Crosstalk between Notch signaling Pathway and Glutamine uptake during Jurkat T cell activation

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Abstract

T cells are a part of the adaptive immune system. The function of T cells is dependent on T cell receptor-based activation, which is accompanied by cell growth, proliferation, and cytokine production. Jurkat cells are an immortalized human cell line derived from the T cell of a patient with acute leukemia. This cell line has been used for understanding many T cell receptor signaling and activation processes. Here we used this cell line to decipher the role of glutamine as a nutrient during the activation process and its cross-talk with the Notch1 signaling pathway. The activation of Jurkat T cells by phytohaemagglutinin (PHA)/phorbol myristate acetate (PMA) leads to a suppression of canonical Notch signaling pathway along with an increase in IL2 production in the presence of glutamine. This also increased the glutamine consumption by cells. Upon removal of glutamine, IL2 production decreased as was observed for murine-derived T effector cells. Interestingly, we found that constitutive expression of Hey1, the downstream effector of Notch pathway, prevented the surge in IL2 secretion upon activation, but the IL2 secretion steadily increased in glutamine-free condition. This observation shows that Notch signaling pathway effectors can modulate the cytokine secretion depending on the presence or absence of exogenous glutamine. This effect may be exploited in studying the modulation of T-cell activation in tumor microenvironment where there is a competition for nutrients between the proliferating tumor cells and the activated T cells.

Introduction

T cells control the adaptive immune response and are critical for immunological memory. When quiescent naive T cells are stimulated by an antigen, they are activated, which causes cell growth, proliferation, and cytokine production [1]. One of the main adaptations during T cell activation is the change in cellular metabolism. As increased proliferation of cells has a different metabolic requirement than quiescent cells, the activated T cells are required to produce more ATP, intermediates for biosynthesis, and propagate anabolic metabolism [2]. Increases in glucose metabolism via PI3K/Akt signaling [3] [4] and glutamine consumption via ERK/MAPK signaling [5] [6] are the two most important metabolic adaptations which that occur during T cell activation. Studies show that the mitochondrial metabolism in the absence of glucose metabolism is sufficient to support interleukin-2 (IL-2) induction [7], whereas unavailability of exogenous glutamine during T cell activation decreases IL-2 secretion [6]. The conserved Notch signaling pathway is known to be involved in a broad spectrum of cell fate decisions and differentiation processes [8]. Notch signaling is essential for initial commitment to the T cell lineage, and it also regulates subsequent steps of T cell development [9]. The regulation of mature T cell activation and differentiation by Notch signaling is ambiguous and the exact mechanisms are not clear [10]. Recently, it has been shown that glutamine consumption is deregulated by Notch signaling pathway [11]. Upon interaction between Notch receptor and its ligand, there is a cleavage of the Notch transmembrane protein releasing the Notch intracellular domain (NICD) which then translocates to the nucleus and cooperates with the DNA-binding factor RBP-Jk (CBF1, Suppressor of Hairless, and Lag-1-CSL) and its co-activators to promote transcription [12].

In this study, we investigated the role of Notch signaling pathway-mediated deregulation of glutamine consumption in the process of Jurkat T cell activation. Jurkat cells are a human lymphoblastic cell line that has been used extensively to study early signal transduction events in T-lymphocyte activation through the T cell receptor (TCR) in vitro [13]. Here we show that TCR-dependent stimulation of Jurkat cells by phytohaemagglutinin (PHA)/phorbol myristate acetate (PMA) causes a decrease in canoni-
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Notch signaling pathway activity along with an increase in glutamine consumption and IL2 production. This increase of IL2 secretion upon PHA/PMA stimulation was, however, prevented by constitutively expressing Notch downstream effectors Hey1 in the presence of glutamine but manifests the increase of IL2 in the absence of exogenous glutamine. Thus, the crosstalk between Notch signaling and glutamine metabolism can regulate cytokine production during T cell activation.

Objective
1. To understand the effect of Jurkat T cell activation on Notch signaling pathway.
2. To understand whether there is a correlation between glutamine uptake and Notch signaling during Jurkat T cell activation.
3. To understand the effect of Notch signaling downstream effectors on Jurkat T cell activation in the presence and absence of exogenous glutamine.

Figure Legend
Figure 1. Notch signaling pathway is downregulated in Jurkat T-cell activation and constitutive expression of Hey1 and its effect on Jurkat T cell activation. (A) Percentage change in glutamine consumption/amount of protein in Jurkat and Jurkat treated with PHA/PMA (n = 3, **p < 0.01). (B) IL2 secretion from Jurkat T cells treated with PHA/PMA grown in complete RPMI (containing glutamine) for 0, 24, 48 and 72 h, expressed in pg/ml/µg of cellular protein (normalized to the total protein obtained from the cells plated) (n = 6, ***p < 0.001 in comparison to 0 h).
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(C) IL2 secretion from Jurkat T cells treated with PHA/PMA and grown in glutamine-free RPMI for 0, 24, 48 and 72 h, expressed in pg/ml/µg of cellular protein (normalized to the total protein obtained from the cells plated) (n = 6, ***p < 0.001, *p < 0.05 in comparison to 0 h).

(D) Western blot of Notch pathway elements. Quantification of change in Hey1 expression (n = 3, ***p < 0.01).

(E) RBP-Jκ promoter activity. (n = 3, ***p < 0.001).

(F) Hey1 expression in Jurkat and Jurkat-Hey1.

(G) Percentage change in glutamine consumption/amount of protein in Jurkat-Hey1 and Jurkat-Hey1 treated with PHA/PMA (ns, not significant).

(H) IL2 secretion from Jurkat-Hey1 cells treated with PHA/PMA and grown in RPMI (containing glutamine) for 0, 24, 48 and 72 h, expressed in pg/ml/µg of cellular protein (normalized to the total protein obtained from the cells plated) (n = 6, ***p < 0.001, in comparison to 0 h).

(I) IL2 secretion from Jurkat-Hey cells treated with PHA/PMA and grown in glutamine-free RPMI for 0, 24, 48 and 72 h, expressed in pg/ml/µg of cellular protein (normalized to the total protein obtained from the cells plated) (n = 6, ***p < 0.001, *p < 0.05, ns-not significant in comparison to 0 h).

Cell culture
Jurkat cells, a CD4+ human lymphoblastoid cell line, were cultured in RPMI 1640 (Gibco) with 10% FBS (Gibco). Cells were stably transfected with pCMV-Hey1 plasmid kindly gifted by Dr. Kristian Helin (Biotech Research and Innovation Centre, Copenhagen, Denmark). For glutamine dependency studies, RPMI media without glutamine (Q-) was used. Jurkat T cells were stimulated by phytohaemagglutinin (PHA)/phorbol myristate acetate (PMA) (Sigma).

Western blotting
For western blotting, cells were lysed in cold radioimmune precipitation assay (RIPA) buffer. Equal amounts of protein were run on SDS-PAGE and blotted on PVDF membranes (GE Healthcare). After incubation with primary and HRP-conjugated secondary antibodies, the blots were developed using an Enhanced Chemiluminescent substrate (Thermo Fischer Scientific). β-actin (Abcam) was used as a loading control. The other antibodies used for the study were RBP-Jκ, SNAT2 (Santa Cruz Biotechnology), cleaved Notch1, and Hey1 (Abcam).

RBP-Jκ reporter assay
We used the RBP-Jκ Cignal kit (Qiagen) for the quantification of Notch canonical pathway signaling. The expression of the firefly luciferase was quantified with a Dual Luciferase Reporter assay system (Promega) according to the manufacturer’s protocol. All transfections were performed in triplicate. The level of the firefly luciferase activity was normalized by the corresponding level of the Renilla luciferase activity, and the values for the negative control were subtracted. Luminescence was measured using a Sirius Luminometer (Berthold Detection Systems).

Glutamine consumption assay
Cells (1×10⁴/well) were cultured in a 24 well plate under stimulated and unstimulated conditions for 48 h. Medium was collected, and cells were lysed for protein estimation. Concentrations of glutamine in the medium were determined using the glutamine/glutamate determination kit (Sigma). The glutamine consumption calculated was normalized to protein level, and then the fold change with respect to the unstimulated cells was obtained.

IL2 enzyme-linked immunosorbent assay
The conditioned medium of the cells was assayed for IL2 secretion using a sandwich ELISA kit (Biovision). The assay was performed according to the manufacturer’s instructions and the results presented as IL2 secreted per µg of cellular protein.

Statistical analysis
Statistical significance of the difference between different conditions was assessed using Student’s two-tailed t-test. All calculations were performed using Microsoft Office Excel. Results & Discussion
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Notch signaling pathway is downregulated upon Jurkat T cell activation

Jurkat T cells are inherently independent on exogenous glutamine for cell survival along with a high expression of Notch1 intracellular domain [14] but upon artificial increase of glutamine consumption, Notch signaling pathway activity was found to decrease [11]. It has also been shown that supplementation of glutamine in exogenous media leads to the maximization of IL2 secretion [15]. In the present study, stimulation of Jurkat T cells in RPMI-1640 containing 6 µg/ml PHA plus 1 ng/ml PMA for 24 h led to increased glutamine consumption (Fig 1A). It was observed that PHA/PMA stimulation increases IL2 secretion in a time-dependent manner (Fig 1B). Upon removal of glutamine from exogenous media, the IL2 secretion decreased similar to murine primary T cells [6] (Fig 1C). Interestingly, the protein expression of canonical Notch pathway elements-cleaved Notch1, DNA-binding protein RBP-Jκ, and downstream effector Hey1-decreased as estimated using western blot analysis (Fig. 1D). A decrease of RBP-Jκ promoter activity was also observed upon stimulation of Jurkat cell with PHA/PMA (Fig. 1E). Thus, Jurkat T cell activation increases glutamine consumption, IL2 secretion along with a decrease in canonical Notch signaling pathway.

Constitutive expression of Hey1 and its effect on Jurkat T cell activation

The canonical Notch signaling pathway was observed to be downregulated with a decrease in the expression of downstream effector Hey1 and RBP-Jκ. So we generated a stable Jurkat cell line constitutively expressing the pathway effector Hey1 (Jurkat-Hey1) to understand its effect on Jurkat T cell activation (Fig. 1F). Hey1 is a member of the basic helix-loop-helix-Orange family of transcriptional repressors that mediate Notch signaling [16], and it is one of the well-characterized target gene of Notch activity [14]. Previous studies have shown that Hey1 overexpression can deregulate glutamine metabolism similar to Notch1 overexpression in K562 [11]. Jurkat-Hey1 cells upon activation by PHA/PMA did not show an increase in glutamine consumption (Fig 1G). Next we monitored the effect of constitutive expression of Hey1 on IL2 secretion upon activation by PHA/PMA. Here IL2 secretion with time was found to decrease moderately when Jurkat-Hey1 cells are activated by PHA/PMA (Fig 1H) in complete media. But when exogenous glutamine was removed, a consistent increase in IL2 secretion with time was observed (Fig 1I). This observation suggests that the Notch pathway may facilitate Jurkat T cell activation in the absence of exogenous glutamine.

In the immune system, Notch signaling is intimately involved in the process of T versus B lymphocyte differentiation from a common lymphocyte progenitor [17]. Earlier studies have shown that activation of naive CD4+ T cells along with simultaneous activation of Notch1 signaling pathway prevented IL2 and INFγ production while inhibiting presenilin-enhanced T cell proliferation upon stimulation [18]. On the other hand, stimulation of Notch3 by Delta1 ligand during activation of CD4+ cells supported the differentiation process in a dose-dependent manner where higher stimulation of Notch3 inhibited T cell activation [19]. But inhibition of Notch pathway in γδ T cells prevented the activation [20]. Again recent studies show that a non-canonical Notch pathway may be involved in regulating the activation of peripheral CD4+ T cells [21]. In the present study, we aimed to understand the role Notch signaling pathway might play in the process of in vitro Jurkat T cell activation. Jurkat T cell line upon stimulation with PHA/PMA resulted in the decrease of Notch signaling pathway elements RBP-Jκ and Notch signaling pathway downstream effectors Hey1 along with the decrease in RBP-Jκ promoter activity. A study based on understanding of HIV latency had shown that upon T cell activation, RBP-Jκ mRNA is among the few genes that are downregulated [22] and here we found a decrease in the protein expression of RBP-Jκ in activated Jurkat cells. Thus, Jurkat T cell activation is associated with a deregulation of the canonical Notch pathway. A recent study has demonstrated that in K562 cells, increase in Notch1 activity deregulates glutamine consumption and makes the cells independent of glutamine for survival or maintenance of ATP/ADP ratio [11]. Increase in glutamine consumption is an important metabolic adaptation during the process of T cell activation, and it can regulate the cytokine release via a ERK-dependent pathway in isolated murine CD4+ T cells [6]. In the present study, we showed that Jurkat T cell activation is dependent on exogenous glutamine and constitutive expression of Notch downstream effector Hey1 in Jurkat cells can prevent the increase in glutamine consumption and IL2.
secretion. Absence of exogenous glutamine has previously shown to decrease IL2 secretion in murine primary T cells [6] and Jurkat T cells [15]. A closer look at the effects of activation of Jurkat and Jurkat-Hey1 cells in the presence and absence of glutamine gives us an interesting insight into the role of Notch pathway and glutamine dependence during Jurkat T cell activation. Comparing the unstimulated Jurkat and Jurkat-Hey1 IL2 secretion shows that there is an initial boost in IL2 secretion provided by the overexpression of Hey1, which is nearly two-fold both in the presence and absence of exogenous glutamine. But with time where Jurkat T cells have a consistent increase of IL2 secretion, Jurkat-Hey1 cells show a decreasing trend in the presence of glutamine. But in the absence of exogenous glutamine, Jurkat T cells upon activation show a decreasing trend in IL2 secretion and Jurkat-Hey1 cells show an increasing trend. Thus, overexpression of Hey1 can positively regulate IL2 secretion in the absence of glutamine but not in the presence of exogenous glutamine. Recently it has been attributed that competition with glucose is a cause of failure of T cells in arresting tumor progression as both require glucose for survival and activity [23]. Another study investigating the role of Notch pathway in CD8+ T cells and its antitumor activity showed that Notch1 gene expression is upregulated upon activation, which is different from that observed by us in Jurkat T cells, but the increase in Notch1 expression is prevented in tumor T cells, resulting in the suppression of antitumor activity. Rescue of Notch1 signaling increased the antitumor activity of activated CD8+ T cells [24]. It would be interesting to explore in the tumor microenvironment the effect of competition with glutamine consumption and whether activation of canonical Notch pathway can support T cell activation in minimal glutamine conditions.

Limitations

1. This work aims at understanding the crosstalk between Notch signaling and a nutrient (glutamine) uptake and utilization in a cellular process like T cell activation. The major limitation of the work is that it is a cell culture-based work and not performed in primary T cells isolated from human or mice, which would have been a more physiologically relevant system.

2. As a T cell model, Jurkat cell line and chemical activation system is used to address this question. Though similar systems have been utilized to study the effect of glutamine, being a tumor cell may have its own shortcomings. But this system was useful for understanding the effect on Notch pathway during T cell activation as it has a constitutively activated Notch1 signaling pathway.

3. Studying the effect of Hes1, another downstream effector of Notch pathway, alongside would have been better as this also have been shown to affect glutamine dependency in other studies.

These observations of Notch and nutrient uptake cross-talking with each other and affecting the fate of a cellular process like T cell activation is very intriguing. The final observation shows that upregulation of downstream effector Hey1 could impart independence from exogenous glutamine during T cell activation for release of IL2, an important cytokine to sustain the activated T cells. The utility of this effect could be tested in a tumor microenvironment where there is a likelihood of competition for glutamine and where Notch-activated T effector cells may respond better.

Additional Information

Methods

Cell culture

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**RBP-Jκ reporter assay**

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**IL2 enzyme-linked immunosorbent assay**

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**Statistical analysis**

Statistical significance of the difference between different conditions was assessed using Student’s two-tailed t-test. All calculations were performed using Microsoft Office Excel.

**Supplementary Material**

Please see https://sciencematters.io/articles/201602000030.

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**Ethics Statement**

Not applicable.

**Citations**


