

Differential Notch1 and Notch2 expression in non-small cell lung cancer

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ABSTRACT

Introduction: NOTCH signaling can be deregulated in non-small cell lung cancer and can have oncogenic or tumor suppressive functions. NOTCH1 is regulated equally with NOTCH2, and the stability of the expression of those Notch receptors could determine the biological functions of lung cancer cells.

Purpose: To estimate expression level of NOTCH2 receptor in diverse NSCLC cell lines. Furthermore, we compared the mRNA level of NOTCH2 expression to the level of NOTCH1 expression.

Materials and methods: We have evaluated the mRNA manifestation of NOTCH1 and NOTCH2 genes by using quantitative real time method (RT-PCR). Moreover, we associated the results from NSCLC

cells with results achieved in non-cancerous human bronchial epithelial cells (HBEpC).

Results: The expression level of NOTCH1 and NOTCH2 was downregulated in NSCLC cell lines, when related to HBEpC. Nevertheless, the decrease in NOTCH1 expression was significant, whereas NOTCH2 was not much different from the expression in control cells.

Conclusions: We conclude that NOTCH1 and NOTCH2 most likely have different biological function in NSCLC. They are active in NSCLC cell lines; nonetheless both are downregulated in lung cancer cells used in this study. Moreover, NOTCH2 expression is comparatively higher than NOTCH1 expression.

Keywords: NOTCH1, NOTCH2, NSCLC, HBEpC

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INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common types of cancer with overall 5-year survival rate around 10-15%. Despite of therapeutic innovations, the prognosis for lung cancer patients has not changed in the last years [1, 2]. Therefore, it is essential to acknowledge molecular mechanisms to identify new therapeutic approaches for lung cancer. Notch signaling is one of the pathways, very often deregulated in NSCLC. It is composed of four Notch receptors (NOTCH1, 2, 3, 4) and five Notch ligands (DLL1, 3, 4 and JAG1, 2) [3,4]. The NOTCH1 receptor is well described in many types of cancer, whereas the NOTCH2 function is not as widely studied. Nevertheless, it is known that those two receptors can have opposite functions in cancer, including NSCLC. According to some studies, NOTCH2 shows a tumor suppressive role by facilitating cell differentiation in lung carcinogenesis. Moreover, the results of immunohistochemical analysis of human NSCLC samples displayed the loss or downregulation of Notch2 associated with non-cancerous lung tissues [5,6]. According to The Human Protein Atlas, most of the malignant cells displayed weak to moderate nuclear positivity and many renal cancers were negative for NOTCH2.

In our previous studies, we showed the differences in NOTCH1 receptor and ligands expression in NSCLC cells and patients, while here we investigated the expression of NOTCH2 and NOTCH1 in order to evaluate the potential relationship in NSCLC.

MATERIALS AND METHODS

Cell lines

The human NSCLC cell lines were achieved from the American Type Culture Collection (ATCC) and were grown in RPMI-1640

(H520, H1299), in EMEM (Calu-3) and in Ham's F12K medium (A549), supplemented with 10% fetal bovine serum (Sigma) in 5%CO₂ at 37°C. Media for NSCLC were bought in ATCC. The HBEpC cell line used in this study was purchased from European Collection of Authenticated Cell Cultures (ECACC) operated by Public Health England and was grown in bronchial epithelial cell serum free growth medium (BEGM™, Lonza), complemented with retinoid acid in 5%CO₂ at 37°C.

RNA extraction and quality control

Total RNA was isolated from all NSCLC cell lines and HBEpC by using the RNeasy Kit following the manufacturer's protocol (Qiagen, USA). The 100 µl RNA extracts were stored at -80°C prior to further processing. Quantity and quality of RNA assessment were performed using a UV/VIS spectrophotometer Nano Drop 2000c (Thermo Fisher Scientific, Inc., USA). The level of integrity required for quantitation was determined for the extracted total RNA using the Agilent RNA 6000 Nano kit on a Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA) according the manufactures' recommendations. Finally, a total of 400 ng of the RNA was reverse transcribed into cDNA in a reaction with High Capacity RNA-to-cDNA Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc., USA) according to the manufacturer's protocol.

Quantitative polymerase chain reaction (qPCR)

The mRNA expression level of NOTCH receptors genes was evaluated in NSCLC and HBEpC cell lines using quantitative real time PCR (RT-PCR). The TaqMan commercially available Gene Expression assays (Applied Biosystems™, USA) were used for real time reaction (Table 1).

Table1. Assays analyzed in the study

According to the ^aHUGO gene nomenclature committee and ^bApplied Biosystems Life Technologies

| Gene symbol | Gene product name | Gene ID ^a | Assay ID ^b |
|-------------|-------------------|----------------------|-----------------------|
| NOTCH1 | notch 1 | 7881 | Hs01062014_m1 |
| NOTCH2 | notch2 | 7882 | Hs01050702_m1 |

The expression of the above-mentioned genes [by the change-in-cycling-threshold ΔC_q method] were normalized to control - ribosomal *18SRNA* gene expression (Hs9999901_s1 *18SRNA*) [7]. The following thermo-cycling conditions were

used: 50°C for 2 min; 95°C for 10 min; 40 cycles of 95°C for 15 sec and 60°C for 60 sec. Each sample was analyzed in triplicate. All reactions were performed using the ABI PRISM® 7900HT Sequence Detection system (Thermo Fisher Scientific, Inc.).

Statistical analysis

Results are expressed as mean \pm SD. Statistical analysis were based on the Mann-Whitney *U* test for nonparametric values and was carried out with GraphPad Prism software (version 5.01; GraphPad Software, Inc., La Jolla, CA, USA). $P < 0.05$ was considered significant.

RESULTS

As a result of mRNA relative expression analysis of Notch signaling genes in non-small cell

lung cancer cell lines (H520, H1299, A549 and Calu-3) and in human bronchial epithelial cells, we obtained NOTCH1 and NOTCH2 receptors to be activated in all chosen cells.

Nevertheless, NOTCH2 and NOTCH1 receptor appeared to be downregulated in all cells, when we compared results from expression analysis for those two genes, we saw the differences. Interestingly, H1299, A549 and Calu-3 had very low NOTCH1 whereas the NOTCH2 mRNA level did not differ much in those cell lines from the control cells, except H1395 and A549 cells (Figure 1).

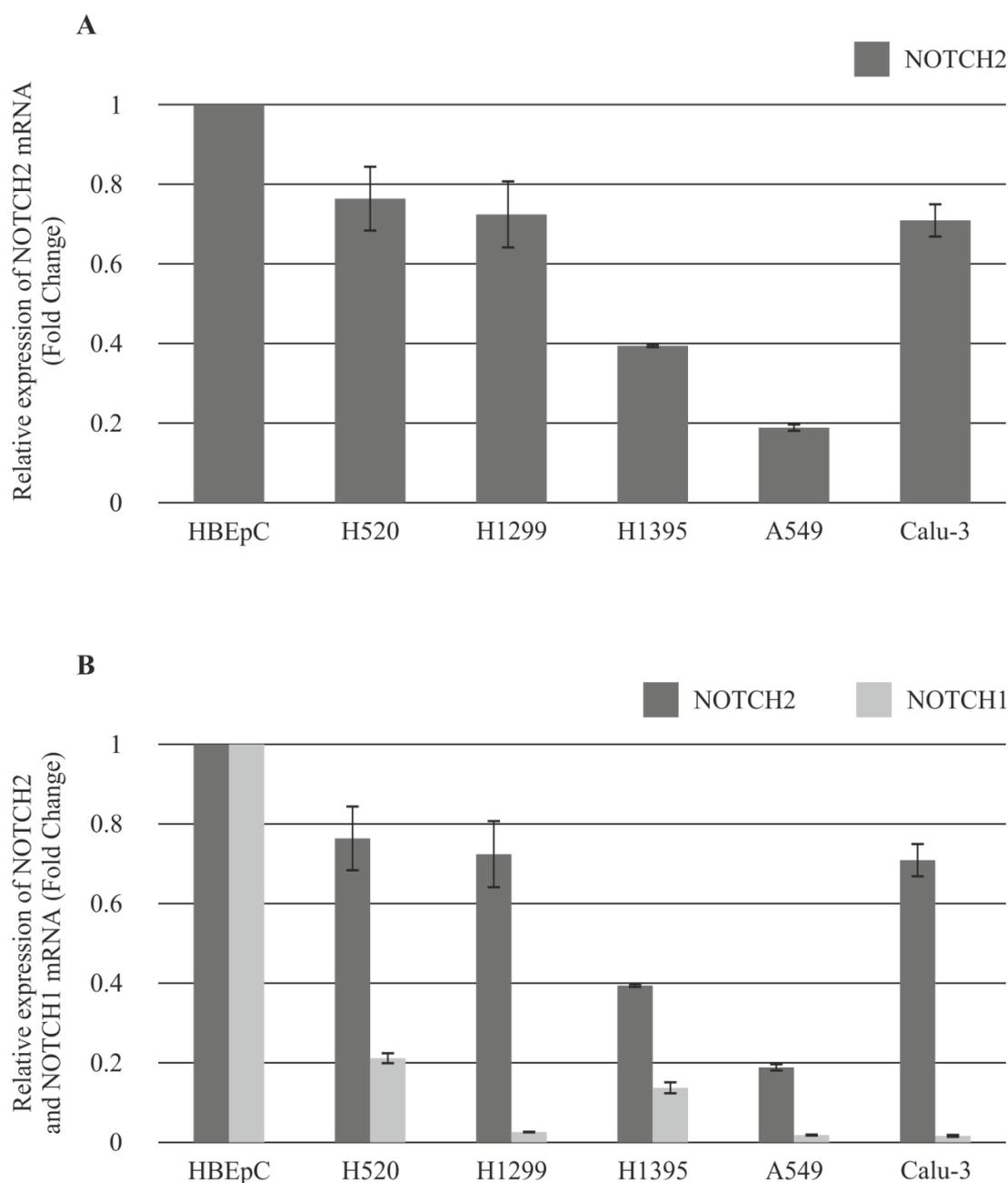


Figure 1. Relative expression of A) NOTCH2 and B) comparison of NOTCH2 and NOTCH1 in NSCLC cell lines. Data were normalized to 18SrRNA levels in quantitative RT-PCR assay, $P < 0.01$ (Mann-Whitney *U* test)

DISCUSSION

Notch1 is transmembrane receptor engaged in apoptosis and cell proliferation in healthy cells [8,9,10]. It is activated through ligand binding and stimulates proteolytic cleavages and release the intracellular domain (N1-ICD). Furthermore, N1-ICD moves into the nucleus and activates target genes. It has been known that Notch signaling is involved in development, progression, and suppression in various types of cancer. Therefore, it can have either tumor activating or suppressing ability [11,12]. Despite the available literature on the notch signaling pathway, there is no definite answer to its function. That is why in our current study, we decided to evaluate NOTCH2 expression level and compare it to NOTCH1 mRNA status in NSCLC cell lines. Our study is continuation of previous experiments on Notch signaling pathway where we noticed, that NOTCH 1 level is significantly reduced in NSCLC. In this study, we find out that NOTCH2 also decreased at mRNA level in NSCLC cells, but it didn't seem substantial. When we studied literature according NOTCH1 and NOTCH2 in different types of cancer, we found inconclusive results. According to Chu and colleagues, NOTCH1 and NOTCH2 have the opposite function in colorectal cancer [13]. The authors suggested that above mentioned receptors are inversely correlated with tumor differentiation, invasion, metastases and survival of patients. Also, Motooka et al. showed that NOTCH1 deficiency in lung cancer caused reduced early tumor formation, whereas NOTCH2 deficiency started tumor formation. In the light of such results, the researchers stating that NOTCH1 acted as an oncogene, but NOTCH2 had tumor suppressor activity [14]. The results according NOTCH2 in cancer are in opposition to those presented by Hayashi et al. The researchers discovered that NOTCH2 acts as an oncogene that endorses bladder cancer growth and metastasis [15]. On the other hand, Hao Liu with co-workers paid attention on target genes for NOTCH1 and NOTCH2 in pancreatic cancer. The researchers revealed that Notch receptors target both oncogenes and tumor suppressor genes; therefore it was unable to describe them as oncogenes or tumor suppressors [16].

CONCLUSION

Based on our results, the differences in the Notch genes expression in NSCLC and in control sample were definitely visible. Moreover, the samples with higher NOTCH2 had lower NOTCH1 expression level. Therefore, we conclude that chosen receptors may have opposite biological role in NSCLC; nevertheless both receptors are regulated equally and evaluation of their expression could be helpful in defining the biological functions of lung cancer cells.

Conflicts of interest

The author declares that there is no conflicts of interest regarding the publication of this study.

Financial Disclosure

None.

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