THE STIMULATING EFFECT OF INTERLEUKIN-33 ON PROLIFERATION OF THE PRIMARY HUMAN LUNG FIBROBLASTS

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Key words: interleukin-33; human lung fibroblasts; proliferation

Interleukin (IL)-33 is a multifunctional cytokine that belongs to the IL-1 cytokine family and expressed by multiple organs and cell types. Recent studies have showed that IL-33 plays an etiological role in several fibrotic disorders and may be involved in the pathogenesis of chronic respiratory diseases. It has been reported that IL-33–induced cutaneous fibrosis is associated with the increased fibroblast proliferation and altered expression of extracellular matrix-modifying genes. However, the role of IL-33 in regulating of functions of lung fibroblasts remains unclear. In the present study we examined the effect of IL-33 on proliferation of human lung fibroblasts. Five primary lines of normal adult human lung fibroblasts were cultured for 3–7 days in the presence of increasing concentrations of IL-33. We have observed that normal human lung fibroblasts responded in a dose-dependent manner to treatment with recombinant human IL-33 by increasing proliferation rates 1.5–2.3 fold compared to the non-stimulated control. The maximum effect of IL-33 on fibroblast proliferation was observed in the cytokine concentrations range from 2 ng/ml to 100 ng/ml. These results suggest that IL-33 may play an important role in the regulation of the human lung fibroblast proliferation. Human lung fibroblasts activated by IL-33 may act as effector cells not only in the pathogenesis of lung diseases, but also in lung remodeling processes.

Materials and Methods

Five primary normal adult human lung fibroblasts lines (NHLF1-NHLF5) were purchased from NIH (Bethesda, MD) and Lonza Walkersville (Walkersville, MD). Fibroblast lines were grown in T75 culture flasks in a humidified atmosphere of 5% CO₂ at 37°C in the high serum tissue culture medium DMEM with glutamine, sodium pyruvate, antibiotic/antimycotic, and 10% bovine calf serum.

NHLF were tested in passage three-seven. Cells were grown to confluency, treated by trypsinization, washed, and replaced in the high serum tissue culture medium at 2x10⁶ cells per well in 96-well flat-bottom tissue culture plates. After overnight incubation in the high serum tissue culture medium the medium in each well was replaced with RPMI 1640 containing all supplements, except the serum concentration was decreased to 0.5% (the low serum tissue culture medium). The fibroblasts were incubated for another 24 h before adding the test substances. Recombinant human IL-33 (R&D Systems) was used in the concentrations of 1, 2, 10, 50, 100 and 300 ng/ml. The low serum tissue culture medium alone was the negative control. Proliferation of fibroblasts was analyzed by the cell proliferation assay (CellTiter Aqueous; Promega) in accordance with the manufacturer’s recommendations, after the fibroblasts were incubated with the test substances for 3–7 days. Changes in the cell proliferation rates were assessed in quintuplicate.

Results and Discussion

Normal human primary pulmonary fibroblasts proliferate in response to stimulation with IL-33.
One-way ANOVA revealed a significant effect of IL-33 (in the concentrations of 2 to 300 ng/ml) on the fibroblast proliferation. Increase in the proliferation rate was observed in four lines (Fig. 1).

One cell line did not respond by a change in proliferation to treatment with IL-33 (data are not shown). The NHLF responded in a dose-dependent manner to treatment with recombinant human IL-33, by increasing proliferation rates 1.5 to 2.3 fold compared to the non-stimulated control fibroblasts. The maximum effect of IL-33 on the fibroblast proliferation was observed in the cytokine concentration of 2 ng/ml with a gradual decline in the concentrations of 100 ng/ml and 300 ng/ml. The average increase in proliferation rates in this concentration was 1.95 ± 0.25-fold compared with the non-stimulated control cultures.

The results obtained suggest that IL-33 may play an important role in the regulation of the human lung fibroblast proliferation.

CONCLUSIONS

Recombinant human interleukin-33 stimulates the primary human lung fibroblasts proliferation in a dose-dependent manner. Human lung fibroblasts activated by IL-33 may act as effector cells not only in the pathogenesis of lung diseases, but also in lung remodeling processes.

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ВЛИЯНИЕ ИНТЕРЛЕЙКИНА-33 НА ПРОЛИФЕРАЦИЮ ПЕРВИЧНЫХ ЛЕГОЧНЫХ ФИБРОБЛАСТОВ

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Ключевые слова: интерлейкин-33; легочные фибробLASTы; пролиферация

Интерлейкин 33 (IL-33, interleukin 33) – многофункциональный цитокин, принадлежащий к семейству провоспалительного интерлейкина 1 (IL-1) и обладающий иммунорегуляторными свойствами. IL-33 стимулирует выработку провоспалительных цитокинов различными по происхождению клетками организма и может функционировать в качестве гистогенеративного ДНК-связывающего белка, стабилизирующего формирование нуклеосомы. IL-33 экспрессируется в различных органах и тканях. Основными источниками данного цитокина являются эндотелиальные и эпителиальные клетки. IL-33, вовлеченный в развитие кардиоваскулярных заболеваний, бронхиальной астмы, болезни Крона, гипергерпезии и гиперплазии тканей. Известно, что данный цитокин играет ключевую роль в этиологии и патогенезе некоторых фиброзных заболеваний (системного склероза, фиброза печени, фиброза кожи). Установлено, что IL-33-индукционный фиброз кожи связан с усиленной пролиферацией фибробластов и измененной экспрессией генов внеклеточного матрикса. В то же время роль IL-33 в регуляции функциональной активности легочных фибробластов остается неизвестной. Целью настоящего исследования являлось изучение влияния рекомбинантного человеческого IL-33 на пролиферацию нормальных фибробластов легких человека. Пять первичных линий нормальных фибробластов легких человека культивировали в течение 3-7 дней в присутствии возрастающих концентраций IL-33. Установлено, что рекомбинантный человеческий IL-33 дозозависимо стимулирует пролиферацию нормальных легочных фибробластов. Было установлено, что после стимуляции легочных фибробластов IL-33 наблюдалось повышение активности пролиферации в 1,5-2,3 раза по сравнению с интактным контролем. Максимальный стимулирующий эффект ИЛ-33 на пролиферацию фибробластов наблюдался в концентрации 2 до 100 нг/мл. Полученные результаты свидетельствуют о важной роли ИЛ-33 в регуляции пролиферации фибробластов легких человека.

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