

# Notch as an Immunologic Basis of Cancer Disparities

Portia LaLiah Thomas<sup>1,2,3</sup> and Anil Shanker<sup>1,3,4,5,\*1</sup>

<sup>1</sup>Department of Biochemistry, Cancer Biology, Neuroscience and Pharmacology, School of Medicine, Meharry Medical College, Nashville, TN, USA <sup>2</sup>Department of Microbiology, Immunology and Physiology, School of Medicine, Meharry Medical College, Nashville, TN, USA <sup>3</sup>School of Graduate Studies and Research, Meharry Medical College, Nashville, TN, USA <sup>4</sup>Host-Tumor Interactions Research Program, Vanderbilt-Ingram Comprehensive Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, USA <sup>5</sup>Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University School of Medicine, Nashville, TN, USA

\*Corresponding author: Email: ashanker@mmc.edu

## ABSTRACT

Inter-individual differences due to racial/ethnic backgrounds may alter host immunity responsible for the cancer immunosurveillance and elimination, leading to disparate cancer incidence and relapse. One basis of disparity in tumor incidence, progression or therapeutic outcomes could lie in the components of Notch intercellular communication system, which provide instructive signals for a variety of pathways regulating cell commitment and differentiation including context-dependent lymphocyte polarization in tumor microenvironment. Notch signaling in hematopoietic cells is perturbed by tumor growth for its advantage, and there are indications that differences in Notch components could underlie poor cancer prognosis in certain populations. Here, we discuss the oncogenic and immunologic aspects of Notch, which should inform on cancer health disparities and therapeutic outcomes.

**KEYWORDS:** Cancer immunity, Health disparity, Notch, Lymphocytes, Immunosurveillance, Immunotherapy

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## INTRODUCTION

### Immunologic basis of racial disparities

Racial disparities in cancer are well documented in various solid cancers. This disparity is particularly evident in breast cancer, where paradoxically, the mortality rates in African-American compared to White American/Caucasian women are considerably higher despite lower lifetime incidence rates of breast cancer (Fregene and Newman, 2005; Howlader et al., 2018). Although many differences in breast cancer incidence/outcome can be explained by socioeconomic disadvantages, recent studies have broadened our understanding of the correlation of genetic mutations and ancestry with race/ethnic-associated disparities. It is crucial to note that genetics not only affects cancer susceptibility but the immune response to cancer as well. It has long been recognized that lymphocytes, specifically natural killer (NK) cells and T cells, differ significantly in their ability to mediate effector responses depending on the genetic constitution of an individual. The major histocompatibility and the leukocyte receptor complexes are the two most polymorphic regions of the immune genome. Individuals with increasingly diverse repertoires of MHC class-I molecules have a greater potential for their NK cells to be more responsive. This is evident from the differential susceptibility of some individuals to HIV infection and their wide range of asymptomatic phase (Kulkarni et al., 2009; Martin et al., 2018; Ramsuran et al., 2018). The underlying mechanism of the differences in lymphocyte effector responses against tumor in disparate populations could involve differential expression of inhibitory and activating receptors on lymphocytes. One of these receptors belongs to the Notch family, which offers a major juxtacrine signaling system that allows cellular crosstalk to program almost every cell type in the body.

Notch signaling is highly conserved evolutionarily as it is important for cell-to-cell communication for tissue patterning during embryonic development. Mammals express four Notch receptors (Notch 1-4), which can bind to five canonical transmembrane ligands from two paralogous gene families– Delta-like (DLL1, DLL3, DLL4) and Jagged (JAG1 and JAG2) (Andersson et al., 2011; Radtke et al., 2010; Yuan et al., 2010). The Delta ligands transactivate Notch amongst neighboring cells and cis-inhibit Notch in its own cells following receptor engagement (Crabtree et al., 2016; Sprinzak et al., 2010; Yaron and Sprinzak, 2012). The receptor-ligand interaction between juxtaposing cells initiates a cascade of events involving transendocytosis, proteolytic cleavages, ubiquitination and deubiquitination transforming the cell surface receptor into a nuclear factor acting on the transcription of several target genes. Briefly, a conformational change in the ligated receptor exposes the S2 cleavage site (12–13 amino acids external to the transmembrane domain) for proteolysis by metalloproteases of the  $\alpha$ -disintegrin-and-metalloproteinase (ADAM) family. The Notch extracellular domain is shed and endocytosed by the ligand-expressing cell (Radtke et al., 2010). After shedding, the transmembrane domain is cleaved at the S3 site by  $\gamma$ -secretase freeing the Notch intracellular domain (NICD). The NICD subsequently traffics to the nucleus, heterodimerizing with DNA binding transcription factor CBF1/suppressor of hairless/LAG-1 (CSL). The C promoter-binding factor (CBF1) in humans is also known as recombination signal binding protein for immunoglobulin  $\kappa$  J region (RBPJ- $\kappa$ ) or  $\kappa$ -binding factor 2 (KBF2) in mice, as suppressor of hairless [Su(H)] in *Drosophila* and longevity-assurance gene-1 (LAG-1) in *Caenorhabditis elegans*. The CSL-bound NICD recruits various coactivators, including mastermind proteins (MAML1-3), inducing transcriptional expression of Notch downstream target genes hairy enhancer of split

(*Hes1*) and hairy related (*Hey1* or *Hrt*). Notch signaling can also crosstalk with other signaling pathways such as NF- $\kappa$ B and TGF- $\beta$ , widening the Notch downstream target genes involved in effector T cells (Kuijk et al., 2013; Radtke et al., 2010; Yuan et al., 2010). Promiscuous receptor-ligand binding makes Notch signaling highly dose- and context-dependent (Previs et al., 2015).

Notch can contribute to tumorigenesis if partnered with another onco-signaling, or can improve antitumor lymphocyte function if presented with the right repertoire of Notch receptor-ligands in the tumor microenvironment (Biktasova et al., 2015; Huang et al., 2011; Lobry et al., 2011). There are indications that differences in Notch components could underlie poor disease prognosis in certain populations. Based on data from the chronic obstructive pulmonary disease (COPD) clinical trial NCT00774176, the expression of mastermind-like protein 1 (MAML1), which affects Notch-dependent angiogenesis in lung, was found to be associated with COPD exacerbation in African Americans (Busch et al., 2016). As per The Cancer Genome Atlas (TCGA) data, a novel Notch protein, Notch 2 N-terminal like protein (NOTCH2NL/N2N), was found to be increased in breast cancer tissue of African-Americans relative to Caucasians. Correspondingly, disparities in Notch signaling can impact cancer development and therapy as discussed below.

## NOTCH IN CANCER DEVELOPMENT

Although Notch signaling plays a crucial role in embryonic development, tissue homeostasis, cell proliferation, apoptosis, hematopoiesis, as well as differentiation and function of various immune cells including lymphocytes, dysregulation of Notch signaling leads to several diseases, including cancer (Radtke et al., 2010; Yuan et al., 2010). For instance,

crosstalk between TGF- $\beta$  and Notch is essential for epithelial-mesenchymal transition (EMT) as Notch signaling is needed to sustain the expression of TGF- $\beta$ -induced Notch target gene *Hey1*, which acts as a transcriptional repressor (Klinakis et al., 2011). Deregulation of the Notch pathway can occur by various mechanisms including overexpression, mutational activation or inactivation, posttranslational modifications and epigenetic regulation (Ntziachristos et al., 2014).

### Notch signaling in breast cancer progression

Notch signaling drives many human hematologic and solid malignancies including breast cancer, medulloblastoma, colorectal cancer, lung cancer and melanoma (Ntziachristos et al., 2014). Notch signaling plays a central role in breast cancer development and progression by promoting tumor growth, invasiveness and metastasis (Previs et al., 2015). Increased expression of NOTCH1 and NOTCH3 receptors have been associated with triple-negative breast cancer (TNBC). NOTCH4 overexpression has been correlated with hormone-receptor positive breast cancer. On the contrary, NOTCH2 has been associated with better survival (Parr et al., 2004). In addition, NOTCH1 has lowered expression in HER-2 positive breast cancers (Touplikioti, 2012). Studies also indicated that high *NOTCH1* and *JAG1* in breast cancer patients correlated with poorer overall survival (Reedijk et al., 2005). Recently, *NOTCH1*, *NOTCH3* and *JAG1* were shown to be at the nexus of a vicious cycle of macrophage infiltration into basal-like breast cancers by regulating the expression of proinflammatory cytokines, IL-1 $\beta$  and CCL2, thus increasing cancer invasiveness (Shen et al., 2017).

### Notch signaling in lung cancer progression

Notch signaling plays an integral role in lung cancer initiation and progression. In non-small cell lung

cancer (NSCLC), Notch signaling crosstalks with various transcription factors to enhance EMT during cancer development. Blockade of Notch signaling inhibits progression and migration of NSCLC by reversing EMT (Xu et al., 2018; Yuan et al., 2014). In a wide variety of lung cancer patients (squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma and large cell carcinoma) expression levels of Delta-like Notch ligands *DLL1* and their target gene *HES1* were significantly altered in hematopoietic compartment by tumor-derived factors. These changes in Notch components were reproduced in various murine cancer models including lung cancer. The decreased expression of Notch components resulted in tumor-induced immunosuppression in T cell function that correlated with poor prognosis (Biktasova et al., 2015; Huang et al., 2011; Thounaojam et al., 2015).

## NOTCH BASIS OF CANCER DISPARITIES

### Expression of Notch in breast cancer is subtype-dependent

Although the role of Notch in cancer initiation and progression is known, its contribution to cancer disparities has not been explored. The plausible effect of Notch on cancer disparities, however, can be seen in breast cancer where more aggressive subtypes known to disproportionately affect minority populations have differential Notch signaling than less aggressive types (hormone-receptor positive). Overexpression of Notch target gene *HES1*,  $\gamma$ -secretase protein presenilin-1 (*PSEN1*), and lunatic-fringe (*LFNG*) – a  $\beta$ 3N-acetylglucosaminyl-transferase, which regulates ligand-mediated activation of the Notch pathway – were found to be favorable for disease-free survival in luminal type A breast cancer. Overexpression of these same Notch genes, however, was unfavorable for disease-free survival in TNBC as analyzed in the TCGA breast cancer cohort

(Orzechowska et al., 2017). In a study using next generation sequencing to identify Notch mutations in solid tumors, only TNBCs showed *NOTCH1* and *NOTCH2* rearrangements which led to constitutive receptor activation (Stoeck et al., 2014). These studies provide evidence of distinct Notch signaling profiles in various breast cancer subtypes, which also have known racial disparities of incidence and outcome. Findings suggest that the differential expression of Notch components can affect breast cancer progression and elucidate response to treatment.

### Expression of Notch2N in breast cancer is race-dependent

A novel Notch protein, Notch 2 N-terminal-like protein (NOTCH2NL/N2N), has been found to be highly upregulated in breast, colorectal, and prostate cancer as reported in The Cancer Genome Atlas (TCGA). Notably, African-Americans show an increase in N2N expression in breast cancer relative to Caucasians ( $p = 0.0037$ ), per TCGA database (Fig. 1).

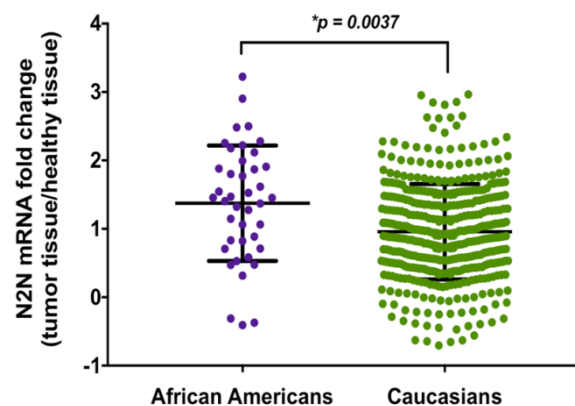


Figure 1. Expression of Notch 2 N-terminal like (N2N) RNA as a predictor of breast cancer disparities.

Genomic data available in The Cancer Genome Atlas (TCGA) were used to analyze N2N gene expression of breast cancer tissues from African Americans ( $n = 41$ ) in comparison with Caucasians ( $n = 423$ ).

\*Unpaired two-tailed *t*-test with Welch's correction.

*In vitro*, N2N has been shown to repress the transcriptional activities of Notch 2 and Notch 1 intracellular domains, which are important for antitumor lymphocyte effector function and memory (Biktasova et al., 2015; Huang et al., 2011; Thounaojam et al., 2015). Thus, N2N could be a possible predictive candidate for cancer disparities and poor prognosis amongst the African-American population. N2N has also been shown to be targeted by neutrophil elastase and implicated in hereditary neutropenia (Duan et al., 2004).

## ROLE OF NOTCH IN CANCER IMMUNITY

### Notch signaling in T lymphocytes

It is well established that tumor-induced immune suppression by multiple mechanisms is a major impediment to the success of cancer therapy. An intact functional immune system is required for the induction of sustained tumor regression upon inactivation of the tumor-driving oncogenes (Rakhra et al., 2010). The generation of effector CD8<sup>+</sup> T cells is imperative for antitumor immunity (Kuijk et al., 2013; Thounaojam et al., 2015; Uzhachenko and Shanker, 2016). The Notch signaling pathway plays an important role in the regulation of differentiation and function of lymphocytes, while being extremely pleiotropic with an interrelated network of receptor-ligand interactions. Most gain-of-function studies indicate that Delta-like ligands promote CD4<sup>+</sup> T cell commitment to Th1 (Amsen et al., 2009; Amsen et al., 2004). Although controversy exists, the bias is that Jag ligands associate with Th2-promoting Notch function (Amsen et al., 2009; Krawczyk et al., 2008). Notch has also been reported to associate with the regulation of *IL17* and *ROR $\gamma$ t* gene promoters to influence Th17 differentiation (Keerthivasan et al., 2011). In addition to promoting Th1, Th2 and Th17 differentiation, some Notch ligands, on the contrary, play an immunosuppressive function. Expression of

Jag ligands by antigen-presenting cells or hematopoietic progenitors favored generation of suppressive T cells *in vitro* and regulatory T cells (Treg) *in vivo* (Kared et al., 2006; Vigouroux et al., 2003; Yvon et al., 2003). In addition, expression of Delta-like Notch ligands in hematopoietic compartment is significantly altered by tumor-derived factors resulting in tumor-induced immunosuppression (Biktasova et al., 2015; Huang et al., 2011; Thounaojam et al., 2015). Systemic blockade of Jag1/2 or DLL1 overexpression overcame tumor-induced T cell tolerance suggesting the involvement of these ligands in anti-tumor T cell function (Huang et al., 2011; Palaga et al., 2003; Sierra et al., 2017). Evidence supports that Notch signaling promotes differentiation of naive CD8<sup>+</sup> T cells into cytotoxic and memory T lymphocytes by upregulating the transcription factor Eomesodermin responsible for regulating expression of effector molecules IFN $\gamma$ , granzymes, and perforins (Biktasova et al., 2015; Palaga et al., 2003; Radtke et al., 2010; Sauma et al., 2012; Thounaojam et al., 2015; Tsukumo and Yasutomo, 2004). Conditional transgenic expression of Notch 1 intracellular domain in CD8<sup>+</sup> T cells induces maturation towards a central memory phenotype (Sierra et al., 2014). In murine CD8<sup>+</sup> T cells, Notch signaling controls activated CD8<sup>+</sup> T cell fate towards terminal effector cell versus memory precursor cell fates (Backer et al., 2014). Studies also noted that Notch1/2 signaling was associated with increased IL-2 synthesis and upregulated expression of IL-2 receptor  $\alpha$  chain, CD25 on T cells and inhibition of Notch signaling resulted in decreased proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes (Adler et al., 2003; Thounaojam et al., 2015). In addition, treatment of tumor-bearing mice with cancer therapeutic drug bortezomib, a proteasome inhibitor, enhanced expression of Notch signaling components in lymphoid tissues resulting in CD8<sup>+</sup> T

cell expression of effector molecules, perforin and granzyme B as well as IFN- $\gamma$  secretion (Thounaojam et al., 2015). Furthermore, there appears to be a consensus in the published data to suggest that Notch 1 and Notch 2 are key players in the induction of cytolytic and memory T cell function (Auderset et al., 2012; Biktasova et al., 2015; Huang et al., 2011; Laky et al., 2015; Sierra et al., 2014; Sugimoto et al., 2010; Thounaojam et al., 2015).

Recently, it was shown that Notch 1 activation could occur in peripheral T cells in a ligand-independent manner through chemical adjustments in the endosome within a few hours post-TCR stimulation (Steinbuck et al., 2018). Alternatively, Notch 1/2 may fine-tune the sensitivity, magnitude and quality of the T cell response by promoting metabolic reprogramming besides specifying lineage choice or controlling expression of regulators following the initial steps of antigen encounter by T cells (Laky et al., 2015). Also, it is known that a transient pulse of a high level of Notch 1/2 cognate Delta-like ligand is capable of inducing *Hes1* expression for a duration that is sufficient to induce a binary cell fate switch. For example, transient DLL-Notch signaling has been shown to be sufficient to induce T cell (Lefort et al., 2006) or NK cell differentiation (Carotta et al., 2006).

### Notch signaling in NK cells

Human studies indicate that Notch 1 signaling is crucial for NK cell maturation and effector function as well, with an increase in Notch 1 signaling leading to an enhanced inhibitory killer immunoglobulin-like receptor (KIR) expression on NK cells. Augmented Notch 1 signaling also induces increased cytolytic effector capacity and cytokine secretion of human peripheral NK cells, enhancing their antitumor functions (Felices et al., 2014). Furthermore, an increase in Notch signaling by miR-181 increases production of IFN- $\gamma$  in primary NK cells (Cichocki et

al., 2011). In murine studies, dendritic cell overexpression of Notch ligand Jag2, which signals through Notch 2, directly enhances NK cytotoxicity, IFN- $\gamma$  production, and proliferation (Kijima et al., 2008). From these studies, it is evident that Notch signaling apparatus is critical not only for T cell effector and memory functions but also for NK cell function.

## OVERCOMING TUMOR INTERFERENCE WITH LYMPHOPOIETIC NOTCH

Given the critical roles of Notch in providing instructive signals for T cell and NK cell differentiation and function, it is logical to consider that tumors will interfere with Notch signaling in lymphocytes to promote and sustain tumor growth. Indeed, tumors downregulate or perturb Notch signaling in lymphocytes to escape immune surveillance. Moreover, tumors tend to alter the expression of Notch ligands as a prominent mechanism of immunosuppression in conjunction with elevated circulating levels of vascular endothelial growth factor (VEGF) (Huang et al., 2011; Novitskiy et al., 2010). In particular, tumors specifically downregulate expression of Delta-like ligands DLL1 and DLL4 in the tumor microenvironment to escape from T cell-mediated immunity (Biktasova et al., 2015; Huang et al., 2011; Thounaojam et al., 2015). Restoring the cognate Notch receptor signaling by enhancing the availability of DLL1 by endogenous overexpression or pharmacological administration of clustered multivalent DLL1 leads to improved tumor rejection (Biktasova et al., 2015; Huang et al., 2011). Furthermore, tumor-bearing mice following treatment with the proteasome inhibitor bortezomib, showed increased CD8<sup>+</sup> T lymphocyte IFN- $\gamma$  secretion and perforin and granzyme B expression by enhanced Notch-NF- $\kappa$ B signaling crosstalk (Thounaojam et al., 2015; Uzhachenko and Shanker,

2016). Thus, for effective treatments and addressal of disparities in cancers, immunotherapeutic strategies would need to overcome tumor-induced Notch-based immunosuppression.

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Although much remains to be learned about key aspects of Notch basis of cancer disparities, available evidence is clear to suggest that Notch signaling is crucial for antitumor lymphocyte effector and memory functions. Notch signaling is perturbed in hematopoietic cells by tumor growth for its advantage, and there are indications that differences in Notch components could underlie poor cancer prognosis in certain racial/ethnic populations. Due to the heterogeneity of Notch signaling in tumors and the potential for Notch to be onco- and tumorigenic on one hand and lymphostimulatory on the other, racial disparities in cancer may be addressed through systematic elucidation of following profiles.

(1) Characterizing the Notch profiles of various cancer subtypes and tumor-infiltrating immune cells in various racial/ethnic populations should serve to provide a useful resource for understanding Notch-based tumor-immune interactions in the tumor microenvironment. (2) Understanding the inter-individual host factors that influence naturally occurring lymphocyte responses will also be an important prerequisite to understand the host immune responsiveness and design a successful immunotherapeutic modality. In contrast to our understanding of the naturally occurring immune responses to many infectious agents, our knowledge of the immunogenetic factors that influence immune responsiveness to tumor-associated antigens is vastly incomplete. (3) An improved understanding of the immunogenetic mechanisms underlying T cell and

NK cell immunity in disparate racial/ethnic populations will be helpful in designing efficient personalized immune strategies against cancer. This knowledge would also be instrumental in the proper evaluation of lymphocyte-based immunotherapy trials as some people could be naturally high responders to adoptive cell immunotherapy, while others could be low responders. This possibility, unless taken into account, could confound the evaluation of immunotherapy trials. (4) Based on the outcomes of these studies, it will, then, be important to develop an immunogenetic signature of Notch signaling components, their receptors, ligands and downstream targets, in various racial/ethnic populations and establish their association with antitumor immune response patterns impacting cancer etiology. The studies could also shed light on the prognostic aspects of Notch in predicting possible cancer health disparities and the outcome of immunotherapy. Findings of these prospective studies would be critical to devise strategies to reverse Notch dysfunction in lymphocytes for effective tumor eradication and durable remission in cancer patients of varied ethnic backgrounds.

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## Conflict of interest

The authors declare that no competing or conflict of interests exist. The funders had no role in study design, writing of the manuscript, or decision to publish.

## Authors' contributions

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## REFERENCES

- Adler, S.H., Chiffolleau, E., Xu, L., Dalton, N.M., Burg, J.M., Wells, A.D., Wolfe, M.S., Turka, L.A., and Pear, W.S. (2003). Notch signaling augments T cell responsiveness by enhancing CD25 expression. *J Immunol* *171*, 2896-2903.
- Amsen, D., Antov, A., and Flavell, R.A. (2009). The different faces of Notch in T-helper-cell differentiation. *Nat Rev Immunol* *9*, 116-124.
- Amsen, D., Blander, J.M., Lee, G.R., Tanigaki, K., Honjo, T., and Flavell, R.A. (2004). Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* *117*, 515-526.
- Andersson, E.R., Sandberg, R., and Lendahl, U. (2011). Notch signaling: simplicity in design, versatility in function. *Development* *138*, 3593-3612.
- Auderset, F., Schuster, S., Coutaz, M., Koch, U., Desgranges, F., Merck, E., MacDonald, H.R., Radtke, F., and Tacchini-Cottier, F. (2012). Redundant Notch1 and Notch2 signaling is necessary for IFN $\gamma$  secretion by T helper 1 cells during infection with *Leishmania major*. *PLoS pathogens* *8*, e1002560.
- Backer, R.A., Helbig, C., Gentek, R., Kent, A., Laidlaw, B.J., Dominguez, C.X., de Souza, Y.S., van Trierum, S.E., van Beek, R., Rimmelzwaan, G.F., *et al.* (2014). A central role for Notch in effector CD8(+) T cell differentiation. *Nat Immunol* *15*, 1143-1151.
- Biktasova, A.K., Dudimah, D.F., Uzhachenko, R.V., Park, K., Akhter, A., Arasada, R.R., Evans, J.V., Novitskiy, S.V., Tchekneva, E.E., Carbone, D.P., *et al.* (2015). Multivalent Forms of the Notch Ligand DLL-1 Enhance Antitumor T-cell Immunity in Lung Cancer and Improve Efficacy of EGFR-Targeted Therapy. *Cancer Res* *75*, 4728-4741.
- Busch, R., Qiu, W., Lasky-Su, J., Morrow, J., Criner, G., and DeMeo, D. (2016). Differential DNA methylation marks and gene comethylation of COPD in African-Americans with COPD exacerbations. *Respir Res* *17*, 143.
- Carotta, S., Brady, J., Wu, L., and Nutt, S.L. (2006). Transient Notch signaling induces NK cell potential in Pax5-deficient pro-B cells. *Eur J Immunol* *36*, 3294-3304.
- Cichocki, F., Felices, M., McCullar, V., Presnell, S.R., Al-Attar, A., Lutz, C.T., and Miller, J.S. (2011). Cutting edge: microRNA-181 promotes human NK cell development by regulating Notch signaling. *J Immunol* *187*, 6171-6175.
- Crabtree, J.S., Singleton, C.S., and Miele, L. (2016). Notch Signaling in Neuroendocrine Tumors. *Front Oncol* *6*, 94.
- Duan, Z., Li, F.Q., Wechsler, J., Meade-White, K., Williams, K., Benson, K.F., and Horwitz, M. (2004). A novel notch protein, N2N, targeted by neutrophil elastase and implicated in hereditary neutropenia. *Mol Cell Biol* *24*, 58-70.
- Felices, M., Ankarlo, D.E., Lenvik, T.R., Nelson, H.H., Blazar, B.R., Verneris, M.R., and Miller, J.S. (2014). Notch signaling at later stages of NK cell development enhances KIR expression and functional maturation. *J Immunol* *193*, 3344-3354.
- Fregene, A., and Newman, L.A. (2005). Breast cancer in sub-Saharan Africa: how does it relate to breast cancer in African-American women? *Cancer* *103*, 1540-1550.
- Howlader, N., Cronin, K.A., Kurian, A.W., and Andridge, R. (2018). Differences in Breast Cancer Survival by Molecular Subtypes in the United States. *Cancer Epidemiol Biomarkers Prev* *27*, 619-626.
- Huang, Y., Lin, L., Shanker, A., Malhotra, A., Yang, L., Dikov, M.M., and Carbone, D.P. (2011). Resuscitating cancer immunosurveillance: selective stimulation of DLL1-Notch signaling in T cells rescues T-cell function and inhibits tumor growth. *Cancer Res* *71*, 6122-6131.
- Kared, H., Adle-Biassette, H., Fois, E., Masson, A., Bach, J.F., Chatenoud, L., Schneider, E., and Zavala, F. (2006). Jagged2-expressing hematopoietic progenitors promote regulatory T cell expansion in the periphery through notch signaling. *Immunity* *25*, 823-834.
- Keerthivasan, S., Suleiman, R., Lawlor, R., Roderick, J., Bates, T., Minter, L., Anguita, J., Juncadella, I., Nickoloff, B.J., Le Poole, I.C., *et al.* (2011). Notch signaling regulates mouse and human Th17 differentiation. *J Immunol* *187*, 692-701.
- Kijima, M., Yamaguchi, T., Ishifune, C., Maekawa, Y., Koyanagi, A., Yagita, H., Chiba, S., Kishihara, K., Shimada, M., and Yasutomo, K. (2008). Dendritic cell-mediated NK cell activation is controlled by Jagged2-Notch interaction. *Proc Natl Acad Sci U S A* *105*, 7010-7015.
- Klinakis, A., Lobry, C., Abdel-Wahab, O., Oh, P., Haeno, H., Buonamici, S., van De Walle, I., Cathelin, S., Trimarchi, T., Araldi, E., *et al.* (2011). A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. *Nature* *473*, 230-233.
- Krawczyk, C.M., Sun, J., and Pearce, E.J. (2008). Th2 differentiation is unaffected by Jagged2 expression on dendritic cells. *J Immunol* *180*, 7931-7937.
- Kuijk, L.M., Verstege, M.I., Rekers, N.V., Bruijns, S.C., Hooijberg, E., Roep, B.O., de Grujil, T.D., van Kooyk, Y., and Unger, W.W. (2013). Notch controls generation and function of human effector CD8+ T cells. *Blood* *121*, 2638-2646.



- Kulkarni, H., Marconi, V.C., He, W., Landrum, M.L., Okulicz, J.F., Delmar, J., Kazandjian, D., Castiblanco, J., Ahuja, S.S., Wright, E.J., *et al.* (2009). The Duffy-null state is associated with a survival advantage in leukopenic HIV-infected persons of African ancestry. *Blood* 114, 2783-2792.
- Laky, K., Evans, S., Perez-Diez, A., and Fowlkes, B.J. (2015). Notch signaling regulates antigen sensitivity of naive CD4+ T cells by tuning co-stimulation. *Immunity* 42, 80-94.
- Lefort, N., Benne, C., Lelievre, J.D., Dorival, C., Balbo, M., Sakano, S., Coulombel, L., and Levy, Y. (2006). Short exposure to Notch ligand Delta-4 is sufficient to induce T-cell differentiation program and to increase the T cell potential of primary human CD34+ cells. *Exp Hematol* 34, 1720-1729.
- Lobry, C., Oh, P., and Aifantis, I. (2011). Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think. *J Exp Med* 208, 1931-1935.
- Martin, M.P., Naranbhai, V., Shea, P.R., Qi, Y., Ramsuran, V., Vince, N., Gao, X., Thomas, R., Brumme, Z.L., Carlson, J.M., *et al.* (2018). Killer cell immunoglobulin-like receptor 3DL1 variation modifies HLA-B\*57 protection against HIV-1. *J Clin Invest*.
- Novitskiy, S.V., Csiki, I., Huang, Y., Johnson, D.H., Harth, E.M., Carbone, D.P., and Dikov, M.M. (2010). Anti-vascular endothelial growth factor treatment in combination with chemotherapy delays hematopoietic recovery due to decreased proliferation of bone marrow hematopoietic progenitor cells. *J Thorac Oncol* 5, 1410-1415.
- Ntziachristos, P., Lim, J.S., Sage, J., and Aifantis, I. (2014). From fly wings to targeted cancer therapies: a centennial for notch signaling. *Cancer Cell* 25, 318-334.
- Orzechowska, M., Jedroszka, D., and Bednarek, A.K. (2017). Common profiles of Notch signaling differentiate disease-free survival in luminal type A and triple negative breast cancer. *Oncotarget* 8, 6013-6032.
- Palaga, T., Miele, L., Golde, T.E., and Osborne, B.A. (2003). TCR-mediated Notch signaling regulates proliferation and IFN-gamma production in peripheral T cells. *J Immunol* 171, 3019-3024.
- Parr, C., Watkins, G., and Jiang, W.G. (2004). The possible correlation of Notch-1 and Notch-2 with clinical outcome and tumour clinicopathological parameters in human breast cancer. *Int J Mol Med* 14, 779-786.
- Previs, R.A., Coleman, R.L., Harris, A.L., and Sood, A.K. (2015). Molecular pathways: translational and therapeutic implications of the Notch signaling pathway in cancer. *Clin Cancer Res* 21, 955-961.
- Radtke, F., Fasnacht, N., and Macdonald, H.R. (2010). Notch signaling in the immune system. *Immunity* 32, 14-27.
- Rakhra, K., Bachireddy, P., Zabuawala, T., Zeiser, R., Xu, L., Kopelman, A., Fan, A.C., Yang, Q., Braunstein, L., Crosby, E., *et al.* (2010). CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. *Cancer Cell* 18, 485-498.
- Ramsuran, V., Naranbhai, V., Horowitz, A., Qi, Y., Martin, M.P., Yuki, Y., Gao, X., Walker-Sperling, V., Del Prete, G.Q., Schneider, D.K., *et al.* (2018). Elevated HLA-A expression impairs HIV control through inhibition of NKG2A-expressing cells. *Science* 359, 86-90.
- Reedijk, M., Odorcic, S., Chang, L., Zhang, H., Miller, N., McCready, D.R., Lockwood, G., and Egan, S.E. (2005). High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. *Cancer Res* 65, 8530-8537.
- Sauma, D., Ramirez, A., Alvarez, K., Roseblatt, M., and Bono, M.R. (2012). Notch signalling regulates cytokine production by CD8+ and CD4+ T cells. *Scand J Immunol* 75, 389-400.
- Shen, Q., Cohen, B., Zheng, W., Rahbar, R., Martin, B., Murakami, K., Lamorte, S., Thompson, P., Berman, H., Zuniga-Pflucker, J.C., *et al.* (2017). Notch Shapes the Innate Immunophenotype in Breast Cancer. *Cancer Discov* 7, 1320-1335.
- Sierra, R.A., Thevenot, P., Raber, P.L., Cui, Y., Parsons, C., Ochoa, A.C., Trillo-Tinoco, J., Del Valle, L., and Rodriguez, P.C. (2014). Rescue of notch-1 signaling in antigen-specific CD8+ T cells overcomes tumor-induced T-cell suppression and enhances immunotherapy in cancer. *Cancer Immunol Res* 2, 800-811.
- Sierra, R.A., Trillo-Tinoco, J., Mohamed, E., Yu, L., Achyut, B.R., Arbab, A., Bradford, J.W., Osborne, B.A., Miele, L., and Rodriguez, P.C. (2017). Anti-Jagged Immunotherapy Inhibits MDSCs and Overcomes Tumor-Induced Tolerance. *Cancer Res* 77, 5628-5638.
- Sprinzak, D., Lakhapanal, A., Lebon, L., Santat, L.A., Fontes, M.E., Anderson, G.A., Garcia-Ojalvo, J., and Elowitz, M.B. (2010). Cis-interactions between Notch and Delta generate mutually exclusive signalling states. *Nature* 465, 86-90.
- Steinbuck, M.P., Arakcheeva, K., and Winandy, S. (2018). Novel TCR-Mediated Mechanisms of Notch Activation and Signaling. *J Immunol* 200, 997-1007.
- Stoeck, A., Lejnine, S., Truong, A., Pan, L., Wang, H., Zang, C., Yuan, J., Ware, C., MacLean, J., Garrett-Engle, P.W., *et al.* (2014). Discovery of biomarkers predictive of GSI response in triple-negative breast cancer and adenoid cystic carcinoma. *Cancer Discov* 4, 1154-1167.
- Sugimoto, K., Maekawa, Y., Kitamura, A., Nishida, J., Koyanagi, A., Yagita, H., Kojima, H., Chiba, S., Shimada, M., and Yasutomo, K. (2010). Notch2 signaling is required for potent antitumor immunity in vivo. *J Immunol* 184, 4673-4678.
- Thounaojam, M.C., Dudimah, D.F., Pellom, S.T., Jr., Uzhachenko, R.V., Carbone, D.P., Dikov, M.M., and Shanker, A. (2015). Bortezomib enhances expression of effector molecules in anti-tumor CD8+ T lymphocytes by

- promoting Notch-nuclear factor-kappaB crosstalk. *Oncotarget* 6, 32439-32455.
- Touplikioti, P. (2012). Expression of Notch Receptors in Primary Breast Cancer and Correlation with Pathological Features. *Clinical and Experimental Pharmacology* 02.
- Tsukumo, S., and Yasutomo, K. (2004). Notch governing mature T cell differentiation. *J Immunol* 173, 7109-7113.
- Uzhachenko, R., and Shanker, A. (2016). Notching tumor: Signaling through Notch receptors improves antitumor T cell immunity. *Oncoimmunology* 5, e1122864.
- Vigouroux, S., Yvon, E., Wagner, H.J., Biagi, E., Dotti, G., Sili, U., Lira, C., Rooney, C.M., and Brenner, M.K. (2003). Induction of antigen-specific regulatory T cells following overexpression of a Notch ligand by human B lymphocytes. *J Virol* 77, 10872-10880.
- Xu, Y., Ren, H., Jiang, J., Wang, Q., Wudu, M., Zhang, Q., Su, H., Wang, C., Jiang, L., and Qiu, X. (2018). KIAA0247 inhibits growth, migration, invasion of non-small-cell lung cancer through regulating the Notch pathway. *Cancer Sci*.
- Yaron, A., and Sprinzak, D. (2012). The cis side of juxtacrine signaling: a new role in the development of the nervous system. *Trends Neurosci* 35, 230-239.
- Yuan, J.S., Kousis, P.C., Suliman, S., Visan, I., and Guidos, C.J. (2010). Functions of notch signaling in the immune system: consensus and controversies. *Annu Rev Immunol* 28, 343-365.
- Yuan, X., Wu, H., Han, N., Xu, H., Chu, Q., Yu, S., Chen, Y., and Wu, K. (2014). Notch signaling and EMT in non-small cell lung cancer: biological significance and therapeutic application. *J Hematol Oncol* 7, 87.
- Yvon, E.S., Vigouroux, S., Rousseau, R.F., Biagi, E., Amrolia, P., Dotti, G., Wagner, H.J., and Brenner, M.K. (2003). Overexpression of the Notch ligand, Jagged-1, induces alloantigen-specific human regulatory T cells. *Blood* 102, 3815-3821.