Nuclear Hormone Receptors and Notch: Their Roles in Lymphocyte Development and Cell Fate Decision

Tanapat Palaga

The nuclear hormone receptor protein family is a group of proteins that play crucial roles in development, organ formation and homeostasis in mammals. Some of the family members have been shown to trigger apoptotic cell death in various organs such as immune cells and the neuronal system. This review will summarize the roles this group of proteins plays in shaping T lymphocyte populations during thymic development. Furthermore, the link between apoptosis induced by the nuclear hormone receptors and another developmental signaling cascade, Notch, will be discussed. Notch is an evolutionarily well conserved signaling pathway playing an important role in cell fate decisions during tissue development. Aberrant Notch signaling has been implied in altered T lymphocyte populations and tumor formation. Finally, the models to explain how Notch interferes with the nuclear hormone receptor mediated cell death will be proposed.

Key words: Nuclear hormone receptor, apoptosis, Notch and thymic development.

Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
Correspondence to: e-mail: tanapat.p@chula.ac.th

นิวเคลียร์ฮอร์โมนรีเซ็บเทอร์และ Notch: หน้าที่ในการพัฒนาการและการตัดสินชะตาของเซลล์ลิมโฟซัยท์

ธนาภัทร ปาลกะ (2547)

วารสารวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **29(2)**

กลุ่มโปรตีนนิวเคลียร์ฮอร์โมนรีเซ็บเทอร์เป็นกลุ่มโปรตีนที่ทำหน้าที่สำคัญระหว่างการพัฒนา การสร้างอวัยวะและการรักษาภาวะธำรงดุลในสัตว์เลี้ยงลูกด้วยนม สมาชิกบางตัวในโปรตีนกลุ่มนี้สามารถกระตุ้นให้เกิดการตายของเซลล์แบบอะพอปโทซิสในอวัยวะหลากหลายประเภท เช่น ในระบบภูมิคุ้มกัน และระบบประสาท บทความนี้จะสรุปหน้าที่ของกลุ่มโปรตีนนี้ในการควบคุมการพัฒนาเซลล์ประเภท T lymphocyte ในระหว่างที่มีการพัฒนาในต่อมไทมัส อีกทั้งจะอภิปรายความสัมพันธ์ระหว่างการตายแบบอะพอปโทซิสที่ถูกเหนี่ยวนำโดยโปรตีนในกลุ่มนี้กับวิถีสัญญาณซึ่งควบคุมพัฒนาการอีกวิถีสัญญาณหนึ่งคือ Notch Notch เป็นวิถีสัญญาณที่ได้มีการอนุรักษ์ไว้ มีหน้าที่หลักในการกำหนดชะตาของเซลล์ระหว่างการพัฒนาของอวัยวะหลายประเภท ความผิดปกติของวิถีสัญญาณของ Notch มีความเกี่ยวข้องกับความเบี่ยงเบนในการพัฒนาของ T lymphocyte และการเกิดมะเร็ง นอกจากนี้จะเสนอตัวแบบเพื่อที่จะอธิบายถึงกลไกซึ่ง Notch ไปรบกวนกระบวนการตายของเซลล์ที่เกิดจากการเหนี่ยวนำโดยสัญญาณของโปรตีนนิวเคลียร์ฮอร์โมนรีเซ็บเทอร์

คำสำคัญ นิวเคลียร์ฮอร์โมนรีเซ็บเทอร์ การตายแบบอะพอปโทซิส Notch
พัฒนาการของต่อมไทมัส

INTRODUCTION

Apoptotic cell death during development is essential for removing excess and no longer needed cells in a highly regulated manner. These phenomena are observed in the developmental processes of all multicellular organisms, ranging from worms to flies, mice and humans. Once the organisms develop to mature adults, apoptosis plays an important role in maintaining homeostasis. Misregulation of apoptosis in any stage during development can give rise to a fatal consequence in the organism. Although the final outcome of apoptosis, whenever it takes place, is death and removal of doomed cells, the early events leading to the final outcome can be diverse and are mediated by a broad range of intracellular signals. Just as pro-apoptotic signalings are diverse, the signals antagonistic to apoptotic events exist and can be mediated similarly by diverse signaling pathways. Balancing the pro- and anti-apoptotic signalings is important in regulating cell death at the physiological level.

Signaling through nuclear hormone receptors (NHRs) is one of many means of regulating cell death.[1] Based on conserved DNA binding, ligand binding and activation domains, NHRs form a large protein family (Figure 1). Two classes of NHRs are identified to date, based on the existance of the endogenous ligands. The ligand-dependent NHRs, such as steroid hormone receptors, *i.e*. estrogen and glucocorticoid receptors, are activated by ligand binding, thus, they are ligand-gated transcription factors.[2] The NHRs, whose ligand has not been identified, classified as orphan nuclear receptors, are activated by protein modification, such as phosphorylation.[3] Activated NHRs function in the nucleus as transcriptional activators to transcribe their target genes. Some activated NHRs such as glucocorticoid receptors function by forming a complex with other transcription factors and interfere with their transcriptional ability.[4] In addition to their biological activities during activation, some of them have been shown to play an important role when they are in an inactive stage as a transcriptional repressor. In an inactive stage, they act as transcriptional repressors by binding to consensus sites within the promoter of the target genes and recruiting the basal transcriptional repressors.[5]

NHRs have been shown to regulate apoptotic cell death during development in many organisms. Insect steroid hormone ecdysone and its receptor, ecdysone receptor, play an important role in regulating apoptotic cell death in tissue remodeling during metamorphosis.[6,7] Thyroid hormone receptor is involved in tissue remodeling during amphibian metamorphosis.[8] An orphan nuclear receptor, Nur77 and its related family members, *i.e*. Nurr1 and Nor1, are essential in T cell receptor (TCR)-mediated cell death in developing T lymphocytes.[1] Recently cloned murine orphan receptor retinoid related orphan receptor gamma (RORgamma) has been shown to regulate survival and differentiation of thymic T cells.[9,10] Retinoic acid receptor (RAR) also plays an important role in regulating cell death of T lymphocytes.[11] Therefore, the functions of NHRs in regulating apoptotic processes are evolutionarily well conserved and widely utilized during development and maintenance of homeostasis.

This review discusses the nature of NHR-mediated cell death during thymic development by focusing on the two NHRs, Nur77 and glucocorticoid receptor (GR). Furthermore, the role of Notch signaling pathways as a common antagonistic signal to the pro-apoptotic activity of NHRs will be discussed. Finally, a hypothesis underlying the molecular events leading to inhibition of cell death by Notch signaling will be proposed.

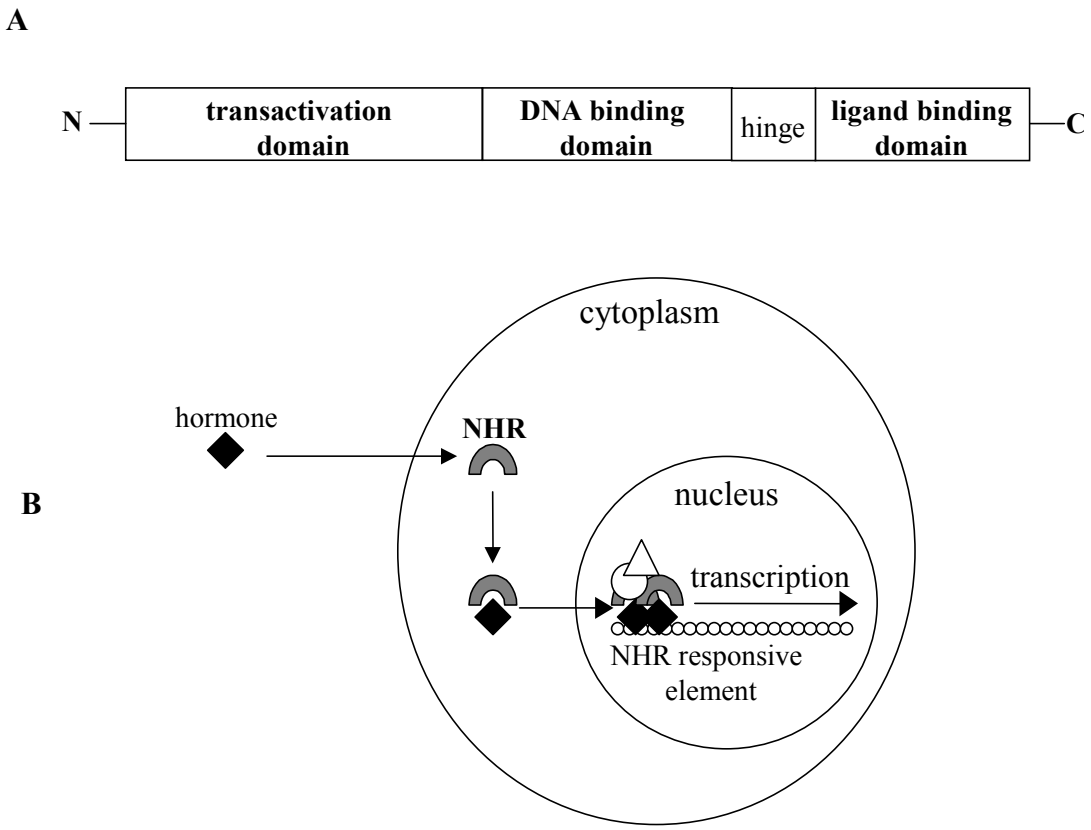

Figure 1. Basic structure of NHR family protein and its activation pathway.

A. Conserved basic structure of the NHR family protein. DNA binding domain and ligand binding domain are well conserved. The transactivation domain binds basic transcriptional activation complex.

B. Activation of NHR by ligand binding. Lipophilic hormone diffuses through the cell membrane and binds specifically to the ligand binding domain of the NHR. This engagement triggers the conformational changes and nuclear translocation of the NHR. In the nucleus, NHR binds to a specific response element and recruits the basic transcriptional activation complex and acts to regulate the transcriptions of target genes.

NHRs and cell death during thymic development

T lymphocytes are major lymphocytes functioning in cell-mediated immune response. T lineage cells develop from common lymphoid progenitors residing in the bone marrow. Developmental processes of T lineage cells involve multiple checkpoints and steps in cell fate decisions leading to the generation of functionally mature T lymphocytes within the thymus.[12] A large excess number of cells is generated at an early stage of T lymphocyte development. Through multiple selection processes, to ensure that only useful and harmless T lymphocytes survive, most cells among this large pool of cells are

induced to die by apoptosis, and only 1 to 2% of the cells survive to become mature T cells that function in the periphery.[13] Therefore, apoptosis during thymic development is crucial in shaping T cell repertoire.

The developmental process of T lineage cells is transcriptionally controlled and often accompanied by changes in expression of cell surface antigens (Figure 2).[12] From the common lymphoid progenitors, the first cell fate decision to be made is whether to become T or B lineage cells. Once they are committed to T lineage cells, cells start to rearrange one of the two chains composing T cell receptor (TCR), a molecule T lymphocytes use to recognize antigens. Influenced by the rearranged TCR and other unknown factors, cells will develop toward either αβ or γδ T cell lineages, two entirely different subsets of T ymphocytes. While αβ T cells are the major T cell population in humans and mice, γδ T cells comprise only 1 to 5% of the total within the lymphoid organs of mature adults. The development of the γδ T cell subset is not entirely understood. After this step, αβ T cells develop further into a $CD4^-$ $CD8^-$ double negative (DN) stage. Only cells expressing functional TCR are selected to proliferate (a process termed β selection) and become $CD4^+CD8^+$ double positive (DP) cells. Most developing thymocytes are induced to die during the DP stage. The random rearrangement of TCR is a major event during the developmental process of T lineage cells. The random rearrangement aims at creating diversification of TCR in order to deal with the unlimited diversity of antigens our bodies encounter in our lives.

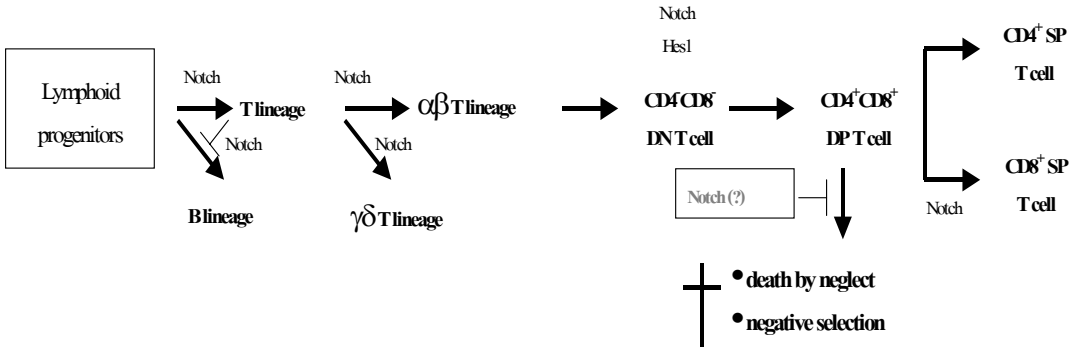

Figure 2. Development of T lymphocytes from common lymphoid precursors to mature functional CD4 or CD8 SP lymphocytes is depicted. The steps whereby Notch has been suggested to play a crucial role are indicated.

During the DP stage, cells go through two important selection processes, negative and positive selection. These selection processes are to ensure that randomly rearranged TCRs expressed on the cell surface of T cells are functional, *i.e*. recognize major histocompatibility complex (MHC) molecule (MHC restriction) and harmless to self (self-tolerance), *i.e*. binding to self antigen with low affinity. The nature of MHC restriction is unique to T lymphocytes, since they recognize antigens only in the context of MHC/peptide complex (a process called antigen presentation). Although the molecular events leading to negative and positive selection are not well understood, multiple signaling pathways, the duration, and strength of the signals have all been shown to play an important role during these complicated processes.[14] T cells bearing TCR with a

high affinity to self-peptide/self MHC complex or self MHC alone, are potentially harmful by developing autoimmune disorders and are removed by apoptosis (negative selection). This process is speculated to be mediated partly by an orphan nuclear hormone receptor of the Nur77 protein family.[15-17] T cells expressing TCR that recognizes self MHC molecules are provided with survival signals through TCR and further develop to mature CD4 or CD8 single positive (SP) T cells (positive selection). T cells bearing TCR with low affinity to self-peptide MHC complex and unable to recognize antigens are left without any input of the survival signal and eventually die off. This process is termed "death by neglect" and is postulated to be induced by the thymic stromal cells-derived steroid hormone glucocorticoid.[18] It is hypothesized that the signals from TCR and glucocorticoid are antagonistic to each other and the balance between the two signals thought is to rescue cells from apoptosis as cells undergo positive selection.[18,19]

Nur77 was originally isolated as an immediate early gene induced in neuronal cells upon treatment with nerve growth factor.[20] By subtractive hybridization of RNA prepared from T cell hybridomas induced to die by TCR cross linking, it has been re-identified as an early response gene necessary for apoptosis induced in thymocytes upon TCR cross-linking.[21,22] Later, it was shown in a transgenic mouse model that overexpression of the Nur77 resulted in massive thymocyte apoptosis.[15] Dominant negative form of Nur77 lacking a transactivation domain, when overexpressed in thymocytes, inhibited antigen-induced negative selection in a mouse model leading to increased self-reactive thymocytes.[23] Because of functional redundancy among the members of the Nur77 family (Nur77, Nurr1 and Nor-1), targeted deletion of Nur77 alone did not yield any obvious phenotype.[24] By overexpressing a dominant negative form of Nur77, which inhibits the transcriptional activity of all members of the family, apoptosis of thymocytes is blocked.[25] In addition, expression of Nur77 is detected in thymocyte subset undergoing clonal deletion in unmanipulated thymus.[26] Taken together, these results imply an essential role of the Nur77 protein family in negative selection and apoptotic cell death of developing thymocytes induced by TCR cross linking. How Nur77 expression leads to cell death of thymocytes is not clear. Interestingly, Nur77 has been shown to localize to the mitochondria in dying cells and this translocation is essential for its pro-apoptotic activity suggesting an unprecedented mechanism for NHRs to regulate cell death directly from the mitochondria.[27]

Steroid hormone glucocorticoid is long known to induce thymic involution upon administration *in vivo*. It is wildly used as an anti-inflammatory agent and immunosuppressant. Glucocorticoid functions as a ligand for GR, which belongs to a steroid hormone receptor protein family, along with estrogen and progesterone receptor. Most developing thymocytes are susceptible to GR-mediated cell death *in vitro*, but thymocytes in different developmental stages show different susceptibility to this hormone. Cells in the DP stages are most susceptible, while cells in the SP stage are less susceptible. Activated GR is postulated to directly or indirectly regulate transcription of genes leading to cell death, but the signaling events down stream of GR activation are not well understood. DNA-binding defective GR completely lost the ability to induce apoptosis of the thymocytes when engaged by its ligands, while it still retained other physiological activities such as anti-inflammatory function.[28] Therefore, DNA binding of GR is necessary for inducing apoptosis in thymocytes. Two lines of evidence imply that GR-mediated cell death may involve degradation of anti-apoptotic protein. GR activation in thymocytes has been shown

to lead to selective degradation through the ubiquitin proteasome pathway of the anti-apoptotic protein, XIAP.[29] In addition, inhibitor specific for the proteasome activity has been shown to block cell death induced by glucocorticoid in thymocytes.[30] Therefore, GR-mediated cell death of thymocytes may utilize the proteasome pathway for selective degradation of the anti-apoptotic proteins leading to cell death. Whether GR plays a key role in the developmental processes of thymocytes at the physiological level are still controversial. While several groups reported the severe effects on thymic development when the level of GR is decreased by using a GR anti-sense mouse model, others have reported that there is no effect on development of thymocytes when GR null fetal thymi was analyzed.[31, 32]

Notch signaling pathway

Notch signaling is a well conserved signaling pathway involved in a broad range of cell fate specifications during development of metazoans (Figure 3).[33] Notch receptor is a single pass transmembrane protein expressed on the cell surface. The extracellular domain is rich in epidermal growth factor like repeats, while the intracellular domain contains several key motifs, including six ankyrin repeats and the PEST motif. There are four Notch proteins identified in mammals (Notch 1-4), while there is only one found in *Drosophila*, and two members are identified in nematode *Caenorhabditis elegans* (Lin12, Glp-1). The signaling pathway is activated by binding of the ligand to the receptor. There are multiple Notch ligands identified across species. There are at least five mammalian Notch ligands identified to date, *i.e*. Jagged 1-2, Delta-like 1, 3, 4. Both ligands and receptors show distinctive patterns of expression temporally and spatially, through the developmental stages. Therefore, it is likely that each receptor and ligand interaction plays a distinctive role in different tissues at different times. The existence of multiple ligands and receptors complicates the studies into the role of Notch. It remains unclear whether each Notch receptor responds similarly to all ligands or if the combination of each receptor-ligand yields a similar outcome or physiological condition.

Multiple lines of evidence suggest that Notch functions in the nucleus using the intracellular domain after activation by ligand binding (Figure 3). In *Drosophila*, when an intracellular form of Notch is overexpressed, it gives rise to the phenotype similar to a constitutively active Notch phenotype and an intracellular domain of Notch is exclusively found in the nucleus suggesting that Notch functions upon being processed.[34,35] Once Notch is bound by its ligand, the successive two proteolitical cleavage events mediated by the tumor necrosis factor α-converting enzyme and γ-secretase, lead to the cleavage of Notch within the transmembrane domain.[36,37] This proteolytic event results in releasing of the intracellular domain of Notch from the rest of the protein. Once released, the intracellular Notch translocates to the nucleus where it forms a heterodimer with a DNA-binding protein of the CSL protein family (CBF-1 (mammals) /Suppressor of Hairless (*Drosophila*) /Lag 1 (*C. elegans*)).[38] CSL and Notch heterodimer act as a transcriptional activator to transcribe Notch target genes. Various genes are identified as the Notch pathway target genes, such as a small basic helix-loop-helix HES protein family.

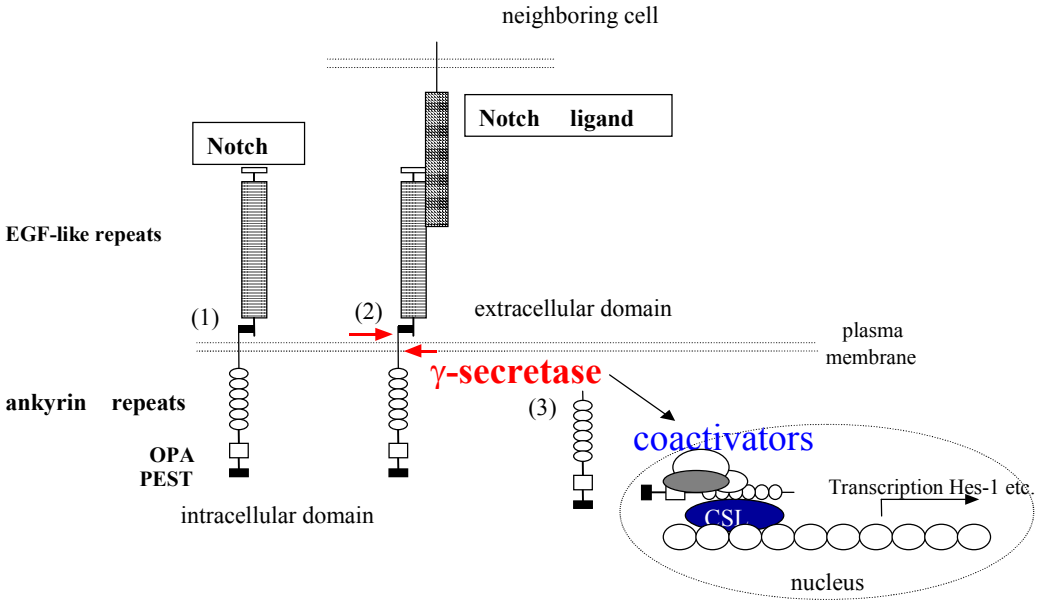

Figure 3. The basic structure of the Notch protein family is shown. Extracellular EGF-like repeats and intracellular ankyrin repeats are well conserved among the members of this family. The signaling pathway is activated by ligand binding and subsequent multiple enzymatic cleavages of the protein to release an intracellular domain. The released intracellular domain migrates to the nucleus and forms a transcriptional activation complex with CSL and transcriptional co-activator.

The most well characterized example of the role Notch plays in regulating cell fate decisions is during neuronal development (Figure 4). During development of *Drosophila* embryos, the neuronal cells and epidermal cells are derived from common precursor cells. Notch signal is active in epidermal cells and necessary for epidermal cell fate specification, while the neuronal cells contain very little Notch activity. When Notch is overexpressed during this stage, neuronal cells failed to develop and excess epidermal cells were observed.[34] In contrast, decreasing Notch activity resulted in excess neuronal cells at the expense of epidermal cells.[34] Therefore, in this instance, Notch signaling is necessary for commitment to epidermal cell fate, while it is dispensable for neuronal cell fate specification. The slight difference in the Notch signaling in two neighboring cells at the beginning of the developmental process is amplified by upregulation of Notch receptor and down regulation of the ligands in the signal receiving cell, while the signal sending cell does the opposite, *i.e*. down regulation of Notch receptor and upregulation of ligands. In this manner, Notch signal receiving cell adopts an epidermal cell fate, while the signal sending cell adopts a neuronal cell fate.

Many examples have been reported where Notch signaling played an essential role in cell fate specification at the branch point of the steps of cell fate decisions, such as during muscle, neuronal and thymic development.[33] During thymic development, both Notch receptors and ligands are expressed dynamically and have been viewed as one of the most crucial signalings regulating cell fate decision in T lymphocytes.[39] Notch1 has been shown to be necessary and sufficient for T cell development.[40] Recently, Notch signaling has been shown to be involved in post-thymic T cell functions.[41]

In general, Notch signaling is perceived as the signal to keep cells in an undifferentiated state until the next developmental cue directs another round of cell fate specification. Misregulation of Notch, therefore, often results in cell fate transformation. An important role of Notch during development is highlighted by the embryonic lethality of targeted gene disruption in the mouse model. Targeted deletion of *Notch1* and *Notch2* in mouse are embryonic lethal at a very early stage of development.[42,43]

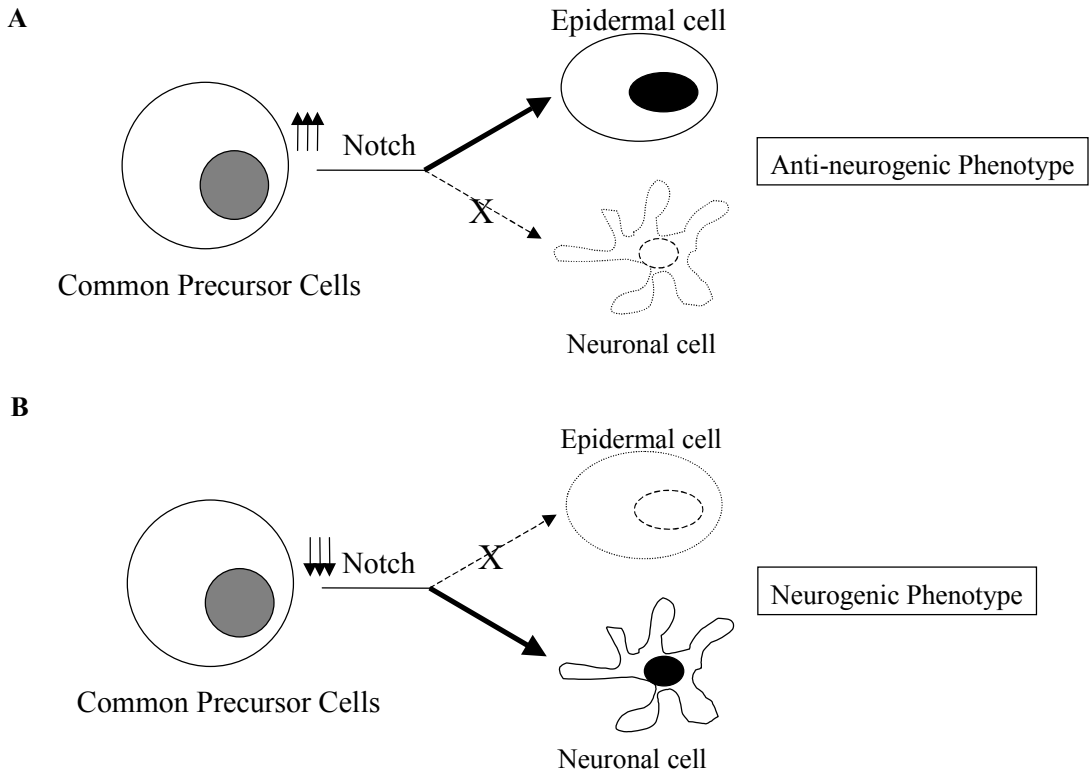

Figure 4. During differentiation of neuronal cell and epidermal cell from common precursor cell, Notch favors an epidermal cell fate over a neuronal cell fate. When Notch is aberrantly expressed, an epidermal cell develops at the expense of a neuronal cell (A). If Notch signaling is disrupted, a neuronal cell develops at the expense of an epidermal cell (B).

Notch signaling and NHR-mediated apoptosis

The efforts to identify anti-apoptotic signal components for apoptosis of developing thymocytes have been made independently in two laboratories. The first one was carried out using expression cloning strategy by infecting thymomas cell line with the retroviral constructs prepared from thymic cDNA library.[44] The selection of infected cells resistance to GR-mediated cell death resulted in identifying a cDNA encoding a truncated form of Notch protein.[44] This study further showed that overexpression of a truncated form of Notch mimicking the processed form of Notch both in T cell hybridomas and thymocytes rescued cells from undergoing apoptosis upon glucocorticoid treatment. The second study was carried out using a yeast two hybrid screen to identify the binding partner for Nur77.[45] This screen yielded multiple positive clones encoding a

truncated Notch protein. It was further shown that overexpression of truncated Notch rescued T cell hybridomas from cell death induced by TCR cross linking or pharmacological reagents phorbol-12-myristate-13-acetate and calcium ionophore treatment.[45] These two studies have shown for the first time that Notch signaling is capable of antagonizing the pro-apoptotic effect of NHRs in T cells. However, the molecular events underlying this anti-apoptotic effect of Notch are largely unclear. In one T cell hybridoma line, an anti-apoptotic gene *Bcl2* is upregulated when Notch is overexpressed.[44] This effect seemed to be a cell line specific effect and not a general mechanism Notch signal utilizes to oppose NHR-mediated cell death.

The role Notch plays in thymocyte apoptosis is studied mostly by overexpressing a truncated active form of Notch proteins. Since Notch signaling is pleiotropic, such an approach may render unknown side effects and the results gaining from such approach may not reflect an *in vivo* function of Notch. To circumvent this problem, we studied the role Notch plays in thymocytes apoptosis by using anti-sense Notch transgenic mice, where the expression of Notch1 is partly decreased.[41] A decrease in Notch1 expression significantly renders thymocytes more susceptible to GR-induced apoptosis, compared to the control wild type (manuscript in preparation). This result is consistent with an anti-apoptotic role of Notch in developing thymocytes. Notch signal has emerged as an anti-apoptotic signal regulating cell death induced by NHRs, a function which may be well conserved through evolution.

In developing thymocytes, Notch signaling negatively regulates the activity of NHRs. The functional interaction between Notch and NHRs may be one of many ways Notch regulates cell fate decisions. To this end, Notch signaling has been shown to regulate the activity of NHRs in some instances. Notch has been shown to regulate the expression of peroxisome proliferation activator receptor (PPAR), an NHR involved in adipogenesis.[46] Decreased Notch expression resulted in less PPAR expression, and as a result, differentiation to adipocytes is inhibited.

In contrast to an anti-apoptotic effect of Notch, some studies have implicated that Notch also positively regulates apoptosis. During *Drosophila* eye development, multiple local signalings direct a highly regulated pattern of *Drosophila* retina.[47] In this tissue, Notch has been shown to be essential for apoptosis of unneeded cells during patterning of the tissue. This pro-apoptotic activity of Notch is counteracted by survival signals generated by the Ras pathway.[47] Notch also induces apoptosis, when overexpressed, in chicken B cell line accompanied by cell cycle arrest.[48] Taken together the studies suggest that Notch signaling is able to manipulate the signaling leading to cell death in a cell-type dependent and context-dependent manner. Notch may not inhibit or induce apoptosis directly, but it may modulate the upstream early signaling, as seen in NHR-mediated cell death in T cells and insect tissues during metamorphosis.

How Notch regulates cell death decisions?

The observations that Notch signaling interferes with NHR-mediated cell death added Notch to the growing list of anti-apoptotic signals. An anti-apoptotic effect of Notch may help explain its involvement in tumor formations.[49,50] However, the molecular events leading to this activity are largely unknown. There are multiple lines of evidence suggesting that Notch may act through various pathways to achieve the outcome as an anti-apoptotic signaling. The potential apoptotic signals that Notch may interact with and regulate are discussed below.

1) Interaction with the NF-κB/Rel pathway

NF-κB pathway plays a significant role in development, differentiation, proliferation and cell death of T lymphocytes. NF-κB/Rel signaling has been shown to be either pro- or anti-apoptotic, depending on the context in which it functions.[51] Notch has been shown to interact, either physically or functionally, with the NF-κB pathway. Notch bound directly to one of the NF-κB family protein, p50.[52] This study postulated that Notch utilized the ankyrin repeats, similar to those of the intrinsic NF-κB inhibitor, IκB protein and sequestered the p50 protein preventing it from functioning as a transcription factor. This protein-protein interaction affects the availability of p50 in the cells. Thus, depending on the amount of p50 and its partner, another NF-κB family protein, p65, Notch can inhibit or enhance the signal of p50/p65 heterodimer. Furthermore, Notch has been shown to interact and inhibit the DNA binding activity of p50, using a different domain besides the ankyrin repeats.[53]

On the other hand, Notch also has been shown to directly promote NF-κB activation. In truncated Notch 3 transgenic mice where Notch 3 is overexpressed specifically in T cells, NF-κB is constitutively active.[54] In another study, Notch has been shown to regulate the NF-κB pathway in hemopoietic progenitor cells.[55] When Notch expression is decreased by expression of an antisense construct, NF-κB activity decreased. This is due to a decrease in an overall expression of NF-κB/Rel proteins. Therefore, in this case, Notch positively regulates the gene expression of NF-κ B/Rel protein family. Interestingly, Notch is shown to directly upregulate one of the NF-κB family proteins, NF-κB2, through CSL.[56] In Epstein-Barr viral transformed B cells, Notch signaling together with TNF pathways are used by the virus to sustain cell survival. In this case, NF-κB is essential for cell survival and inhibition of NF-κB leads to apoptosis of transformed cells.[57] Therefore, Notch signaling, by modulating NF-κB activity or gene expression, regulates the cell survival and other biological processes governed by NF-κB. Apart from being regulated by Notch signaling, NF-κB pathway also regulates the activity of Notch signaling. This is achieved by regulating the expression at the transcriptional level of Notch ligand, Jagged 1 in T lymphocytes.[58] The intricate interaction between Notch and NF-κB/Rel signaling components may render Notch both pro- and anti-apoptotic effect, depending on the cell types and operating NF-κB pathway components in the cells.

2) Interfering with the function of apoptotic proteins

Notch has been identified through the yeast two hybrid assay as a binding partner of Nur77 in T cell hybridoma system.[45] A recent study analyzed protein-protein interaction between Nur77 and anti-apoptotic protein Bcl-2 and showed a potential mechanism by which Nur77 kills cells. Binding of Nur77 to Bcl-2 triggered the conformational changed of Bcl-2 and turned it into a pro-apoptotic molecule.[59] Under the condition when Notch is activated, Notch may compete with Bcl-2 to bind to Nur77 and, thus, disrupt the fatal interaction between Nur77 and Bcl-2. Currently, Nur77 is the sole NHR that is shown to interact physically with Notch. Therefore, this proposed mechanism can only explain the anti-apoptotic effect of Notch against Nur77-mediated cell death. Further investigations into the physical interaction among NHRs, Notch and Bcl-2 apoptotic family proteins are needed to confirm this possible mechanism.

Several groups have shown in recent years that Notch and tumor suppressor gene *p53* may interact genetically and functionally. Notch activation induced

upregulation of p53 in hepatocellular carcinomas and resulted in p53-dependent apoptosis of neuronal progenitor cells.[60,61] On the other hand, loss of p53 in thymomas resulted in activation of Notch 1 and altered thymic development.[62] Therefore, crossregulation between Notch and p53 may be another strategy Notch uses to regulate cell death decision.

3) Regulation of cell cycles and cell proliferation

Notch signaling, when activated, has a dramatic effect on cell cycle regulation. The interference with cell cycle may be a way Notch regulates apoptosis. In one study, overexpression of activated Notch in RKE cell lines resulted in an abrupt induction of cell cycle regulator cyclinD1 and increased CDK2 activity, resulting in promotion of cell cycle entry.[60] The presence of the CBF1 binding sites within the promoter of cyclin D1 also suggests that this activity of Notch is mediated in a CBF1-dependent manner. Notch also promotes cell proliferation through one of its target genes, Hes1. This has been shown in developing T cells by targeted deletion of *Hes1*, and in cerebellar granule neuron precursors by overexpressing Hes1.[61, 62]

In contrast, Notch has also been shown to induce cell cycle arrest in some systems.[63-65] In some of these studies, Notch activation leads to induction of $p21^{WAF1/Cip1}$ in small cell lung cancer cells and in keratinocytes, which results in cell cycle arrest.[63,65] Therefore, the ability to control cell cycle by Notch is context-dependent, which may lead to different outcomes regarding cell death.

Implications and future directions

If one regards cell death as one of cell fates, Notch signaling as a regulator of cell death induced by NHRs emerges as one way Notch utilizes to interact with cell fate decisions. Apart from the cell death, the interaction between the two pathways is of interest. Both Notch and NHRs have been shown separately to have pleiotropic effects during development and are essential in maintaining homeostasis in the mature adult. The cross talk between these two important pathways highlights the fact that a single signaling pathway does not operate independently, but rather the intricate and complex interaction between two pathways cause cells to interpret developmental cues differently. Therefore, misregulation of one pathway is likely to affect the other and, hence, affect cell fate decision.

The largely unknown mechanisms of Notch in interfering with NHR activity need further investigation. Since both Notch and NHRs operate in many tissues in different manners, the tissue specific nature of each pathway needs to be taken into consideration. Furthermore, until now, overexpression of activated Notch was a common tool to investigate the role of Notch. The results gained from such studies are informative but need to be revisited as the superphysiological expression of Notch may not at all reflect its *in vivo* activity. To this end, decreasing or targeted deletion of Notch is a better way to gain an insight into the physiological role Notch plays in regulating NHR activity. In mammals, as four Notch proteins exist, the functional redundancy among the members also needs to be taken into consideration.

Notch pathways have been linked to tumor formation of many tissues, such as lymphoid systems and breast tissues.[49, 54, 66] It has been shown *in vitro* that activated Notch is able to induce neoplastic formation by cooperation with other oncogenes, such as adenoviral *E1A*, *c-myc* and *E2A-PBX1*.[50, 67, 68] Considering the role Notch plays in tumorigenesis in many tissues, it is likely that the ability of hyperactivation of Notch to inhibit cell death contributes to some extent to tumor formation. Interfering with the Notch signaling, such as through inhibition of the processing of Notch or using Notch "decoy" receptors may open up a novel

approach to the treatment of cancer in a tumor and tissue specific manner.

ACKNOWLEDGMENTS

I thank Professor Dr. Barbara Osborne for the critical reading of this manuscript. This work was supported in part by the Grant for Development of New Faculty Staff at Chulalongkorn University.

REFERENCES

1. Winoto, A. and Littman, D. (2002) "Nuclear hormone receptors in T lymphocytes" *Cell* **109**, s57-s66.
2. Evans, R. (1988) "The steroid and thyroid hormone receptor superfamily" *Science* **240**, 889-895.
3. Giguere, V. (1999) "Orphan nuclear receptors: from gene to function" *Endocr. Rev.* **20**, 689-725.
4. Karin, M. (1998) "New twists in gene regulation by glucocorticoid receptor" Is DNA binding dispensable? *Cell* **93**, 487-490.
5. Chen, J. and Evans, R. (1995) "A transcriptional co-repressor that interacts with nuclear hormone receptors" *Nature* **377**, 454-457.
6. Thummel, C. (1995) "From embryogenesis to metamorphosis : The regulation and function of Drosophila nuclear receptor superfamily members" *Cell* **83**, 871-877.
7. Baehrecke, E. (2000) "Steroid regulation of programmed cell death during *Drosophila* development" *Cell Death Differ* **7**, 1057-1062.
8. Tata, J. (1996) "Metamorphosis: an exquisite model for hormonal regulation of post-embryonic development" *Biochem. Soc. Symp.* **62**, 123-126.
9. Sun, Z., Unutmaz, D., Zou, Y., Sunshine, M., Pierani, A., Brenner-Morton, S., Mebius, R. and Littman, D. (2000) "Requirement for RORgamma in thymocyte survival and lymphoid organ development" *Science* **288**, 2369-2373.
10. Kurebayashi, S., Ueda, E., Sakaue, M., Patel, D., Medvedev, A., Zhang, F. and Jetten, A. (2000) "Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis" *Proc. Natl. Acad. Sci. USA.* **97**, 10132-10137.
11. Szondy, Z., Reichert, U. and Fesus, L. (1998) "Retinoic acids regulate apoptosis of T lymphocytes through an interplay between RAR and RXR receptors" *Cell Death Differ* **5**, 4-10.
12. Osborne, B. (2000) "Transcriptional control of T cell development" *Curr. Opin. Immunol.* **12**, 301-306.
13. Osborne, B. (1995) "Induction of genes during apoptosis: examples from the immune system" *Semin Cancer Bio.* **6**, 27-33.
14. Hoaquist, K. (2001) "Signal strength in thymic selection and lineage commitment" *Curr Opin Immunol* **13**, 225-231.
15. Calnan, B. J., Szychowski, S., Chan, F. K. M., Cado, D. and Winoto, A. (1995) "A role for the orphan steroid receptor Nur77 in apoptosis accompanying antigen-induced negative selection" *Immunity* **3**, 273-282.
16. Xue, Y., Chomez, P., Castanos-Velez, E., Biberfeld, P., Perlmann, T. and Jondal, M. (1997) "Positive and negative thymic selection in T cell receptor-transgenic mice correlate with Nur77 mRNA expression" *Eur. J. Immunol.* **27**, 2048-2056.
17. Amsen, D., Calvo, C. R., Osborne, B. A. and Kruisbeek, A. M. (1999) "Costimulatory signals are required for induction of transcription factor Nur77 during negative selection of CD4+CD8+ thymocytes" *Proc. Natl. Acad. Sci. USA.* **96**, 622-627.
18. Ashwell, J. D., Lu, F. W. M. and Vacchio, M. S. (2000) "Glucocorticoids in T cell development and function" *Annu. Rev. Immunol.* **18**, 309-345.
19. Jamieson, C. A. M. and Yamamoto, K. R. (2000) "Crosstalk pathway for inhibition of glucocorticoid-induced apoptosis by T cell receptor signaling" *Proc. Natl. Acad. Sci.* **97**, 7319-7324.
20. Milbrandt, J. (1988) "Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene" *Neuron* **1**, 183-188.
21. Liu, Z., Smith, S. W., McLaughlin, K. A., Schwartz, L. M. and Osborne, B. A. (1994) "Apoptotic signals delivered through the T-cell receptor of a T-cell hybrid require the immediate-early gene nur77" *Nature* **367**, 281-284.

22. Woronicz, J., Calnan, B., Ngo, V. and Winoto, A. (1994) "Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas" *Nature* **367**, 277-281.
23. Zhou, T., Cheng, J., Yang, P., Wang, Z., Liu, C., Su, X., Bluethmann, H. and Mountz, J. D. (1996) "Inhibition of Nur77/Nurr1 leads to inefficient clonal deletion of self-reactive T cells" *J. Exp. Med.* **183**, 1879-1892.
24. Lee, S. L., Wesselschmidt, R. L., Linette, G. P., Kanagawa, O., Russell, J. H. and Milbrandt, J. (1995) "Unimpaired thymic and peripheral T cell death in mice lacking the nuclear receptor NGFI-B (Nur77)" *Science* **269**, 532-535.
25. Cheng, L. E. C., Chan, F. K. M. C., Cado, D. and Winoto, A. (1997) "Functional redundancy of the Nur77 and Nor-1 orphan steroid receptors in T-cell apoptosis" *EMBO J.* **16**, 1865-1875.
26. Cho, H., Edmondson, S., Miller, A., Sellars, M., Alexander, S., Somersan, S. and Punt, J. (2003) "Identification of the targets of clonal deletion in an unmanipulated thymus" *J. Immunol.* **170**, 10-13.
27. Li, H., Kolluri, S., Gu, J., Dawson, M., Cao, X., Hobbs, P., Lin, B., Chen, G., Lu, J., Lin, F., Xie, Z., Fontana, J., Reed, J. and Zhang, X. (2000) "Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3" *Science* **289**, 1159-1164.
28. Reichardt, H., Kaestner, K., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., Gass, P., Schmid, W., Herrlich, P., Angel, P. and Schutz, G. (1998) "DNA binding of the glucocorticoid receptor is not essential for survival" *Cell* **93**, 531-541.
29. Yang, Y., Fang, S., Jensen, J. P., Weissman, A. M. and Ashwell, J. D. (2000) "Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli" *Science* **288**, 874-877.
30. Grimm, L. M., Goldberg, A. L., Poirier, G. G., Schwartz, L. M. and Osborne, B. A. (1996) "Proteasomes play an essential role in thymocyte apoptosis" *EMBO J.* **15**, 3835-3844.
31. King, L., Vacchio, M., Dixon, K., Hunziker, R., Margulies, D. and Ashwell, J. (1995) "A targeted glucocorticoid receptor antisense transgene increases thymocyte apoptosis and alters thymocyte development" *Immunity* **3**, 647-656.
32. Purton, J., Boyd, R., Cole, T. and Godfrey, D. (2000) "Intrathymic T cell development and selection proceeds normally in the absence of glucocorticoid receptor signaling" *Immunity* **13**, 179-186.
33. Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. J. (1999) "Notch signaling: cell fate control and signal integration in development" *Science* **284**, 770-776.
34. Lieber, T., Kidd, S., Alcamo, E., Corbin, V. and Young, M. Y. (1993) "Antineurogenic phenotypes induced by truncated Notch proteins indicate a role in signal transduction and may point to a novel function for Notch in nuclei" *Genes & Development* **7**, 1949-1965.
35. Rebay, I., Fehon, R. G. and Artavanis-Tsakonas, S. (1993) "Specific truncations of *Drosophila* Notch define dominant activated and dominant negative forms of the receptor" *Cell* **74**, 319-329.
36. Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, Roux, P., Black, R. A. and Israel, A. (2000) "A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE" *Mol. Cell* **5**, 207-216.
37. De Strooper, B. D., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M., Ray, W. J., Goate, A. and Kopan, R. (1999) "A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain" *Nature* **398**, 518-522.
38. Fortini, M. E. and Artavanis-Tsakonas, S. (1994) "The Suppressor of Hairless protein participates in Notch receptor signaling" *Cell* **79**, 273-282.
39. Radtke, F., Wilson, A., Mancini, S. and MacDonald, H. (2004) "Notch regulation of lymphocyte development and function" *Nat. Immunol.* **5**, 247-253.

40. Radtke, F., Wilson, A., Stark, G., Bauer, M., van Meerwijk, J., MacDonald, H. and Aguet, M. (1999) "Deficient T cell fate specification in mice with an induced inactivation of Notch1" *Immunity* **10**, 547-558.
41. Palaga, T., Miele, L., Golde, T. and Osborne, B. (2003) "TCR-mediated Notch signaling regulates proliferation and IFN-gamma production in peripheral T cells" *J. Immunol.* **171**, 3019-3024.
42. Swiatek, P. J., Lindsell, C. E., Franco del Amo, F., Weinmaster, G. and Gridley, T. (1994) "Notch1 is essential for postimplantation development in mice" *Genes & Dev.* **8**, 707-719.
43. Hamada, Y., Kadokawa, Y., Okabe, M., Ikawa, M., Coleman, J. R. and Tsujimoto, Y. (1999) "Mutation in ankyrin repeats of the mouse Notch2 gene induces early embryonic lethality" *Development* **126**, 3415-3424.
44. Deftos, M. L., He, Y.-W., Ojala, E. W. and Bevan, M. J. (1998) "Correlating Notch signaling with thymocyte maturation" *Immunity* **9**, 777-786.
45. Jehn, B. M., Bielke, W., Pear, W. S. and Osborne, B. A. (1999) "Protective effect of Notch-1 on TCR-induced apoptosis" *J. Immunol.* **162**, 635-638.
46. Garces, C., Ruiz-Hidalgo, M. J., De Mora, J. F., Park, C., Miele, L., Goldstein, J., Bonvini, E., Porras, A. and Laborda, J. (1997) "Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis" *J. Biol. Chem.* **272**, 29729-29734.
47. Miller, D. and Cagan, R. (1998) "Local induction of patterning and programmed cell death in the developing Drosophila retina" *Development* **125**, 2327-2335.
48. Morimura, T., Goitsuka, R., Zhang, Y., Saito, I., Reth, M. and Kitamura, D. (2000) "Cell cycle arrest and apoptosis induced by Notch1 in B cells" *J. Biol. Chem.* **275**, 36523-36531.
49. Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D. and Sklar, J. (1991) "TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in Tlymphoblastic neoplasms" *Cell* **66**, 649-661.
50. Capobianco, A. J., Zagouras, P., Blaumeller, C. M., Artavanis-Tsakonas, S. and Bishop, J. M. (1997) "Neoplastic transformation by truncated alleles of human *Notch1/TAN1* and *Notch2*" *Mol. Cell. Bio.* **17**, 6265-6273.
51. Barkett, M. and Gilmore, T. (1999) "Control of apoptosis by Rel/NF-kB transcription factors" *Oncogene* **18**, 6910-6924.
52. Guan, E., Wang, J., Laborda, J., Norcross, M., Baeuerle, P. A. and Hoffman, T. (1996) "T cell leukemia-associated human Notch/translocation-associated Notch homologue has IkB-like activity and physically interacts with nuclear factor-kB proteins in T cells" *J. Exp. Med.* **183**, 2025-2032.
53. Wang, J., Shelly, L., Miele, L., Boykins, R., Norcross, M. A. and Guan, E. (2001) "Human Notch-1 inhibits NF-kB activity in the nucleus through a direct interaction involving a novel domain" *J. Immunol.* **167**.
54. Bellavia, D., Campese, A. F., Alesse, E., Vacca, A., Felli, M. P., Balestri, A., Stoppacciaro, A., Tiveron, C., Tatangelo, L., Giovarelli, M., Gaetano, C., Ruco, L., Hoffman, E. S., Hayday, A. C., Lendahl, U., Frati, L., Gulino, A. and Screpanti, I. (2000) "Constitutive activation of NF-kappaB and T-cell leukemia/lymphoma in Notch3 transgenic mice" *EMBO J.* **19**, 3337-3348.
55. Cheng, P., Zlobin, A., Volgina, V., Gottipati, S., Osborne, B., Simel, E. J., Miele, L. and Gabrilovich, D. I. (2001) "Notch-1 regulates NF-kB activity in hemopoietic progenitor cells" *J. Immunol.* **167**, 4458-4467.
56. Oswald, F., S., L., Adler, G. and Schmid, R. M. (1998) "NF-kB2 is a putative target gene of activated Notch-1 via RBP-Jκ" *Mol. Cell Biol.* **18**, 2077-2088.
57. Cahir-MacFarland, E., Davidson, D., Schauer, S., Duong, J. and Kieff, E. (2000) "NF-kappa B inhibition causes spontaneous apoptosis in Epstein-Barr virus-transformed lymphoblastoid cells" *Proc. Natl. Acad. Sci. USA.* **97**, 6055-6060.

58. Bash, J., Zong, W.-X., Banga, S., Revera, A., Ballard, D. W., Ron, Y. and Gelinas, C. (1999) "Rel/NF-kB can trigger the Notch signaling pathway by inducing the expression of Jagged1, a ligand for Notch receptors" *EMBO J.* **18**, 2803-2811.
59. Lin, B., Kolluri, S., Lin, F., Liu, W., Han, Y. H., Cao, X., Dawson, M., Reed, J. and Zhang, X. (2004) "Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3" *Cell* **116**, 527-540.
60. Qi, R., An, H., Yu, Y., Zhang, M., Liu, S., Guo, Z., Cheng, T. and Cao, X. (2004) "Notch1 signaling inhibits growth of human hepatocellular carcinoma through induction of cell cycle arrest and apoptosis" *Cancer Res.* **63**, 8323-8329.
61. Yang, X., Klein, R., Tian, X., Cheng, H., Kopan, R. and Shen, J. (2004) "Notch activation induces apoptosis in neuronal progenitor cells through a p-53-dependent pathway" *Dev. Biol.* **269**, 81-94.
62. Laws, A. and Osborne, B. (2004) p53 regulates thymic Notch1 activation. *Eur. J. Immunol.* **34**, 726-734.
63. Ronchini, C. and Capobianco, A. (2001) "Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch (ic)" *Mol. Cell Biol.* **21**, 5925-5934.
64. Tomita, K., Hattori, M., Nakamura, E., Nakanishi, S., Minato, N. and Kageyama, R. (1999) "The bHLH gene *Hes1* is essential for expansion of early T cell precursors" *Genes Dev.* **13**, 1203-1210.
65. Solecki, D., Liu, X., Tomoda, T., Fang, Y. and Hatten, M. (2001) "Activated Notch2 signaling inhibits differentiation of cerebella granule neuron precursors by maintaining proliferation" *Neuron* **31**, 557-568.
66. Morimura, T., Goitsuka, R., Zhang, Y., Saito, I., Reth, M. and Kitamura, D. (2000) "Cell cycle arrest and apoptosis induced by Notch1 in B cells" *J. Biol. Chem.* **275**, 36523-36531.
67. Sriuranpong, V., Borges, M., Ravi, R., Arnold, D., Nelkin, B., Baylin, S. and Ball, D. (2001) "Notch signaling induced cell cycle arrest in small cell lung cancer cells" *Cancer Res.* **61**, 3200-3205.
68. Rangarajan, A., Talora, C., Okuyama, R., Nicolas, M., Mammucari, C., Oh, H., Aster, J., Krishna, S., Metzger, D., Chambon, P., Miele, L., Aguet, M., Radtke, F. and Dotto, G. (2001) "Notch signaling is a direct determination of keratinocyte growth arrest and entry into differentiation" *EMBO J.* **20**, 3427-3436.
69. Gallahan, D. and Callahan, R. (1997) "The mouse mammary tumor associated gene INT3 is a unique member of the NOTCH gene family (NOTCH4)" *Oncogene* **14**, 1883-1890.
70. Girad, L., Hanna, Z., Beaulieu, N., Hoemann, C. D., Simard, C., Kozak, C. A. and Jolicoeur, P. (1996) "Frequent provirus insertional mutagenesis of *Notch1* in thymomas of $MMTV^{D}$/myc transgenic mice suggests a collaboration of c-myc and Notch1 for oncogenesis" *Genes Dev.* **10**, 1930-1944.
71. Feldman, B. J., Hampton, T. and Cleary, M. L. (2000) "A carboxy-terminal deletion mutant of Notch1 accelerates lymphoid oncogenesis in E2A-PBX1 transgenic mice" *Blood* **96**, 1906-1913.

Received: May 3, 2004
Accepted: July 7, 2004