Stable cell line of postnatal dermal fibroblasts as a new experimental model for biomedical skin research
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Introduction
The ability to restore tissue after damage is vital to any organism. Deviations from the normal healing process leads to formation of pathological scars and contractures. Despite the advances in modern medicine, scar complications remain one of the most complex and discussed problems in biomedicine.

Fibroblasts are the main resident cells of connective tissues, including the dermis. Excessive contractile activity of fibroblasts in healing skin wounds and burns is associated with pathological scars. Traditionally, primary cells or a mouse embryonic fibroblast cell line 3T3/NH have been used for experimental studies of dermal pathologies. Unfortunately, both models have significant drawbacks. However, the use of primary cells is limited by their low pausing activity, while 3T3/NH cell line does not fully recapitulate the phenotype of dermal fibroblasts. It is also known that before a certain stage of embryonic development the fetus does not form scars, therefore, embryonic fibroblasts should significantly differ from postnatal fibroblasts (Moulin et al., 2001). Thus, a new cell line effectively combining the properties of both primary and immortalized cell lines is highly needed.

Methods

Isolation and cultivation of mouse dermal fibroblasts: Dermal fibroblasts were isolated from C57BL/6 mice (P1) as described earlier (Takashima A., 2001). Mouse dermal fibroblast cells (MDF) were immortalized according to the 3T3-protocol: 3-day transfer, inoculum 3x10^4 cells (Todderm, Green, 1985).

Results I: Obtaining and characterization of dp3T3 fibroblast cell line

Fibroblasts were seeded on coverslips of 50,000 cells, cultured for 2 days and fixed using a 4% paraformaldehyde solution in phosphate buffered saline (PBS) for 10 minutes. Antibody staining and phalloidin staining were performed according to the Abcam protocol (Takashima A., 2001). Mouse dermal fibroblast cells (MDF) were immortalized according to the 3T3-protocol: 3-day transfer, inoculum 3x10^4 cells per well.

Results II: Comparison of contractility, αSMA and collagen I expression in 3T3/NH, MDF and dp3T3 cell lines

A. dp3T3, MDF and 3T3/NH fibroblasts morphology comparison after two days in culture. (×100 magnification, scale bar 100 μm). B. Results of the MTT test for dermal fibroblasts at different passages and the 3T3/NH line, the mean and standard deviation is shown. C. Chromosome number. Mean, the mean and standard deviation is shown.

Results III: Expression analysis of fibronectin and profibrotic cytokines (LOX and CTGF) in 3T3/NH, MDF and dp3T3 cell lines

A. Western blot data for αSMA, standardization on β-actin.

Conclusions

1. The karyotype of newly obtained dp3T3 line (median 52 chromosomes) is closer to the normal karyotype of the mouse (40) than the karyotype of the 3T3/NH line (mean 75).

2. The dp3T3 fibroblast line has morphological characteristics more similar to the MDF line than the classical 3T3/NH fibroblast line which is closer to the 3T3/NIH line including fibrillar actin microarchitecture.

3. In 3T3/NH fibroblasts, the expression of αSMA is lower than in dp3T3 fibroblasts and in MDF. Immunocytochemistry data confirmed the data of PCR and Western blot. In 3T3/NH fibroblasts, the αSMA protein is diffusely located in the cytoplasm of cells, and in dp3T3 and in MDF it is found in stress-fibrils.

4. The dp3T3 line is similar in its contractility to MDF: both have a higher contractile ability than the 3T3/NH line including derivative fibroblast line.

5. According to our data, levels of profibrotic cytokines Lox and CTGF, the dp3T3 line is much more similar to mouse dermal fibroblasts than the 3T3/NH line.

To summarize, dp3T3 is more similar to MDF than to 3T3/NH that indicate the preservation of tissue-specific characteristics.

The work on creating a new cell line is a complex and multicomponent task, requiring a large number of tests. We successfully carried out the first stage of this work - obtaining a line of immortalized dermal postnatal fibroblasts. The next stage will focus on the validation of its tumorigenic potential, the migration properties and overall suitability of this new cell line for skin research and bioengineering.

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References