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ANALYSIS OF THE EXPRESSION AND PRODUCTS ACCUMULATION OF CD-MARKER GENES IN CULTURES OF LUNG AND SKIN RATS FIBROBLASTS IN ONTOGENESIS

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Introduction. Products of CD genes are not only indicators of cell membership to a particular population, but also play an important role in the adhesion cells process and their perception of various signals. The purpose of our work was to determine the dynamics of the expression and products accumulation intensity of specific CD genes in the lung and skin of rat fibroblast cultures in ontogenesis.

Methods. The tissues were ground in DMEM medium containing 1% trypsin. After 30 min incubation at 37 °C, the cells were harvested and sown in ventilated culture flasks in a DMEM medium containing 10% FBS and cultured them (37 °C and a humidity of 95% in the presence of 5% CO_2). Cell attachment and cell culture density were monitored using an inverted microscope, using 3rd passage fibroblasts, the gene expression analysis was performed on DNA – microchips (Arrayit) and Affymetrix 428 Scanner. The total RNA from the cells was isolated on spin columns with a set of RNeasy Mini Kit (Qiagen). Synthesis of cDNA by reverse transcription was performed using QIAGEN OneStep RT-PCR Kit (Qiagen). The amplification was carried out using a BIO-RAD iCycler. The final amount of the produced protein product was measured immunochemically on antibody-conjugated ELISA-microchips using the Antibody Array Assay Kit (Full Moon BioSystems, Inc.) reagent kits.

Results and Discussion. An analysis of the expression of CD-marker genes has shown that the cells we are investigating have a set of molecular data that is characteristic of mature fibroblasts. The results of measuring genes expression suggest that in all the age groups of lungs and skin fibroblasts there is practically no expression of markers, which are characteristic exclusively of mesenchymal stem cells (MSC). At the same time, markers common to mature fibroblasts and MSC are expressed in cells of both types of tissues in all age groups, indicating the "purity" of the fibroblasts culture. CD-marker genes expression intensity indicators in lung fibroblasts with age vary insignificantly, as opposed to skin cells, where this index in the case of all genes has a maximum in cells of 1-month-old animals, and in the future ontogenesis tends to decrease. The products accumulation of the CD-markers genes is much more intense in the skin fibroblasts than in the lung cells.

Conclusions. The obtained results indicate that, despite the phenotypic homogeneity of skin and lungs fibroblasts, the composition and number of surface cell markers in the course of ontogenesis vary unevenly.

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