Proliferation of alveolar type II pneumocytes is stimulated by Jagged-1 in vitro

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Background

Notch receptors are key regulators of development by controlling cell-fate determination in many multicellular organisms. However, there are controversial results concerning the effects of notch signalling on proliferation, differentiation and cell death of various cell lines, including lung specific cells. Although there is accumulated evidence of a role of notch in the developing lung, it is still unclear how notch might influence adult pulmonary cell lines, e.g. pneumocytes. As in human lung tissue up to 10% of pneumocytes were found to show expression of notch receptors, investigations of potential effects of Jagged-1 on alveolar typ II cells appear to be legitimate. Furthermore, TGF-ß induced epithelial-mesenchymal transition (EMT) as seen in lung fibrosis development is known to be mediated at least in part through notch signalling, emphasising once again the importance of a clarification of the role of the notch pathway in pulmonary diseases.

Aim

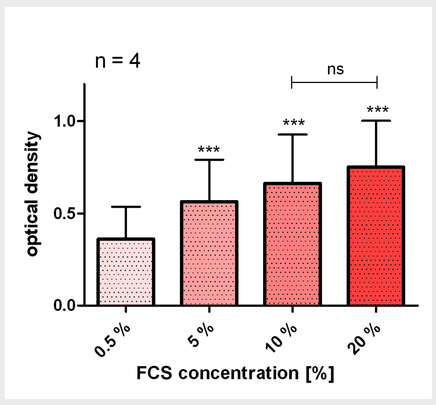
The potential role of the notch/jagged-1 signaling pathway in pulmonary diseases led us to evaluate its effect on rat alveolar type II cells, namely the RLE-6TN. To date, there is still no available data on the effects of Jagged-1 on the proliferation of this specific cell line.

Methods

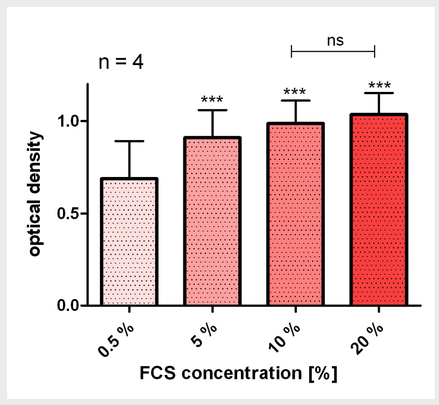
Rat alveolar type II cells (RLE 6TN) were obtained from the American Type Culture Collection (ATCC no. CRL-2300; Manasas, VA, USA) and cultured in DMEM/Ham´s F12 containing 10% fetal calf serum (FCS) and L-glutamine. After 24h cells were washed and then incubated with either 0.5%FCS (negative control), 10%FCS (positive control) or with the test substances in medium containing 0.5% FCS for 24 h at 37°C and 5% CO_2. Cell proliferation was finally measured by direct microscopic cell count and fluorometrically by the proliferation assay EZ4U basing on tetrazolium salt reduction. Proliferation experiments were performed by EZ4U assay and the number of seeded cells was 2500c/well (96 well microplate), whereas for direct cell count by microscopy (12.5 amplification) a 12 well microplate with 100000c/well was utilised. Statistic analysis was performed by non-parametric testing (Mann-Whitney-U Test).

Results

▼ Figure 1.

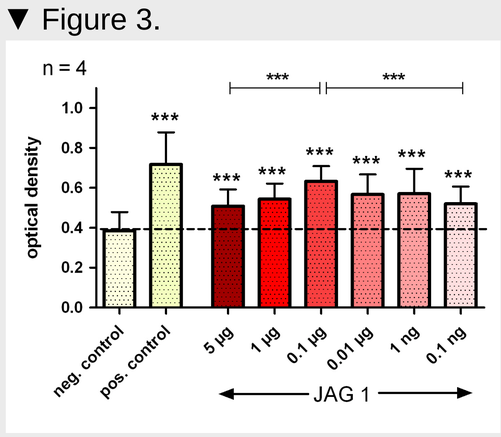


▼ Figure 2.

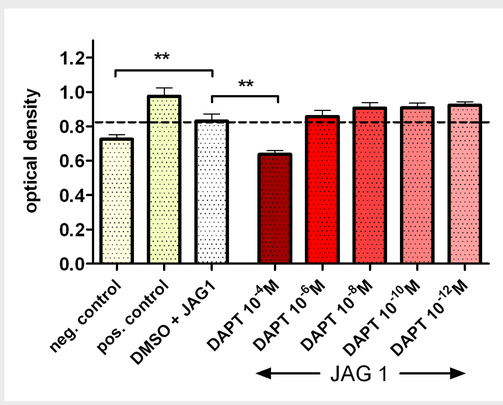


Dependency of RLE-6TN proliferation on FCS concentration:
Different concentrations of FCS were used [0.5% to 20%] to analyze the effect of this nutrient on RLE-6TN proliferation. The same experiment was performed with 2500c/well (Figure 1) and 5000c/well (Figure 2). After 24 h of incubation cell proliferation was measured by fluorometer (Tecan®) using the EZ4U assay. 5%, 10% and 20% FCS concentrations showed a strong significant difference in proliferation compared to the serum starving control of 0.5%FCS (p<0.001).

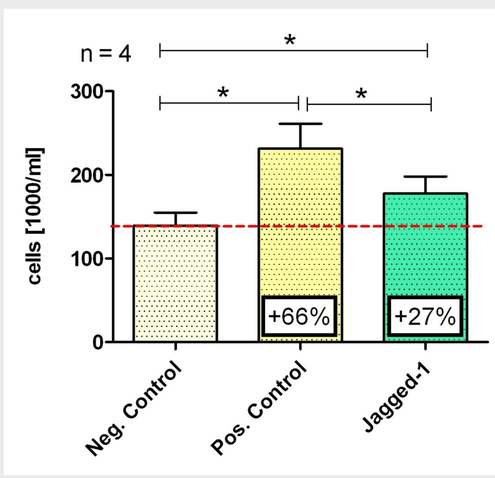
▼ Figure 3.



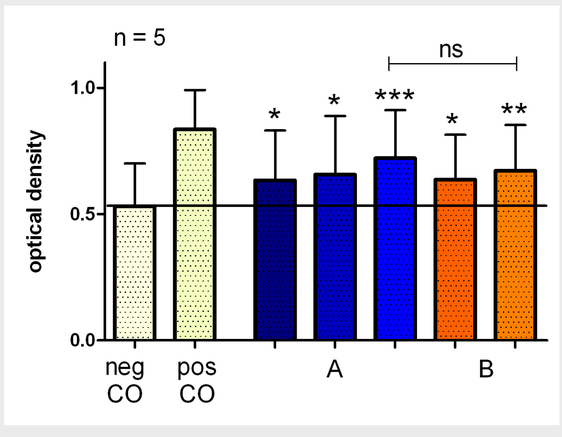
▼ Figure 4.



Effects of Jagged-1 on RLE-6TN proliferation by fluorometry
After 24h of preincubation at optimal conditions cells were washed and incubated for further 24h with different concentrations of the notch ligand Jagged-1 [5 µg/ml to 0.1 ng/ml] as well as with 0.5%FCS as negative control and 10%FCS as positive control. Cells showed a concentration dependent significant increase in proliferation, with the maximal effect at 0.1 µg/ml (Figure 3). We then tested the inhibitory effect of the specific γ-secretase inhibitor DAPT on the Jagged-1 [0.1µg/ml] triggered proliferation, showing a significant inhibitory effect of DAPT at 10^{-4}M (Figure 4).



◀Figure 5.
Effects of Jagged-1 on RLE-6TN proliferation by direct microscopic cell count
Validation of the fluorometrically obtained data regarding cell proliferation after exposure to Jagged-1. Again, we detected a significant effect on cell proliferation by Jagged-1 (+27%).



◀Figure 6.
Preincubation and its effect on proliferative actions of Jagged-1:
In this experimental setting we investigated the effects of preincubation on Jagged-1 mediated proliferation of RLE-6TN.
A: cells were preincubated with 0.5% FCS for 45min, washed and incubated for 24h with Jagged-1 [5µg/1µg/0.1µg/ml]
B: cells were preincubated with Jagged-1 [1µg/0.1µg/ml] for 45min, washed and incubated with 0.5% FCS for 24h
These data show that preincubation with Jagged-1 for 45min elicited a significant response of cell proliferation in comparison to the respective control.

Conclusion

Herewith, we report for the first time that the Jagged-1/notch signaling pathway is affecting rat alveolar type II cell proliferation in vitro. The notch signalling pathway might therefore represent a promising target for therapeutic strategies in pulmonary diseases.

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