1 A Snapshot of the Molecular Biology of Notch Signaling: Challenges and Promises

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Abstract

Evolutionary conserved Notch signaling is of high importance for embryogenesis and adult tissues, representing one of the most fascinating pathways that regulate key cell fate decisions and other core processes. This chapter gives a short introduction to the first volume of the book entitled *Notch Signaling in Embryology and Cancer*, that 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. On a first look, Notch signaling, that first developed in metazoans and that was first discovered in a fruit fly, seems fallaciously simple, with its key feature being a direct link between an extracellular signal and transcriptional output without the requirement of an extended chain of protein intermediaries as needed by the majority of other signaling pathways. However, on a second, closer look, this obvious simplicity hides remarkable complexity. Notch signaling, that relies on an extensive collection of mechanisms that it exerts alongside of its core transcriptional machinery, orchestrates and governs cellular development by inducing and regulating communication between adjacent cells. In general, a cell expressing the Notch receptor can be activated in trans by ligands on an adjacent cell leading to alteration of transcription and cellular fate. However, ligands also have the ability to inhibit Notch signaling and this can be accomplished when both receptor and ligands are co-expressed in cis on the same cell. The so called non-canonical Notch pathways further diversify the potential outputs of Notch, and allow it to coordinate regulation of many aspects of cell biology. Fortunately, the generation and investigation of knockout mice and other animal models have in recent years resulted in a huge volume of new scientific informations concerning Notch gene function, allowing to dissect the role of specific Notch components for human development and health, and showing promise in opening new avenues for prevention and therapy of a broad variety of independent diseases, including cancer, although this goal is still challenging.

Keywords

Notch · Notch signaling · Notch pathway · Embryonic development · Jagged · Delta like ligand

Evolutionary conserved Notch signaling, that first developed in metazoans (Gazave et al. 2009; Richards and Degnan 2009) and that was first discovered in a fruit fly (*Drosophila melanogaster*), represents one of the most fascinating pathways

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that govern both embryonic development and adult tissue homeostasis. A huge volume of scientific evidence, that 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). At first glance, the Notch pathway seems fallaciously simple, with its key feature being a direct link between an extracellular signal and transcriptional output without the requirement of an extended chain of protein intermediaries as needed by so many other signaling pathways (Hunter and Giniger 2020). However, on a second, closer look, this obvious simplicity hides remarkable complexity, and consistent with its central role in many aspects of development, it has to be noted that Notch signaling has an extensive collection of mechanisms that it exerts alongside its core transcriptional machinery (Hunter and Giniger 2020). There is no doubt that the enormous scientific progress in unraveling the molecular mechanisms of Notch signaling that has been made recently has shown promise in opening new avenues for prevention and therapy of a broad variety of independent diseases, including cancer, although this goal is still challenging.

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 1914). 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). In the following years, many additional alleles were identified, that were associated with the Notch phenotype (Morgan 1928). 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. 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).

During the last decades, a broad variety of independent inherited diseases linked to defective Notch signaling has been identified, highlighting its clinical relevance. The discovery of these congenital diseases started in 1996 in patients diagnosed with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; an autosomal dominant hereditary stroke disorder resulting in vascular dementia) (Joutel et al. 1996), with the linkage analysis-based discovery of heterozygous *NOTCH3* mutations on chromosome 19. Since these pioneer investigations, several other inherited disorders, including Adams–Oliver, Alagille, and Hajdu–Cheney syndromes, and several types of cancer, have convincingly been linked to defective Notch signaling (Li et al. 1997; Oda et al. 1997).

Interestingly, there is an emerging role of Notch as a promising therapeutic target in various malignancies, inherited diseases, and other disorders. In this context, it is of interest that in a mouse model (Notch3$^{tm1.1Ecan}$) of lateral meningocele syndrome (LMS), cancellous bone osteopenia was no longer detected after intraperitoneal administration of antibodies directed against the negative regulatory region (NRR) of Notch3 (Yu et al. 2019). In that study, anti-Notch3 NRR antibody suppressed expression of Hes1, Hey1, and Hey2 (Notch target genes), and decreased Tnfsf11 (receptor activator of NF Kappa B ligand) messenger RNA in Notch3$^{tm1.1Ecan}$ osteoblast cultures (Yu et al. 2019). This study indicates that cancellous bone osteopenia of Notch3$^{tm1.1Ecan}$ mutants can be reversed by anti-Notch3 NRR antibodies, thereby opening new avenues for treatment of bone osteopenia in LMS patients (Yu et al. 2019). Another example an emerging role of Notch as a promising therapeu-

tic target is the unilateral ureteral obstruction (UUO) mouse model, where treatment with the g-secretase inhibitor DAPT showed an amelioration of renal fibrosis including lower fibrotic levels and collagen deposition (Marquez-Exposito et al. 2020). However, the direct effect of Notch signaling pathway activation in the regulation of the ECM proteins has not been confirmed yet (Marquez-Exposito et al. 2020).

In 2012, when the first edition of "Notch Signaling in Embryology and Cancer" was published by Landes and Springer in the prestiguous 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 health care professionals) with up to date information in a comprehensive, highly readable format. Since that time, a huge mountain of new scientific findings has been build up, that, at one side underlines the many facettes and the high biological/clinical relevance of Notch signaling and at the other, further unravels the underlying molecular mechanisms. Therefore, we have 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 in three separate volumes to include additional chapters. 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).

This first volume gives an overview on the molecular mechanisms that mediate the biological effects of the highly conserved Notch signaling system. As outlined above, 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). 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 its core transcriptional machinery. In many biological processes, including morphological events during embryogenesis and during pathogenesis and progression of cancer, Notch-mediated coordination of the activity of gene expression with regulation of cell morphology is of high importance. Notably, Notch signaling orchestrates and governs cellular development by inducing and regulating communication between adjacent cells (Fleming 2020). In general, a cell expressing the Notch receptor can be activated in trans by ligands on an adjacent cell leading to alteration of transcription and cellular fate. However, ligands also have the ability to inhibit Notch signaling and this can be accomplished when both receptor and ligands are coexpressed in cis on the same cell. Notably, the manner in which cis-inhibition is accomplished is not entirely clear but it is known to involve several different protein domains of the ligands and the corresponding Notch receptor. While some of the protein domains involved in trans-activation are also used for cis-inhibition, others are used uniquely for each process. Other important aspects for the regulation of both canonical and noncanonical Notch signaling are phosphorylation and proteolytic cleavage of Notch (Hunter and Giniger 2020). The so-called noncanonical Notch pathways diversify the potential outputs of Notch, and allow it to coordinate regulation of many aspects of the biology of cells. Special attention should be given to the role of posttranslational modifications of Notch for noncanonical Notch signaling. 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 the most current and clearest understanding of the molecular mechanisms that mediate Notch signaling with an authoritative day-to-day source of the same.

In Chap. 2, Brendan McIntyre and coworkers give an excellent overview of Basic Mechanisms of Notch Signaling in Development and Disease (McIntyre et al. 2020). They underline that the evolutionary conserved Notch signaling pathway is associated with the development and differentiation of all metazoans, that it is needed for proper germ layer formation and segmentation of the embryo and that it controls the timing and duration of differentiation events in a dynamic manner. As these authors further briefly summarize, perturbations of Notch signaling may result in blockades of developmental cascades, developmental anomalies, and cancers. Brendan McIntyre and coworkers conclude that an in-depth understanding of Notch signaling is thus required to comprehend the basis of development and cancer, and can be further exploited to understand and direct the outcomes of targeted cellular differentiation into desired cell types and complex tissues from pluripotent or adult stem and progenitor cells. In their chapter, Brendan McIntyre and coworkers explicitly summarize the molecular, evolutionary, and developmental basis of Notch signaling, focussing on understanding the basics of Notch signaling and its signaling control mechanisms, its developmental outcomes and perturbations leading to developmental defects, as well as have a brief look at mutations of the Notch signaling pathway causing human hereditary disorders or cancers.

In Chap. 3, Robert J. Fleming discusses explicitly the role of an extracellular region of Serrate for Ligand-induced cis-inhibition of Notch signaling (Fleming 2020). As the author points out, cellular development can be controlled by communication between adjacent cells mediated by the highly conserved Notch signaling system. He explicitly summarizes that a cell expressing the Notch receptor can be activated in trans by ligands on an adjacent cell leading to alteration of transcription and cellular fate. Robert J. Fleming further explains that ligands also have the ability to inhibit Notch signaling and that this can be accomplished when both receptor and ligands are coexpressed in cis on the same cell. Notably, the manner in which cis-inhibition is accomplished is not entirely clear but it is known to involve several different protein domains of the ligands and the corresponding Notch receptor. While some of the protein domains involved in trans-activation are also used for cis-inhibition, others are used uniquely for each process. In the chapter, the involvement of various ligand regions and the receptor are discussed in relation to their contributions to Notch signaling.

In Chap. 4, Hunter and Giniger discuss other important aspects for the regulation of canonical and noncanonical Notch signaling, namely, phosphorylation and proteolytic cleavage of Notch (Hunter and Giniger 2020). As they point out, the Notch signaling pathway seems deceptively simple, with its key feature being a direct connection between extracellular signal and transcriptional output without the need for an extended chain of protein intermediaries as required by so many other signaling paradigms. However, they discuss that this apparent simplicity hides considerable complexity and that Notch signaling, consistent with its central role in many aspects of development, has an extensive collection of mechanisms that it employs alongside its core transcriptional machinery. They convincingly summarize that these so-called noncanonical Notch pathways diversify the potential outputs of Notch, and allow it to coordinate regulation of many aspects of the biology of cells. In their chapter, Hunter and Giniger review noncanonical Notch signaling with special attention to the role of posttranslational modifications of Notch. Moreover, they also consider the importance of coordinating the activity of gene expression with regulation of cell morphology in biological processes, including axon guidance and other morphological events during embryogenesis.

In Chap. 5, Bhawana Maurya and coworkers summarize how Maheshvara a conserved RNA helicase regulates Notch signaling in *Drosophila melanogaster* (Maurya et al. 2020). They explain that gene expression is regulated at multiple steps after generation of primary RNA transcripts, including mRNA processing, stability, transport, along with co- and posttranscriptional regulation and that these processes are all controlled via involvement of multitude of RNA

binding proteins (RBPs). As they further discuss, innumerable human diseases have been associated with altered expression of these RNA binding proteins. In their chapter, the authors focus on *maheshvara* (*mahe*), which encodes a putative DEAD box RNA helicase protein in *Drosophila* and plays an important role in regulation of Notch signaling. Fine tuning of Notch signaling is required at multiple steps, since its misregulation leads to a variety of human diseases. Additionally, the authors explain that mutations in *DDX59*, a human homolog of *mahe* results in orofaciodigital syndrome associated with broad neurological phenotypes, and that drosophila *mahe* mutants show abnormal peripheral and central nervous system development that resembles neuropathology of patients harboring mutations in the *DDX59* gene. In summary, this chapter explicitly presents recent advances in our knowledge as to how *mahe* regulates Notch signaling and nervous system development.

In Chap. 6, Laura Marquez-Exposito and coworkers summarize our present understanding, how Gremlin, a member of the transforming growth factor-β (TGF-β) family, regulates Notch signaling (Marquez-Exposito et al. 2020). The authors conclude that the axis Gremlin-1/Notch plays a significative role in the embryonary development as well as some adult tissue injury, such as kidney failure. Nevertheless, more studies are needed in order to determine the intrincate functions of these signaling pathways in development and adult homeostasis.

The following chapters (7 and 8) focus on other, selected aspects of the molecular regulation of Notch signaling. In Chap. 7, Debdeep Dutta and coworkers explain the role of the Heterogeneous Nuclear Ribonucleoprotein Hrp48 and Deltex for the regulation of Notch Signaling in *Drosophila melanogaster* (Dutta et al. 2020). The authors point out that, due to its involvement in numerous developmental events, Notch signaling requires tight spatial and temporal regulation. Deltex is a cytoplasmic protein that physically binds to Notch and regulates its signaling activity in a context-dependent manner. However, as Debdeep Dutta and coworkers explain, the biology of Deltex in regulation of Notch signaling is not well explored. The authors report that Hrp48, an RNA-binding protein, was identified as an interacting partner of Deltex, and that interaction of these two proteins seemed to regulate the Notch signaling outcome in the epithelial tissue. Additionally, it was found that coexpression of Deltex and Hrp48 can lead to cell death as well as JNK activation. Debdeep Dutta and coworkers conclude that, considering the well-conserved nature of Notch, Hrp48, and Deltex, this interaction can be helpful to understand the regulation of Notch signaling both in development and disease condition.

In Chap. 8, Amanda Salviano-Silva and coworkers explicitly summarize the relevance of the interaction of long noncoding RNAs and Notch signaling for tissue homeostasis (Salviano-Silva et al. 2020). They shortly explain that Notch signaling is a crucial pathway involved in cellular development, progression, and differentiation and that deregulation of Notch signaling commonly impacts tissue homeostasis, being highly associated with proliferative disorders. As they point out, the long noncoding RNAs (lncRNAs), which are transcripts with more than 200 nucleotides that do not code for proteins, were already described as Notch signaling pathway-interacting molecules. Many of them act as important transcriptional and posttranscriptional regulators, affecting gene expression and targeting other regulatory molecules, such as miRNAs. Due to their strong impact on function and gene expression of Notch-related molecules, lncRNAs influence susceptibility to cancer and other diseases, and can be regarded as potential biomarkers and therapeutic targets. In this chapter, the authors summarize the cross talk between the Notch signaling pathway and their most important modulating lncRNAs, as well as the pathological consequences of these interactions, in different tissues.

In Chap. 9, Rajaguru Aradhya and Krzysztof Jagla report and discuss the relevance of Insulin-dependent noncanonical activation of Notch in *Drosophila*: a fascinating story of Notch-induced muscle stem cell proliferation (Aradhya and Jagla 2020). As they point out, *Notch* plays multiple roles both in development and in adult tissue

homeostasis with flagship functions being its capacity to keep precursor and stem cells in a nondifferentiated state but also its ability to activate cell proliferation that in some contexts could lead to cancer. In general, both these functions involve canonical, ligand-dependent Notch activation. However, as the authors explain a ligand-independent Notch activation has also been described in a few cellular contexts. In their chapter, Rajaguru Aradhya and Krzysztof Jagla focus on one of such contexts, *Drosophila* muscle stem cells, called AMPs, and discuss how insulin-dependent noncanonical activation of Notch pushes quiescent AMPs to proliferation.

In Chap. 10, Tsaouli and coworkers discuss the impact of NF-κB for molecular mechanisms of Notch signaling in lymphoid cell lineages development (Tsaouli et al. 2020). As they point out, Notch is a ligand–receptor interaction-triggered signaling cascade highly conserved, that influences multiple lineage decisions within the hematopoietic and the immune system, representing a recognized model of intercellular communication that plays an essential role in embryonic as well as in adult immune cell development and homeostasis. Four members belong to the family of Notch receptors (Notch1–4), and each of them plays nonredundant functions at several developmental stages. They explain that canonical and noncanonical pathways of Notch signaling are multifaceted drivers of immune cells biology and that increasing evidence highlighted Notch as an important modulator of immune responses, also in cancer microenvironment. The authors discuss, that in these contexts, multiple transduction signals, including canonical and alternative NF-κB pathways, play a relevant role. In this chapter, they first describe the critical role of Notch and NF-κB signals in lymphoid lineages developing in thymus: T natural killer cells, thymocytes, and thymic T regulatory cells. The authors also address the role played by ligand expressing cells, and given the importance of Notch/NF-κB cross talk, discuss its role in T-cell leukemia development and progression.

Last but not least, we would like to thank all authors for their excellent contributions to this book. We hope that this volume will provide a broad audience (ranging from medical students to basic scientists, physicians, and all other health care professionals) who seek the most current and clearest understanding of the molecular mechanisms of Notch signaling with up-to-date information in a comprehensive, highly readable format and with an authoritative day-to-day source of the same.

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