1 Notch Signaling in Prevention And Therapy: Fighting Cancer with a Two-Sided Sword

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Abstract

The evolutionary conserved Notch pathway that first developed in metazoans and that was first discovered in fruit flies (*Drosophila melanogaster*) governs fundamental cell fate decisions and many other cellular key processes not only in embryonic development but also during initiation, promotion, and progression of cancer. On a first look, the Notch pathway appears remarkably simple, with its key feature representing a direct connection between an extracellular signal and transcriptional output without the need of a long chain of protein intermediaries as known from many other signaling pathways. However, on a second, closer look, this obvious simplicity exerts surprising complexity. There is no doubt that the enormous scientific progress in unraveling the functional mechanisms that underlie this complexity has recently greatly increased our knowledge about the role of Notch signaling for pathogenesis and progression of many types of cancer. Moreover, these new scientific findings have shown promise in opening new avenues for cancer prevention and therapy, although this goal is still challenging. Vol. III of the second edition of the book *Notch Signaling in Embryology and Cancer,* entitled *Notch Signaling in Cancer*, summarizes important recent developments in this fast-moving and fascinating field. Here, we give an introduction to this book and a short summary of the individual chapters that are written by leading scientists, covering the latest developments in this intriguing research area.

Keywords

Angiogenesis · Cancer · Cancer stem cells · Cancer treatment · Notch · Non-melanoma skin cancer · Notch signaling · Notch pathway · Skin cancer · Tumor angiogenesis

Abbreviations

BCC	Basal cell carcinoma
CSC	Cancer stem cell
Dll	Delta-like
ESCC	Esophageal squamous cell carcinoma
Hes	Hairy and enhancer of split

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HNSCCs	Head and neck squamous cell cancers
HPV	Human papillomavirus
Hrt	Hes-related transcription factor
JAG	Jagged
NID	Notch intracellular domain
NMSC	Non-melanoma skin cancer
SCC	Squamous cell carcinoma

Evolutionary conserved Notch signaling that first developed in metazoans (Gazave et al. 2009; Richards and Degnan 2009) and that was first discovered in the fruit fly *Drosophila melanogaster* represents one of the most fascinating pathways that govern both embryonic development and adult tissue homeostasis and an essential element of the defense line against cancer. Notably, the fascinating tale that earned the gene the name *Notch* began over a century ago, when the American scientist John S. Dexter discovered at Olivet College (Olivet, Michigan, USA) the typical notched-wing phenotype (a nick or notch in the wingtip) in his stock of mutant fruit flies *Drosophila melanogaster* (Dexter 2014; Reichrath and Reichrath 2020a, b). The alleles responsible for this phenotype were identified 3 years later at Columbia University (New York City, New York, USA) by another American scientist, Thomas Hunt Morgan (1866–1945) (Morgan 1917; Reichrath and Reichrath 2020a, b). In the following years, many additional alleles were identified that were associated with the Notch phenotype (Morgan 1928; Reichrath and Reichrath 2020a, b). In subsequent decades, notwithstanding the extensive research on the *Notch* locus, researchers struggled to identify the function for the *Notch* gene due to the lethality early in embryogenesis and the broad variety of phenotypic consequences of Notch mutants (Reichrath and Reichrath 2020a, b). Despite these challenges, the observations of John S. Dexter, Thomas Hunt Morgan, and others were finally confirmed by cloning and sequencing of the mutant *Notch* locus in the research laboratories of Spyros Artavanis-Tsakonas and Michael W. Young, more than half a century later (Wharton et al. 1985; Kidd et al. 1986). A huge mountain of new scientific evidence, which has been constantly growing during the last decades, has now convincingly shown that the Notch pathway governs, from sponges, roundworms, *Drosophila melanogaster*, and mice to humans, many key cell fate decisions and other core processes that are of high importance both for embryogenesis and in adult tissues (Andersson et al. 2011). Moreover, it has now been demonstrated that Notch signaling represents in humans an essential element of the defense line against cancer.

In 2012, when the first edition of *Notch Signaling in Embryology and Cancer* was published by Landes and Springer in the prestigious series *Advances in Experimental Medicine and Biology*, it was the benchmark on this topic, providing a broad audience (ranging from medical students to basic scientists, physicians, and all other healthcare professionals) with up-to-date information in a comprehensive, highly readable format. Since that time, a huge mountain of new scientific findings has been built up that underlines the many facets and the high biological/clinical relevance of Notch signaling for health and many diseases, including various types of cancer (Reichrath and Reichrath 2020a, b). Therefore, we decided that it is now the right time to publish an updated and extended version. The second edition of this book has been expanded substantially to cover all aspects of this fast-growing field and has been divided into three separate volumes to include additional chapters (Reichrath and Reichrath 2020a, b). In this new edition, leading scientists provide a comprehensive, highly readable overview on molecular mechanisms of Notch signaling (Volume I), Notch's role in embryonic development (Volume II), and, last but not least, its relevance for cancer (Volume III) (Reichrath and Reichrath 2020a, b).

This third volume gives an overview on Notch's role for selected types of cancer. As outlined previously, it must be emphasized that the Notch pathway seems delusorily simple, with one of its key features being a direct link between an extracellular signal and transcriptional output without the requirement for an extended chain of

protein intermediaries as needed by so many other signaling pathways (Hunter and Giniger 2020; Reichrath and Reichrath 2020a, b). However, this apparent simplicity hides remarkable complexity, and, consistent with its important role in many aspects of development, it has to be noted that Notch signaling has an extensive collection of mechanisms that it exerts alongside of its core transcriptional machinery (Hunter and Giniger 2020; Reichrath and Reichrath 2020a, b). In many biological processes, including morphological events during pathogenesis and progression of cancer, Notch-mediated coordination of the activity of gene expression with regulation of cell morphology is of high importance (Hunter and Giniger 2020; Reichrath and Reichrath 2020a, b). Fortunately, the generation and investigation of knockout mice and other animal models have in recent years resulted in a huge mountain of new informations concerning Notch gene function, allowing to dissect the role of specific Notch components in human development and disease. This volume is intended to provide both basic scientists and clinicians who seek today's clearest understanding of the molecular mechanisms that mediate Notch signaling with an authoritative day-to-day source.

In the first chapter, Benedetto Daniele Giaimo, Ellen Kolb, Rhett A. Kovall, and Tilman Borggrefe convincingly demonstrate the importance of the transcription factor RBP-J as a molecular switch in regulating the Notch response (Benedetto Daniele Giaimo et al. 2020). As they explain, the Notch signal transduction cascade requires cell-to-cell contact and results in the proteolytic processing of the Notch receptor and subsequent assembly of a transcriptional coactivator complex containing the Notch intracellular domain (NID) and transcription factor RBP-J. In the absence of a *Notch* signal, RBP-J remains at Notch target genes and dampens transcriptional output. Like in other signaling pathways, RBP-J is able to switch from activation to repression by associating with corepressor complexes containing several chromatin-modifying enzymes. In their chapter, Giaimo et al. focus on the recent advances concerning RBP-J corepressor functions, especially in regard to chromatin regulation. The authors put this into the context of one of the best-studied model systems for Notch, blood cell development. They elaborate that alterations in the RBP-J corepressor functions can contribute to the development of leukemia, especially in the case of acute myeloid leukemia (AML). The versatile role of transcription factor RBP-J in regulating pivotal target genes like *c-MYC* and *HES1* may contribute to the better understanding of the development of leukemia.

In the following chapter, Tobias Reiff, Miriam Baeumers, Christine Tibbe, and Thomas Klein provide a review on the role of the tumor suppressor lethal (2) giant discs (Lgd)/CC2D1, Notch signaling, and cancer (Reiff et al. 2020). They state that the endosomal pathway plays a pivotal role upon signal transduction in the Notch pathway and that recent work on *lethal (2) giant discs* (*lgd*) points to an additional critical role in avoiding uncontrolled ligand-independent signaling during trafficking of the Notch receptor through the endosomal pathway to the lysosome for degradation. In their chapter, the authors line out the fascinating journey of Notch through the endosomal system and present an overview of the current knowledge about Lgd and its mammalian orthologs Lgd1/CC2D1b and Lgd2/CC2D1a. They further discuss how Notch is activated in the absence of *lgd* function in *Drosophila* and ask whether there is evidence that a similar ligand-independent activation of the Notch pathway can also happen in mammals if the orthologs are inactivated.

In the next chapter, Violeta Jonusiene and Ausra Sasnauskiene summarize the relevance of Notch for endometrial cancer (Jonusiene and Sasnauskiene 2020). They explain that human endometrium is a unique, highly dynamic tissue that undergoes cyclic changes of cell proliferation, differentiation, and death. Endometrial cancer is the most common malignancy among women in developed countries. Importantly, the incidence of endometrial cancer is rising in high-income countries. Currently histological classification is used for subtyping of endometrial cancer, while ongoing research is evaluating markers for more accurate molecular classification. As the authors point out, accumulating evi-

dence links aberrant Notch signaling with diseases such as hyperplasia and endometrial cancer. This chapter summarizes the current state of Notch signaling investigations in the endometrium, endometriosis, and endometrial cancer.

In the following chapter, Yong Li, Yahui Li, and Xiaoxin Chen review our scientific knowledge of Notch's role in esophageal squamous cell carcinoma (ESCC) (Li et al. 2020). The authors explain that ESCC is a deadly disease that requires extensive research on its mechanisms, prevention, and therapy. Recent studies have shown that *NOTCH* mutations are commonly seen in human ESCC. This chapter summarizes our current understanding of the Notch pathway in normal esophagus and in ESCC. The authors explain that in normal esophagus, Notch pathway regulates the development of esophageal squamous epithelium, in particular, squamous differentiation. Exposure to extrinsic and intrinsic factors, such as gastroesophageal reflux, alcohol drinking, and inflammation, downregulates the Notch pathway and thus inhibits squamous differentiation of esophageal squamous epithelial cells. In ESCC, Notch plays a dual role as both a tumor suppressor pathway and an oncogenic pathway. In summary, further studies are warranted to develop Notch activators for the prevention of ESCC and Notch inhibitors for targeted therapy of a subset of ESCC with activated Notch pathway.

In the next chapter, Kazunori Kawaguchi and Shuichi Kaneko report on the relevance of Notch signaling for liver cancer (Kawaguchi and Kaneko 2020). They point out that interactions between liver cells are closely regulated by Notch signaling. Notch signaling has been reported clinically related to bile duct hypogenesis in Alagille syndrome, which is caused by mutations in the Jagged1 gene. Notch activation and hepatocarcinogenesis are closely associated since cancer signaling is affected by the development of liver cells and cancer stem cells. Gene expression and genomic analysis using a microarray revealed that abnormalities in Notch-related genes were associated with the aggressiveness of liver cancer. This pattern was also accompanied with α-fetoprotein- and EpCAM-expressing phenotypes in vitro, in vivo, and in clinical tissues. Hepatitis B or C virus chronic infection or alcohol- or steatosis-related liver fibrosis induces liver cancer. Previous reports demonstrated that HBx, a hepatitis B virus protein, was associated with Jagged1 expression. The authors report their finding that the Jagged1 and Notch1 signaling pathways were closely associated with the transcription of covalently closed circular hepatitis B virus DNA, which regulated cAMP response element-binding protein, thereby affecting Notch1 regulation by the E3 ubiquitin ligase ITCH. This viral pathogenesis in hepatocytes induces liver cancer. The authors conclude that Notch signaling exerts various actions and is a clinical signature associated with hepatocarcinogenesis and liver context-related developmental function.

In the next contribution, Cristina Porcheri and Thimios A. Mitsiadis report on Notch's role in head and neck cancer (Porcheri and Mitsiadis 2020). Head and neck cancer is a group of neoplastic diseases affecting the facial, oral, and neck region. It is one of the most common cancers worldwide with an aggressive, invasive evolution in the late stages of malignancy. Due to the heterogeneity of the tissues affected, it is particularly challenging to study the molecular mechanisms at the basis of these tumors, and to date we are still lacking accurate targets for prevention and therapy. The authors explain that Notch signaling is involved in a variety of tumorigenic mechanisms, such as regulation of the tumor microenvironment, cell-to-cell communication, and metabolic homeostasis. Moreover, they provide an up-to-date review of the role of Notch in head and neck cancer and draw parallels with other types of solid tumors where the Notch pathway plays a crucial role in emergence, maintenance, and progression of the disease. Additionally, the authors give a perspective view on the importance of the pathway in neoplastic development in order to define future lines of research and novel therapeutic approaches.

In the following chapter, Trianth Das, Rong Zhong, and Michael T. Spiotto explain the rele-

vance of Notch signaling for human papillomavirus-associated oral tumorigenesis (Das et al. 2020). They point out that the Notch pathway is critical for the development of many cell types including the squamous epithelium lining of cutaneous and mucosal surfaces. In genetically engineered mouse models, Notch1 acts as one of the first steps to commit basal keratinocytes to terminally differentiate. Similarly, in human head and neck squamous cell cancers (HNSCCs), Notch1 is often lost consistent with its essential tumor-suppressive role for initiating keratinocyte differentiation. However, constitutive Notch1 activity in the epithelium results in expansion of the spinous keratinocyte layers and impaired terminal differentiation which is consistent with the role of Notch1 as an oncogene in other cancers, especially T-cell acute lymphoblastic leukemia. The authors also report their previous observation that Notch1 plays a dual role as both a tumor suppressor and oncogene depending on the mutational context of the tumor. Namely, gain or loss or Notch1 activity promoted the development of human papillomavirus (HPV)-associated cancers. The additional HPV oncogenes likely disrupted the tumor-suppressive activities of Notch and enable the oncogenic pathways activated by Notch to promote tumor growth. In this review, the authors detail the role of Notch pathway in head and neck cancers with a focus on HPV-associated cancers.

In their contribution, Sandra and Jörg Reichrath summarize the impact of Notch signaling for carcinogenesis and progression of non-melanoma skin cancer (Reichrath and Reichrath 2020c). They explain that, since many decades, non-melanoma skin cancer (NMSC) is the most common malignancy worldwide. Basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) are the major types of NMSC, representing appr. 70% and 25% of these neoplasias, respectively. Because of their continuously rising incidence rates, NMSCs represent a constantly increasing global challenge for healthcare, although they are in most cases nonlethal and curable (e.g., by surgery). The authors elaborate that, while at present, carcinogenesis of NMSC is still not fully understood, the relevance of genetic and molecular alterations in several pathways, including evolutionary highly conserved Notch signaling, has now been shown convincingly. Choosing NMSC as a model, the authors give in this review a brief overview on the interaction of Notch signaling with important oncogenic and tumor suppressor pathways and on its role for several hallmarks of carcinogenesis and cancer progression, including the regulation of cancer stem cells (CSCs), tumor angiogenesis, and senescence.

In the next contribution, Rachael Guenter, Zeelu Patel, and Herbert Chen summarize the role of Notch signaling in thyroid cancer (Guenter et al. 2020). They explain that thyroid cancer is the most common malignancy of the endocrine system with a steadily rising incidence. The term thyroid cancer encompasses a spectrum of subtypes, namely, papillary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, and medullary thyroid cancer. Each subtype differs histopathologically and in degrees of cellular differentiation, which may be in part due to signaling of the Notch pathway. The Notch pathway's role in cancer biology is controversial, as it has been shown to play both an oncogenic and tumor-suppressive role in many different types of cancer. This discordance holds true for each subtype of thyroid cancer, indicating that Notch signaling is likely cell type and context dependent. The authors explain that, whether oncogenic or not, Notch signaling has proven to be significantly involved in the tumorigenesis of thyroid cancer and has thus earned interest as a therapeutic target. The authors conclude that advancement in the understanding of Notch signaling in thyroid cancer holds great promise for the development of novel treatment strategies to benefit patients.

In the following chapter, Zacharias Fasoulakis, George Daskalakis, Marianna Theodora, Panos Antsaklis, Michael Sindos, Michail Diakosavvas, Kyveli Angelou, Dimitrios Loutradis, and Emmanuel N Kontomanolis elaborate on the relevance of Notch signaling in cancer progression (Fasoulakis et al. 2020). As they point out, the Notch signaling pathway controls cell prolifera-

tion, fate, differentiation, and cell death, by short-range signaling between nearby cells that come in contact. Fibroblasts, representing an essential for tumor growth component of stroma, have also been shown to be affected by Notch regulation. Notch gene mutations have been identified in a number of human tumors revealing information on the progression of specific cancer types, such as ovarian cancer and melanoma, immune-associated tumors such as myeloid neoplasms, but especially lymphocytic leukemia. The authors further explain that activation of Notch can be either oncogenic or it may contain growth-suppressive functions, acting as a tumor suppressor in other hematopoietic cells, hepatocytes, and skin and pancreatic epithelium.

In the next contribution, Qiang Shen and Michael Reedijk elaborate on the role of Notch signaling for the breast cancer microenvironment (Shen and Reedijk 2020). They explain that Notch promotes breast cancer progression through tumor-initiating cell maintenance, tumor cell fate specification, proliferation, survival, and motility. In addition, Notch is recognized as a decisive mechanism in regulating various juxtacrine and paracrine communications in the tumor microenvironment (TME). In this chapter, we review recent studies on stress-mediated Notch activation within the TME and sequelae such as angiogenesis, extracellular matrix remodeling, changes in the innate and adaptive immunophenotype, and therapeutic perspectives.

Last but not least, Giulia Monticone and Lucio Miele present a journey from notching phenotypes to cancer immunotherapy (Monticone and Miele 2020). The authors point out that Notch is a remarkable evolutionary conserved pathway, which has fascinated and engaged the work of investigators in an uncountable number of biological fields, from development of metazoans to immunotherapy for cancer. Nowadays Notch is the protagonist of some of the most cutting-edge fields including immunotherapy and synthetic biology. In their chapter, Monticone and Miele provide a comprehensive overview of the Notch field, with particular focus on the newest mechanistic and therapeutic advances and the future challenges of this constantly evolving field.

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