IN THE SPOTLIGHT

Prometastatic NOTCH Signaling in Colon Cancer

Onno Kranenburg

Summary: Dysregulation of NOTCH signaling contributes to the development of colorectal cancer, but how this pathway regulates metastasis has so far remained unclear. Sonoshita and colleagues identified a novel NOTCH-driven metastasis pathway that is amenable to therapeutic intervention and generated a companion diagnostic tool that allows analysis of pathway activity in human tumor tissue sections. *Cancer Discov; 5(2); 115–7. ©2015 AACR.*

See related article by Sonoshita et al., p. 198 (1).

Colorectal cancer is one of the most common malignancies in men and women. It is a devastating disease that causes more than half a million deaths yearly worldwide. Moreover, the incidence and mortality rate of colorectal cancer are rising with the aging population. Mortality is largely due to the consequences of metastatic tumor growth, which predominantly occurs in the liver and the lungs. Although surgery alone is usually sufficient to cure the disease locally, treatment of metastatic disease is a huge challenge. The median overall survival time of patients with metastatic colorectal cancer is less than two years with the current standard of care. Therefore, more effective treatment strategies are urgently needed, and these need to be based on a thorough understanding of disease progression and metastasis.

Dysregulation of NOTCH signaling has long been known to contribute to colorectal cancer development, but its role in the regulation of metastasis has remained largely obscure. In this issue of *Cancer Discovery*, Sonoshita and colleagues (1) have identified a novel signaling pathway that promotes colon cancer metastasis. Building on their previous finding that the endogenous NOTCH signaling inhibitor AES blocks colon cancer metastasis (2), they now delineate a novel NOTCH-driven, AES-suppressed, metastasis-promoting signaling pathway.

NOTCH receptor signaling is initiated by binding to Jagged or Delta-like ligands expressed by neighboring cells. In colon cancer, these ligands are produced by stromal cell types, notably tumor endothelial cells (3). Following ligand binding, the NOTCH receptor is cleaved by ADAM (A Disintegrin And Metalloprotease) and subsequently by the γ-secretase complex within the transmembrane domain. This causes release of the NOTCH intracellular domain (NICD) from the membrane and translocation of the NICD into the nucleus. Nuclear NICD subsequently binds $RBPJ_K$ (also known as CSL) to stimulate expression of NOTCH target genes, resulting in enhanced proliferation (*MYC*; *CCND1*) survival (*BCL2*, NF-κB) and suppression of secretory cell differentiation (HES1; ref. 4). The Taketo group had previously identified AES1 as a negative regulator of the $RBPJ_K$ complex. AES1 binds $RBPJ_K$, resulting in transcriptional repression, presumably by recruitment of histone deacetylase 4 (HDAC4) into the complex (2). They also showed that AES-mediated inhibition of NOTCH signaling resulted in an inability of tumor cells to invade and metastasize. However, the mechanisms underlying the regulation of invasion and metastasis by NOTCH signaling and by AES were still unclear and are now, at least in part, revealed in this article (1).

By using a mouse genetics approach, the authors first established that the invasive phenotype of APC/AES-deficient tumors depends on the NOTCH-activated transcription factor $RBPJ_K$. They subsequently identified the *Dab1* gene as a novel NOTCH-activated $RBPJ_K$ target gene. DAB1 had previously been implicated in the activation of SRC family tyrosine kinases. The authors show that in colorectal cancer cells, DAB1 binds c-ABL to stimulate its activity, whereas activated c-ABL phosphorylates DAB1, which further enhances c-ABL activation in a positive feedback loop. The authors then go on to show that ABL phosphorylates the guanine nucleotide exchange factor TRIO, resulting in enhanced RHO activity. Genetic and pharmacologic intervention studies further show that the DAB1–ABL–TRIO pathway is essential for NOTCH-induced tumor cell invasion in colorectal cancer cell lines and tumors. In addition, the authors have generated a novel phospho-specific antibody recognizing ABL-phosphorylated TRIO on tyrosine 2681 (TRIO pY2681). This antibody was subsequently used to demonstrate that patients with TRIO pY2681–high colorectal cancer tumors have a significantly shorter disease-specific survival.

From a scientific perspective, the data presented are intriguing, as they reveal a previously unknown connection between NOTCH and RHO signaling that regulates colon cancer invasion and metastasis. From a clinical perspective, the data are equally interesting, as the pathway provides multiple opportunities for therapeutic intervention. In the article, the authors have used the ABL/KIT/platelet-derived growth factor receptor (PDGFR) inhibitor imatinib that had previously been shown to suppress colon cancer metastasis in a mouse xenograft model (5). Interestingly, in that article, imatinib targeted the tumor stroma through PDGFR inhibition. Therefore, imatinib may target both tumor and

University Medical Center Utrecht, Utrecht, the Netherlands.

Corresponding Author: Onno Kranenburg, University Medical Center Utrecht, Heidelberglaan 100, 3584CX Utrecht, the Netherlands. Phone: 31-887558632; Fax: 31-2541944; E-mail: o.kranenburg@umcutrecht.nl

doi: 10.1158/2159-8290.CD-14-1456

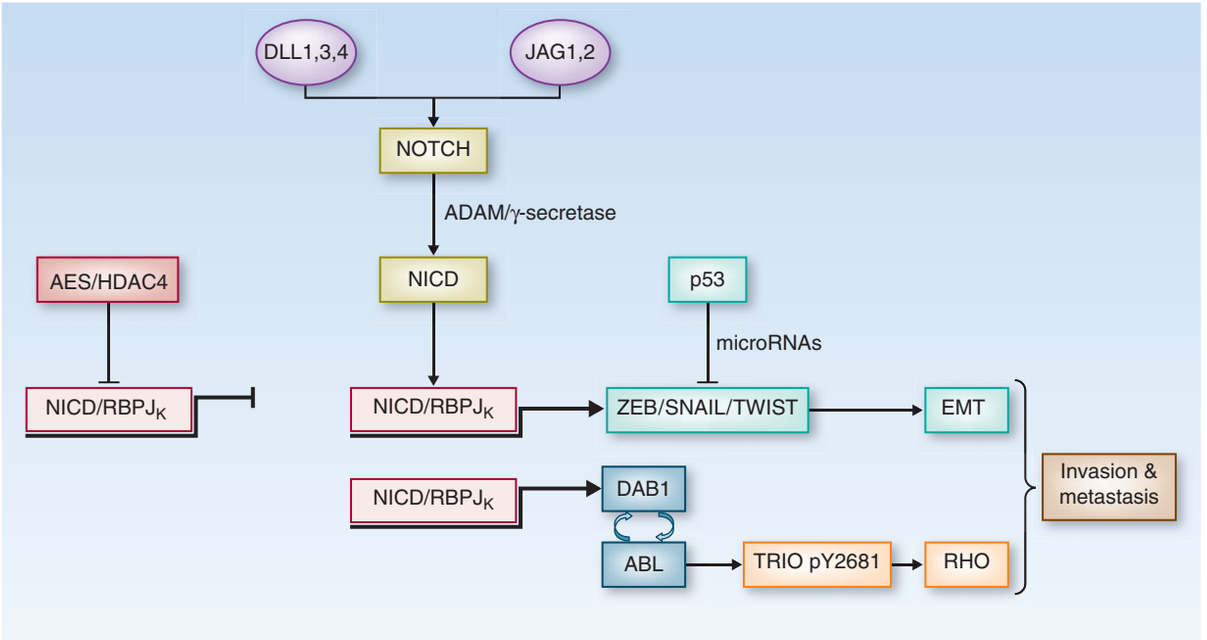

Figure 1. Prometastatic signaling by NOTCH. Following NOTCH activation by Jagged or Delta-like ligands (JAG1,2; DLL1,3,4), NOTCH receptors are proteolytically activated by the subsequent action of ADAM and the γ-secretase complex. The NICD translocates into the nucleus where it binds to and activates the $RBPJ_K$ transcription factor in cooperation with the transcriptional coactivator mastermind-like 1 (MAML1; not shown in the figure). Binding of AES1 counteracts $RBPJ_K$ activation, presumably by recruiting HDAC4 into the complex. $RBPJ_K$ activation stimulates expression of multiple target genes involved in tumor cell proliferation, suppression of differentiation, and survival (not shown), but also in a prometastatic program involving DAB1 and EMT drivers, such as ZEB1, SNAIL, and TWIST. Although RHO activity promotes collective cell migration, EMT results in a single-cell invasive phenotype. Whether NOTCH activation in human colorectal cancer primarily drives single cell or collective migration and how this is regulated are currently not known. See also Fig. 7 by Sonoshita et al. (1).

stromal cells in colorectal cancer tumors—by inhibiting ABL and PDGFR, respectively—and could be an excellent candidate for the treatment of colorectal cancer tumors, in which metastasis is driven by the NOTCH–DAB1–ABL–TRIO pathway. The phospho-TRIO antibody could serve as a companion diagnostic tool for the preselection of patients with such tumors and for monitoring pathway suppression during treatment.

Strikingly, a recently published study describes the generation of genetically engineered mice that develop highly metastatic intestinal tumors driven by conditional expression of the NICD in the intestinal epithelium in a p53-deficient background (6). This article focuses on NOTCH-induced epithelial–mesenchymal transition (EMT) in the promotion of metastasis. EMT inducers, such as ZEB, SNAIL, and TWIST, are direct transcriptional targets of the NICD but are suppressed by p53 family–regulated miRNAs. In the absence of p53, the NICD is capable of inducing EMT, resulting in tumor invasion and metastasis. EMT couples enhanced invasive capability to the cancer stem cell (CSC) phenotype, encompassing increased clone- and tumor-initiating potential. It is conceivable that the concomitant activation of EMT and the DAB1–ABL–TRIO pathway by the NICD synergizes in the generation of aggressive tumor cells with metastatic potential (Fig. 1). The NICD/$p53^{-/-}$ model would be ideally suited to test whether therapeutic targeting of the DAB1–ABL–TRIO pathway has an impact on local tumor invasion, spontaneous metastasis, and survival.

Gene expression profiling has recently been used to identify molecularly distinct colon cancer subtypes. Strikingly, tumors belonging to the consensus molecular subtype 4 (CMS4; ref. 7) are prone to metastasize and express high levels of gene signatures that reflect a CSC phenotype and EMT, thus providing an independently discovered link between the CSC phenotype, EMT, and metastasis in colorectal cancer. Whether NOTCH signaling, and in particular the DAB1–ABL–TRIO pathway, is overactive in these tumors and whether it plays a role in establishing the aggressive tumor phenotype have so far not been tested. Interestingly, the study by Sonoshita and colleagues (1) provides evidence that the DAB1–ABL–TRIO pathway could be connected to EMT by showing that the anti–TRIO pY2681 signal is high in invasive tumor buds together with markers of EMT and CSCs. In addition, NOTCH ligands produced by endothelial cells had previously been shown to be essential for the maintenance of colon CSCs (3). Therefore, it will be highly relevant to establish whether the DAB1–ABL–TRIO pathway is overactive in CMS4 tumors that are characterized by EMT and a CSC phenotype. If so, this would provide alternative targets for therapeutic intervention in a metastasis-prone colorectal cancer subtype that is refractory to current systemic therapy.

A key issue that needs to be addressed is whether metastasis formation in colorectal cancer requires tumor cells to undergo a real and complete EMT, generating single, invasive, stem-like, stress- and immune-resistant tumor cells, or whether metastases are initiated from less autonomously operating clusters of tumor cells, potentially even containing stromal elements from the tumor of origin and/or blood-borne factors such as

platelets. This is essential because these alternative models for metastasis formation involve molecularly distinct processes and hence would require inhibition of different targets. For instance, collective tumor cell invasion requires high RHOA activity (8), whereas single-cell invasiveness requires metalloproteinase-induced ECM degradation and is associated with high RAC and RHOC activities. A complicating issue is that tumor cells may switch between modes of invasion, depending on the microenvironmental challenges that they are faced with. As NOTCH signaling drives both an EMT program and a RHO-dependent invasion program via distinct transcriptional targets (Fig. 1), it will be interesting to establish how the specific modes of cell invasion are controlled by distinct NOTCH-activated target gene sets. Novel platforms for translational research are in place for answering such questions, including patient-derived organoids, a mouse model for NOTCH-driven spontaneous metastasis formation (6), innovative intravital imaging techniques to visualize local tumor progression in the colon and metastasis formation in the liver (9), and techniques to isolate and study tumor cell clusters in patients with cancer. Eventually, the answers to such questions should steer the design of clinical proof-of-concept studies aiming to establish NOTCH signaling intermediates as *bona fide* drug targets for the treatment of aggressive metastasis-prone colorectal cancer. An important issue to consider in the design of such trials is to assess whether targeting the core NOTCH pathway (anti-NOTCH and anti-Jagged antibodies; γ-secretase inhibitors) or targeting specific NOTCH-activated transcriptional programs could be more effective. In addition, it should be assessed whether inhibition of the newly identified pathway sensitizes colorectal cancer cells to commonly used chemotherapeutic agents. If demonstrated in preclinical studies, this could rapidly be translated into clinical trials seeking to improve survival benefit. Phase I studies have already shown that the addition of imatinib to a standard chemotherapy regimen is safe and potentially effective (10, 11). The anti–TRIO pY2681 antibody could be used as a selection tool to identify the patients whose tumors have an active DAB1–ABL–TRIO pathway and who may therefore benefit from imatinib therapy or from other inhibitors of the pathway.

In summary, the article by Sonoshita and colleagues (1) identifies a novel prometastatic branch of the NOTCH signaling network involving c-ABL and RHO, and thereby provides novel leads for the targeted therapy of metastasis-prone colorectal cancer. The anti–TRIO pY2681 antibody provides a novel companion diagnostic tool for future clinical studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online February 5, 2015.

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