

Notch signaling pathway is activated dynamically during evolution playing significant role in cell fate determination and differentiation. It has been known that alterations of this pathway may lead to human malignancies, including gastric cancer. Despite a decline in the overall incidence, this disease still remains an important global health problem. Therefore, a better understanding of the molecular alterations underlying gastric cancer may contribute to the development of rationally designed molecular targeted therapies. It has been reported that Notch1 receptor could become a prognostic marker of gastric cancer and novel target for gastric cancer therapy. Among the novel and targeted approaches for the treatment of gastric cancer is also the process of Notch receptors regulation by specific microRNA. γ -secretase inhibitors are also taken into consideration.

Key words: gastric cancer, Notch, miR-34, COX-2, γ -secretase inhibitors.

Role of Notch signaling pathway in gastric cancer pathogenesis

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Introduction

Despite a decline in the overall incidence, gastric cancer still remains an important global health problem. About 95% of gastric cancers are caused by adenocarcinoma originating from the glandular cells of the stomach lining [1]. According to Lauren's classification there are two main types of gastric adenocarcinoma: intestinal and diffuse [2]. The intestinal type of gastric cancer is characterized by cohesive neoplastic cells forming gland-like tubular structures and is closely associated with environmental and dietary risk factors such as *Helicobacter pylori* infection and high-salt diet. The well-known and accepted paradigm for pathogenesis of this type of gastric cancer is a multistep progression from chronic gastritis to gastric atrophy to intestinal metaplasia to dysplasia [2]. The histology of diffuse gastric cancer is characterized by poorly differentiated cells and no glandular structures. It seems that the major etiologic risk factor is also *H. pylori* infection [2].

In westernized countries, a large number of gastric cancer patients are diagnosed when the tumor is at an unresectable stage. Currently, the only solution for these patients is systemic chemotherapy which prolongs survival without quality of life compromise. Unfortunately, survival of patients with advanced gastric cancer treated with palliative chemotherapy remains low. Therefore, a better understanding of the molecular alterations underlying gastric cancer pathogenesis is important from the clinical point of view. It may contribute to development of the rationally designed molecular targeted therapies, which interfere with the multiple signaling pathways involved in cancer cell biology [3–7]. One of these pathways – the Notch signaling pathway – is activated dynamically during evolution and plays a crucial role in the fate of cell differentiation during embryonic development. On the other hand, alterations of this pathway may lead to abnormalities including malignant diseases, e.g. gastric cancer [8].

In this paper we review the role of the Notch signaling pathway in gastric cancer pathogenesis.

The Notch signaling pathway

The Notch pathway is an evolutionarily conserved cell signaling mechanism that participates in many cellular processes including proliferation, differentiation, apoptosis and stem cell maintenance [8] (Fig. 1). There are four Notch receptors: Notch1, 2, 3 and 4. Each of them is synthesized as a precursor form composed of extracellular, transmembrane and intracellular domains. Within the Golgi apparatus, the precursor Notch protein is cleaved by a furin-like convertase to generate two subunits. One subunit contains most of the extracellular domain and the second subunit consists of the rest of the extracellular and transmembrane domains. The Notch ligand family comprises five members: Jagged1/2 and Delta-like 1/3/4 (DLL1/3/4), which are also single-

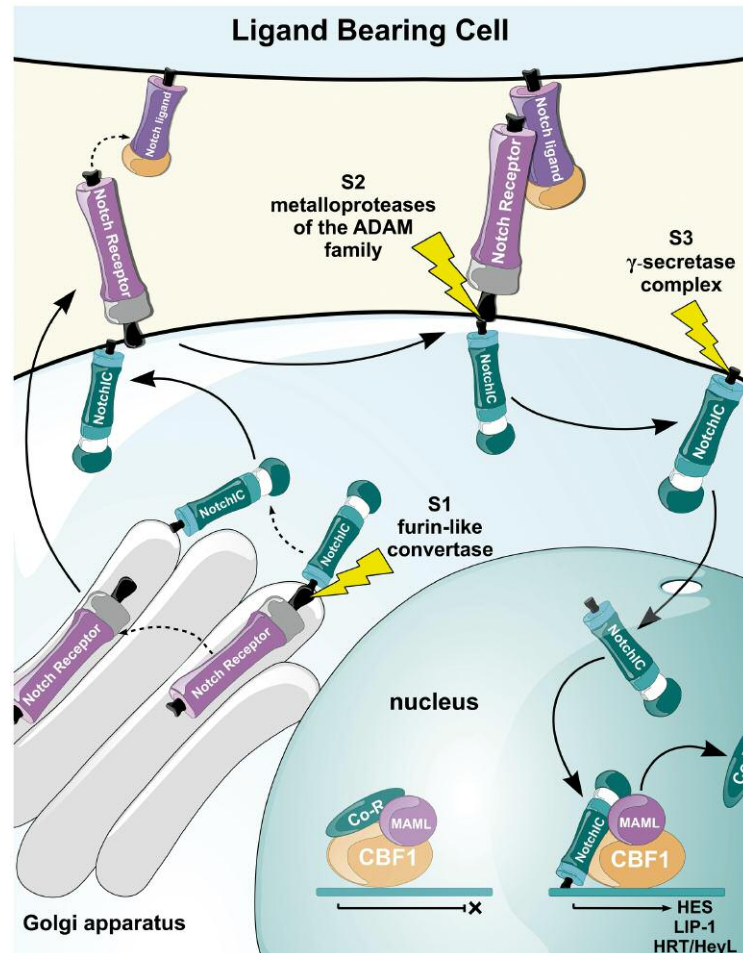


Fig. 1. Notch signaling pathway – details in the text

pass type I transmembrane proteins. The extracellular domain of the Notch receptor has been shown to contain 36 EGF-like repeats [8, 9]. Ligand binding to EGF-like repeats unfolds the negative regulatory region (NRR) permitting the next cleavage by metalloproteases of the ADAM family [8]. During the next step, γ -secretase complex executes an intramembrane cleavage releasing the Notch intracellular domain (NotchIC or NICD) which undergoes translocation to the nucleus [10]. It has been reported that for activation of Notch signaling the Mastermind-like family of proteins (MAML1/2/3) are required. MAML forms a ternary complex with CBF1-NotchIC via direct interaction with NotchIC. Then, the ternary complex composed of CBF1-NotchIC-MAML acts as a transcriptional activator, resulting in Notch target gene transcription. Among the primary targets there are several genes belonging to the basic helix-loop-helix (bHLH) family. Following Notch activation at least two families of bHLH proteins are induced: the Hairy/Enhancer-of-Split (HES) family and the Hairy-Related Transcription factor (HRT) family, which are known to be transcriptional repressors [11].

Although a great number of Notch cellular responses occur as a result of activation of the canonical Notch pathway described above, there are other proteins that may also act as Notch ligands and trigger Notch induction. In this non-canonical pathway other transmembrane proteins are involved. It is worth noting that these proteins have EGF-like

repeats too. Among them we may list Dner, F3/contactin-1 and NB-3/contactin-6. However, these Notch ligands bind Notch receptors with less affinity than the conventional Notch ligands because they do not have a DSL region in their structure [12].

The oncogenic role of the Notch signaling pathway in gastric cancer pathogenesis

The latest studies have revealed that in normal gastric mucosa Notch signaling is involved in the process of differentiation of gastric epithelium into foveolar glands. The results of these studies have demonstrated that expression of Notch1, Notch3, Jagged1, Jagged2 and Hes1 was detected in the isthmus area of normal mucosa where putative gastric stem cells are found [13] (Table 1). It should be noted here that Notch signaling is associated with glandular differentiation not only of normal gastric mucosa but also of gastric carcinoma cells. Notch receptors, e.g. Notch1, Notch2 and Notch3, and Notch ligands such as Jagged1 and Jagged2 have been detected in samples of human gastric cancer tissues too [13]. For example, expression of Notch1 appears in both premalignant and cancer tissues. It is especially observed in samples of intestinal metaplasia and well-differentiated intestinal type of gastric cancer. Thus it is reasonable to consider that Notch1 may play a crucial role in both promoting metaplastic tran-

Table 1. Notch expression in normal gastric mucosa

Notch receptors and Notch ligands	Tissue-specific expression	The role in gastric histology
Notch1 Notch2 Jagged1 Jagged2	isthmus area of normal mucosa where putative gastric stem cells are found	differentiation of gastric epithelium into foveolar glands

Table 2. Notch expression in different types of gastric cancer

Notch receptors and Notch ligands	Type of gastric cancer	The role of Notch proteins in gastric cancer pathogenesis
Notch1	pre-malignant and cancer tissue: intestinal metaplasia and well differentiated intestinal type of gastric cancer	crucial role in promoting metaplastic transition of gastric epithelial cells and in maintaining constant proliferation of intestinalized epithelial cells
Notch2	intestinal and diffuse type	–
DLL1	diffuse and mixed type	–
Notch3 Jagged	intestinal type	–

sition of gastric epithelial cells and in maintaining constant proliferation of intestinalized epithelial cells [14, 15]. The activated form of the Notch1 receptor has promoted colony-forming ability and xenografted tumor growth of human stomach adenocarcinoma SC-M1 cells [16]. Piazzini *et al.* have analyzed the expression of Notch1 and its ligand DLL1 in eight gastric cancer cell lines. It is worth noting that DLL1 expression was not detected in KATOIII, SNU601, SNU719 or AGS cells, which was probably associated with promoter hypermethylation. In contrast, 5-aza-2'-deoxycytidine caused up-regulation of DLL1. The increase in DLL1 expression was correlated with activation of Notch1 signaling, with an increase in cleaved NICD domain and Hes1, and also with down-regulation in Hath1. Moreover, in gastric cancer patients a strong correlation between expression of DLL1 and Hes1 has been observed. It is also worth noting that DLL1 methylation and Hath1 expression were associated with the diffuse and mixed type of gastric cancer. What is important, none of the samples from INS-GAS mice infected with *H. pylori*, a model of the intestinal type of gastric cancerogenesis, demonstrated promoter methylation of DLL1 [16]. In contrast, expression of Notch3 and Jagged2 is connected with the intestinal type of gastric cancer and better histological differentiation [13] (Table 2). Paradoxically, patients with over-expression of Notch3 and Jagged2 genes have shown a better outcome than those with cancers demonstrating lower expression. Why? Kang *et al.* thought that the first effects of Notch signaling may take place when cells undergo malignant transformations. During the second step, cancer cells escape from an undifferentiated progenitor state to become differentiated cells, followed by cell death [13]. Yeah *et al.* revealed that another ligand, Jagged1, is also connected with gastric cancer pathogenesis. Expression of this protein was clearly correlated with aggressiveness, which means that patients with Jagged1 expression had a poor survival rate [17]. Among other signaling pathways involved in gastric cancer progression STAT3 and Twist pathways are worthy of consideration. In SC-M1 cells Twist and phosphorylated STAT3 levels were promoted by the activated Notch1 receptor. Moreover, overexpression of N1IC enhanced the interaction

between nuclear STAT3 and Twist promoter in cancer cells. Colony formation, migration and invasion abilities of SC-M1, AGS and KATOIII cells were also promoted by N1IC by the way of STAT3 phosphorylation and Twist expression. It should also be noted that the N1IC-promoted tumor growth and lung metastasis of SC-M1 cells in mice were suppressed by the STAT3 inhibitor JSI-124 and Twist knockdown [18]. In endoscopic biopsies of gastric noncancerous mucosa with mild inflammatory changes Notch2 expression and its translocation to the nucleus remain at a very low level. In gastric cancer lines it became frequent, which may suggest a correlation of Notch2 expression and its proteolytic activation with pathogenesis of gastric cancer [14]. It has been reported that constitutive expression of Notch2 intracellular domain (N2IC) impacts on migration, invasion, colony formation and wound-healing abilities of SC-M1 cells. These mentioned abilities are inhibited by Notch2 knockdown. In contrast, a study with the use of AGS and AZ521 cancer cells demonstrated that knockdown of Notch2 may be associated with cancer progression [19]. The importance of the Notch signaling pathway in the growth and survival of Notch2-activating gastric cancer cells was evaluated by cleavage inhibition of Notch receptors with two γ -secretase inhibitors (GSIs), L685,458 and DAPT [13]. As revealed by the studies, the GSIs may inhibit cell growth and induce apoptosis in such cancers as hepatoma, breast cancer, pancreatic cancer and myeloma [20–22]. In this context it should be emphasized that the Notch signaling pathway widely participates in cellular physiology. Therefore, it is likely that inactivation of γ -secretases may lead to pathological dysfunction of different tissues and organs. Moreover, GSIs do not target the proteins connected with Notch signaling only. It must be mentioned that GSIs may also target proteases and therefore may have widespread adverse effects *in vivo* because proteases are implicated in a wide array of cellular processes [20, 23]. The studies of Sun *et al.* have revealed that although Hes1 expression and its nuclear translocation are inhibited by GSIs, both gastric cancer cells AGS and BGC823 showed neither growth arrest nor cell death. This suggests that Notch2 signaling may not be the crucial molecular machinery for the

maintenance and survival of gastric cancer cells. Probably, other powerful signaling pathways may compensate Notch signaling when its activity is suppressed. In this context, simply blocking Notch signaling may not achieve the promising inhibitory outcome in an experimental system of gastric cancer [14].

Activation of the Notch1 signaling pathway could promote progression of gastric cancer at least in part through cyclooxygenase-2 (COX-2) [24, 17]. The colony forming, migration, and invasion abilities enhanced by N1IC were suppressed in SC-M1 cells after treatment with the COX-2 inhibitor NS-398 or COX-2 knockdown. These processes were restored by prostaglandin PGE2 or exogenous COX-2 [17]. Murata *et al.* have demonstrated that COX-2 overexpression in gastric cancer is highly correlated with tumor invasion into the lymphatic vessels in the gastric wall and metastasis to the lymph nodes [25]. Fosslien *et al.* reported that COX-2 produced both by cancer cells and by cells from the tumor microenvironment contribute to new vessel formation. In other words, it may have an impact on the process of tumor angiogenesis [26]. Therefore, it may explain how selective COX-2 inhibition reduces tumor growth, where the tumor COX-2 gene has been silenced by methylation [24]. The last studies of Tseng *et al.* have revealed that the active intracellular domain of Notch2 (N2IC) can bind to COX-2 promoter and may induce COX-2 expression through a CBF1-dependent manner in SC-M1 cells [19]. The capacity of N2IC to enhance the tumor progression in SC-M1 cells may be inhibited by COX-2 knockdown. Another possibility to enhance tumor progression is treatment with NS-398, which is a known COX-2 inhibitor [19].

Ji *et al.* suggest that regulation of the Notch receptors by specific microRNA could become a novel approach for gastric cancer treatment. Their studies have demonstrated that miR-34 restoration in gastric cancer Kato III cells reduces expression of target genes, e.g. Notch, Bcl-2 and HMGA2 [27, 28]. miR-34 is composed of three processed miRNAs. In this family miR-34a is encoded by its own transcript, whereas miR-34b and miR-34c share a common primary transcript. It is now recognized that expression of miR-34a was uncommon in such cancers as pancreatic cancer, osteosarcoma, breast cancer and non-small cell lung cancer [29–32]. The inactivation of miR-34a was also identified in cell lines derived from some tumors including lung, breast, colon, kidney, bladder, pancreas and melanoma. In malignant melanoma, colorectal cancer and oral squamous cell carcinoma the inactivation of miR-34b and miR-34c has been observed – probably by the mechanism of CpG methylation [33–35]. Recent studies have revealed that ectopic miR-34 is associated with inhibition of cell proliferation, colony formation and cell cycle G1 arrest [35, 36]. Re-expression of miR-34a may induce apoptotic cell death, which was probably suppressed by inactivation of the p53 gene. It is worth noting that miR-34a could target several mRNAs, e.g. SIRT1, Bcl-2, N-myc, or cyclin D1, which leads to the translational repression of these genes [36, 37].

In summary, Notch signaling is clearly involved in the pathogenesis of gastrointestinal tumors such as gastric and colon cancer. Similarly as in the differentiation process, its effect in carcinogenesis in the gastrointestinal tract is

probably cell type specific and circumstance dependent. Why? Because Notch proteins suppress malignant transformation in some tissues and promote malignancy in others. An improved understanding of the Notch-mediated signaling pathway in tissues under physiological conditions as well as in those undergoing malignant transformation is essential from the clinical point of view because it may lead to novel cancer therapeutics.

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Submitted: 6.03.2012

Accepted: 26.10.2012