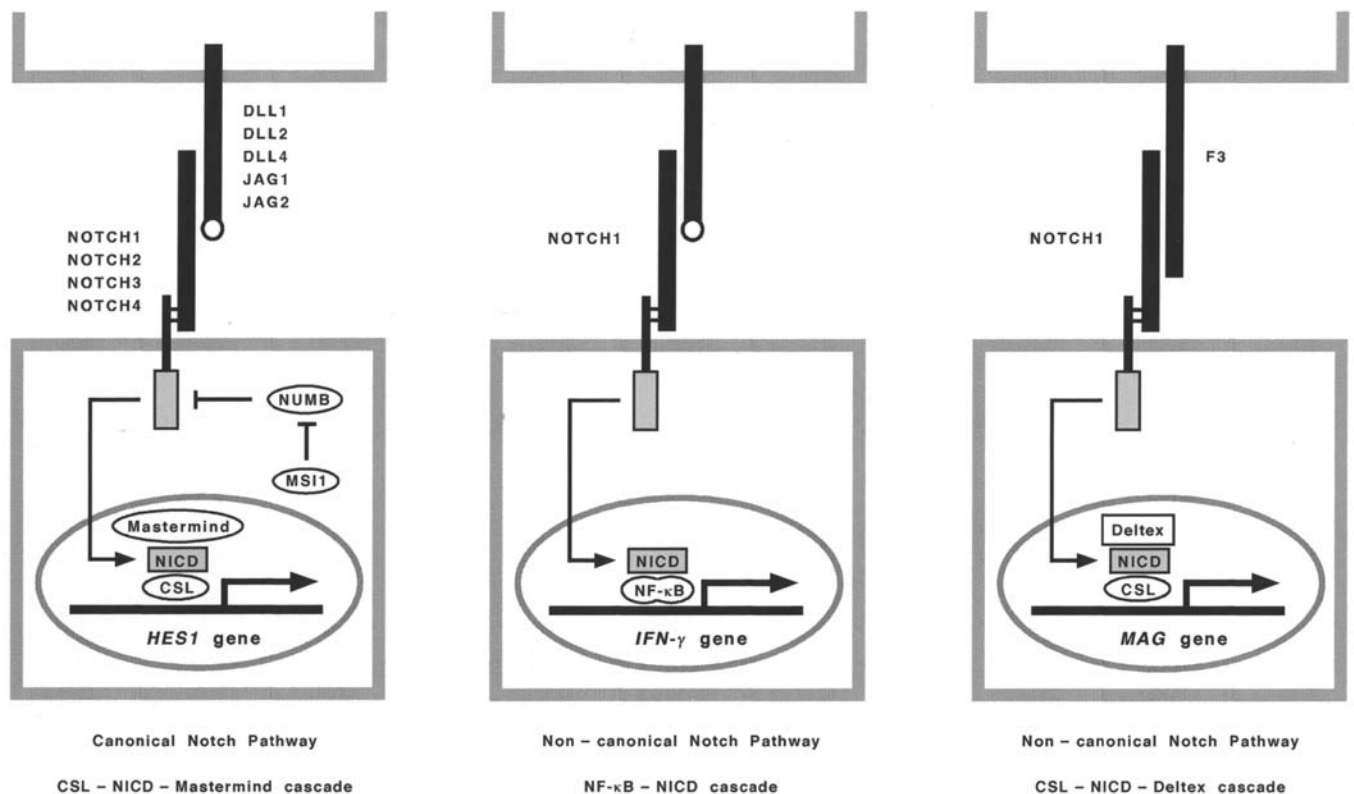
Figure 1. Notch signaling pathway. Notch signals are transduced to the canonical pathway (CSL-NICD-Mastermind signaling cascade) or the non-canonical pathway (NF-κB-NICD and CSL-NICD-Deltex signaling cascades) based on the expression profile of Notch ligands, Notch receptors, and Notch signaling modifiers. (Left), Binding of DLL1, DLL3, DLL4, JAG1 and JAG2 with DSL domain (open circle) to Notch receptor leads to the release of Notch intracellular domain (NICD) for transcriptional activation of Notch target genes, such as *HES1*, through the CSL-NICD-Mastermind complex. (Middle), Notch signaling activation also leads to transcriptional activation of NF-κB target genes, such as *IFN-γ* through direct association between NICD and NF-κB. (Right), Binding of DNER, F3/Contactin and NB-3 without DSL domain to Notch receptor leads to transcriptional activation of differentiation-associated genes, such as *MAG*, through the CSL-NICD-Deltex complex.

transduce signals of DLL-type ligands, JAG-type ligands, and atypical ligands in the context-dependent manner.

Canonical Notch signaling. NICD, released after ligand-binding to Notch receptor, is translocated into the nucleus to associate with CSL (RBPSUH) transcription factor (5,16). CSL-NICD complex is activated by Mastermind family co-activators MAML1, MAML2 and MAML3 (17) for transcriptional activation of *HES1, HES5, HES7, HEY1, HEY2* and *HEYL* genes, encoding bHLH/orange domain transcriptional repressors (4,18-20). Because HES/HEY family members repress the transcription of tissue specific transcription factors, Notch signaling to the CSL-NICD-Mastermind complex results in the maintenance of stem or progenitor (transit-amplifying) cells through the inhibition of differentiation (Fig. 1, left). Signaling transduction from typical Notch ligands to the CSL-NICD-Mastermind cascade is also known as the 'canonical' Notch signaling pathway.

Non-canonical Notch signaling. NICD also interacts with p50 or c-Rel in the nucleus to enhance the NF-κB activity (21). Notch signaling to the NF-κB-NICD complex augments transcriptional activation of NF-κB target gene, such as *IFN-γ* (Fig. 1, middle). On the other hand, binding of atypical Notch ligands to Notch receptor in the absence of typical Notch ligands gives rise to the association of CSL, NICD and

Deltex (11-13). Because Deltex affects the DNA-binding preferentiality of CSL, the CSL-NICD-Deltex complex activates the transcription of unique target genes, such as *MAG* (Fig. 1, right). Because MAG is a tissue specific transcription factor to induce terminal differentiation, atypical Notch signaling to the CSL-NICD-Deltex complex results in differentiation of progenitor cells. Notch signaling to the NF-κB-NICD cascade and the CSL-NICD-Deltex cascade are called the 'non-canonical' Notch signaling (Fig. 1).

Notch signaling regulator. *Drosophila* Neuralized and Mindbomb are RING-type E3 ubiquitin ligases inducing ubiquitination and internalization of Delta, which results in canonical Notch signaling activation in the neighboring cells (22,23). NEURL1/NEURL and NEURL2 are human homologs of *Drosophila* Neural, while MIB1 and MIB2 are human homologs of *Drosophila* Mindbomb. Mouse Mib1 and Mib2 interact with *Xenopus* Delta, and Mib2 induces Delta ubiquitination. However, effects of human NEURL1, NEURL2, MIB1 and MIB2 on DLL-dependent Notch signaling remain to be elucidated (24). Lunatic Fringe (LFNG), Manic Fringe (MFNG) and Radical Fringe (RFNG) are Notch glycosyl-transferase, which enhance Notch binding to DLL rather than JAG (25).

NUMB and NUMBL are docking proteins with phospho-tyrosine-binding (PTB) domain and SH3-binding proline-rich
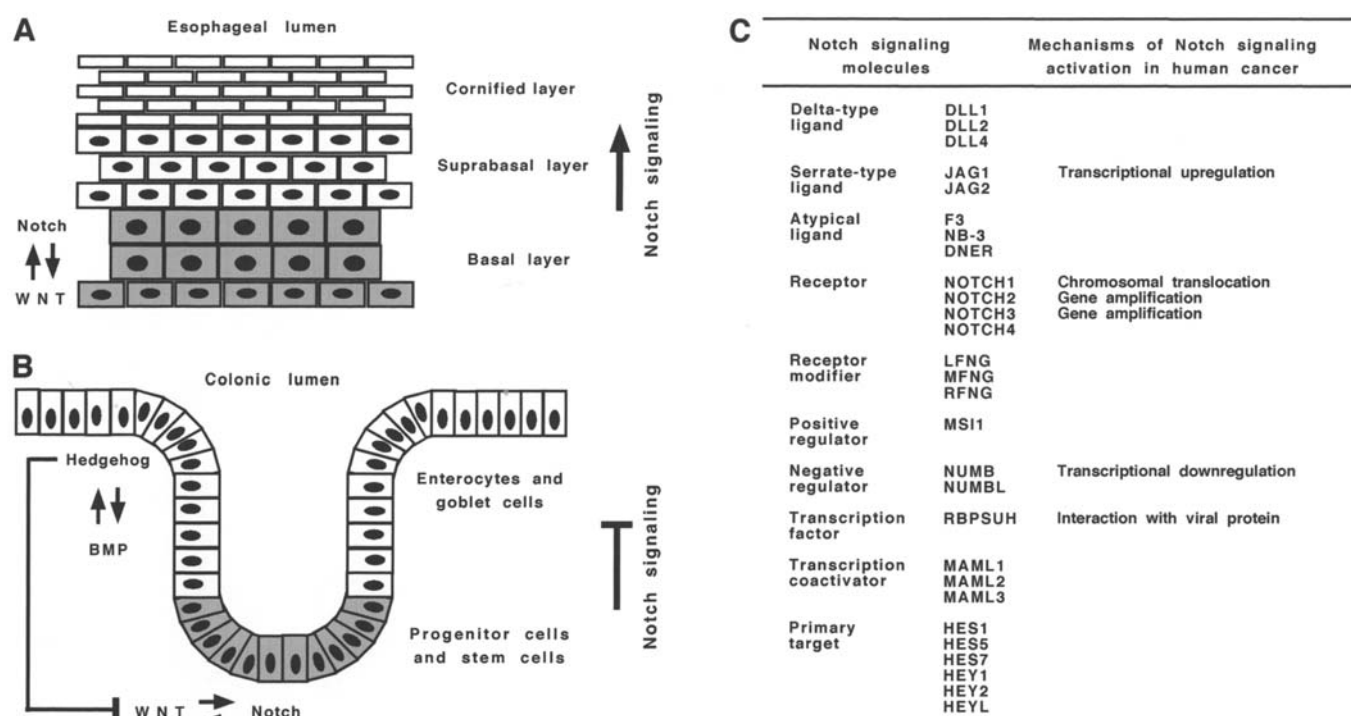
Figure 2. Notch signaling in the gastrointestinal tract and human cancer. (A), Esophagus. Notch and WNT signals are activated in the stem and progenitor cells (gray) in the basal layer. (B), Colon. Notch and WNT signals are activated in the stem and progenitor cells (gray) in the lower part of the crypt. (C), Human Notch signaling molecules and mechanisms of Notch signaling activation in human cancer. Notch signaling is aberrantly activated in a variety of human cancer due to chromosomal translocation of *NOTCH1, NOTCH3*, gene amplification of *NOTCH2, NOTCH3*, upregulation of *JAG1, NOTCH1*, and down-regulation of *NUMB*.

region, functioning as cytoplasmic Notch signaling inhibitors (26-28). MSI1, binding to *NUMB* mRNA, represses NUMB translation to activate Notch signaling (29). FBXW7/SEL10 with F-box and WD-repeats are NICD-binding protein, functioning as a component of ubiquitin ligase to degrade nuclear NICD (30).

2. Notch signaling network

Stem cell signaling network. Notch, WNT, FGF, Hedgehog and BMP signaling pathways constitute the stem cell signaling network regulating the balance of self-renewal, proliferation and differentiation among stem and progenitor (transit-amplifying) cell population (33-42). Notch signaling plays a key role in the stem cell signaling network through the interaction with other signaling pathways. Notch signaling interaction with WNT signaling pathway will be further described.

WNT signaling pathway. WNT signals are context-dependently transduced to the canonical and non-canonical WNT signaling pathways (43-45). Canonical WNT signals are transduced through Frizzled family receptor and LRP5/LRP6 co-receptor to the ß-catenin signaling cascade for cell-fate determination. *JAG1* and *NUMB* genes are predicted as evolutionarily conserved targets of the canonical WNT signaling pathway (10,28). JAG1 expressed on progenitor cells activates canonical Notch signaling pathway in the neighboring stem or progenitor cells. JAG1-dependent canonical Notch signaling pathway maintains the homeostasis of stem and progenitor cells synergistically with the canonical WNT signaling pathway.

3. Notch signaling in normal gastrointestinal tract

Esophagus. Esophagus is lined with stratified squamous epithelium, consisting of basal layer, suprabasal layer and cornified layer. Esophageal epithelial stem cells, located at the bottom of the basal layer, give rise to neighboring progenitor cells within the basal layer. Progenitor cells are differentiated to keratinocytes in the suprabasal layer, and then to de-nucleated cells in the cornified layer. *Jag1, Jag2, Notch1* and *Notch2* are highly expressed in the basal layer of mouse esophagus, and weakly expressed in the suprabasal layer (46). *JAG1, DLL1, NOTCH1, NOTCH2* and *NOTCH3* are highly expressed in the basal layer of human skin epidermis, and weakly expressed in the suprabasal layer (47). Together, these facts indicate that canonical Notch signaling is activated mainly in the basal layer to maintain the balance of stem and progenitor cells (Fig. 2A).

Stomach. Fundic glands occupy fundus and body, corresponding to the proximal two thirds of the stomach. Fundic gland consists of the surface pit area with mucus cells, the isthmus/neck area with stem cells, progenitor cells and parietal cells, and the base area with parietal cells, chief cells and entero-endocrine cells. Stem cells in the isthmus/neck area give rise to progenitor cells for mucous-, parietal- and chief-cell lineage. *Jag1, Jag2, Dll1, Notch1, Notch2* and *Notch3* are expressed in mouse fundic mucosa (46). *NOTCH1, NOTCH2, NOTCH3* and *HES1* are expressed in human gastric mucosa (48).

Colon. Colon epithelium consists of a flat absorptive surface and the crypt. Stem and progenitor cells located around the

lower part of the crypt (or proliferating crypt) give rise to enterocytes, goblet cells, and Paneth cells. Among *Jag1, Jag2, Dll1, Notch1* and *Notch3* expressed in colon, *Jag1, Jag2* and *Notch1* are expressed in the lower half of the crypts (46). Canonical Notch signaling activation leads to Hes1 up-regulation, and Atoh1/Hath1/Math1 down-regulation (49). Atoh1 bHLH transcription factor induces differentiation to the goblet cells. Canonical Notch signaling in colon is implicated in the maintenance of stem cells and progenitor cells, and also in the inhibition of goblet cell differentiation (Fig. 2B).

4. Notch signaling in gastrointestinal cancer

General view of Notch signaling in carcinogenesis. Notch signaling is aberrantly activated due to chromosomal trans-location of *NOTCH1* in acute lymphoblastic leukemia (14), amplification and overexpression of *NOTCH2* in medullo-blastoma (50), chromosomal translocation of *NOTCH3* in lung cancer (51), amplification and overexpression of *NOTCH3* in ovarian cancer (52), and upregulation of *JAG1/NOTCH1* or down-regulation of *NUMB* in breast cancer (53,54) (Fig. 2C). Together these facts indicate that Notch signaling is oncogenic in a variety of human tumors.

On the other hand, Notch signaling is anti-oncogenic for squamous cell carcinoma (SCC) of skin and cervical uterus and also for basal cell carcinoma (BCC) of skin (2,47,55), partially due to the interference with canonical WNT signaling.

Esophageal cancer. Esophageal cancer is classified into SCC, adenocarcinoma, and others. Alcohol drinking and tobacco smoking are risk factors for esophageal SCC, which is the major type of esophageal cancer in developing countries and Japan. *Helicobacter pylori* eradication and Barret's esophagus are risk factors for esophageal adenocarcinoma, incidence of which is gradually increasing in developed countries. Oral SCC, head and neck SCC, and esophageal SCC are similar types of cancer. Although *JAG1* and *RBPSUH* are rarely amplified and overexpressed in oral SCC (56), *JAG1* expression in head and neck SCC is significantly lower than in adjacent non-cancerous tissue (57). Canonical WNT signaling is activated in SCC, while Notch signaling is inactivated in SCC. Because Notch signaling promotes the terminal differentiation of keratinocytes (Fig. 2A), Notch signaling is anti-oncogenic in esophageal SCC.

Gastric cancer. *NOTCH1, NOTCH2* and *NOTCH3* were expressed in all of the eight gastric cancer cell lines, *HES1* in seven gastric cancer cell lines, and *ATOH1* and *MUC6* in three gastric cancer cell lines (48). *HES1* is a target gene of the canonical Notch signaling pathway, and HES1 represses transcription of *ATOH1*. MUC6 is a marker of chief-cell lineage. Together, these facts indicate that canonical Notch signaling pathway to inhibit chief cell differentiation is frequently activated in gastric cancer.

Colorectal cancer. *NOTCH1, NOTCH2,* and *NOTCH3* were up-regulated in colon cancer, while *ATOH1* expression was down-regulated in colorectal cancer (49). Because Notch signaling inhibits the terminal differentiation of goblet cells

in colorectal mucosa (Fig. 2B), Notch signaling is oncogenic in colorectal cancer.

5. Clinical application

Diagnostics and prognostics. High-throughput technologies and bioinformatics supervised by human intelligence is the driving force for the pharmacogenomics and pharmaco-genetics in the post-genome era (58). Single nucleotide poly-morphism (SNP), epigenetic change, and genetic alteration of genes encoding Notch signaling-associated molecules will be utilized as biomarkers for gastrointestinal cancer.

Preventive and therapeutics. γ-Secretase inhibitors, such as LY450139 and LY411,575, block the S3 cleavage of Notch receptors to inhibit Notch signaling activation (59,60). Despite the side effects, such as thymus atrophy and intestinal goblet cell hyperplasia, γ-secretase inhibitors are promising anti-cancer drugs. Therefore, γ-secretase inhibitors will be applied as anti-cancer drugs for human cancer, such as gastric cancer and colorectal cancer.

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