

ERK activation in murine fibroblasts under various growth conditions

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Introduction

Raf/MEK/ERK pathway is known to regulate numerous cellular events such as cellular proliferation, senescence, differentiation, apoptosis, and transformation. It is known that differences in duration and intensity of ERK activation signals effect these decisions of different biological responses in fibroblasts (1). For intensity, it is known that strong prolonged activation of ERK leads the cells to go in senescence (2). On the other hand, weaker ERK signal leads cellular proliferation. Even though it remains unclear how the activation of one signaling molecule ERK leads to specific cellular responses (3), accurate regulation of ERK signal could be the key to balance these responses (1). By regulating the ERK activation signals, the cells could have totally different cellular outcomes such as senescence or cell growth. For further research of the pathway, analysis of ERK activation for the growth induction of murine fibroblasts was done. The aim of the research was to see if the cellular outcomes activated by ERK could be controlled by conditional Raf using different activation kinetics, and to adjust parameters to induce accelerated cell growth in 3T3BXB-ER cells by activation of Raf constructs.

Materials and Methods

Cell Culture

Murine immortalized fibroblasts (NIH3T3) and engineered cells (3T3BXB-ER) were used. 3T3BXBER cells are NIH3T3 cells, expressing Raf-1 kinase domain-estrogen receptor fusion proteins, which could be regulated by tamoxifen or estrogen (4). The cells were kept in a humidified incubator at 37°C with 5.0% carbon dioxide in the DMEM medium supplemented with 10% fetal bovine serum (SIGMA), 1% 200mM L-glutamine (SIGMA), and 1% penicillin streptomycin.

Proliferation Rate/Cell Counting

The cells were counted on the 4th to 5th day of the experiments. Images were taken with Canon IXY 14.1 mega pixels with an inverted light microscope.

Western Blot

Samples were loaded to 12% acrylamide gels for sodium dodecyl sulfate polyacrylamide gel electrophoresis. The following antibodies were used.［Primary antibody: P-p44/42 MAPK (T202/Y204) (Cell Signaling) / Anti-MAP Kinase (ERK-1, 2) (SIGMA), Secondary antibody: antibody rabbit (Stabilized Peroxidase Conjugated Goat Rabbit (Thermo)）］ Luminata Crescendo/Classico Western HRP Substrate (Milipore) was used for protein detection.

Results and Discussion

Using the fibroblasts NIH3T3 and 3T3BXB-ER, ERK activation for the growth induction was analyzed. It was tested to see if the cellular outcomes could be controlled by changing intensity of ERK activation, investigating parameters which may stimulate accelerated cell growth by BXB-ER activation. Using Western blots and cell counting, differences of ERK signals between activation by serum and by BXB-ER were compared. As expected, the induction of ERK seemed to be optimal in fibroblasts at various concentrations of serum, but BXB-ER activation by 100nM tamoxifen induced robust ERK activation signal which led cells to go in senescence. To change the cellular outcome, activation levels of ERK were adjusted using different concentrations of tamoxifen/estrogen which acutely regulate BXB-ER activation. First, tamoxifen/estrogen concentrations were decreased to see the signal intensity and cell growth. By lowering, it produced the similar growth rates as the control, but BXB-ER activation could not induce additional growth. Second, MEK inhibitor, PD184352, was used to regulate the intensity of ERK activation with various serum concentrations. When ERK activation was inhibited by PD184352 (0.2μM-4μM), the cells grew slower than the control. Then, BXB-ER activation was induced by tamoxifen 100nM in addition to PD184352.Under the condition, the additional BXB-ER activation could restore ERK phosphorylation levels, and also growth rates to the levels they were before MEK inhibition, but not more. Therefore, the cells seemed to control the level of ERK activity very tightly, to be optimal for growth. It is possible that additional research should have been completed with smaller intervals for PD184352 concentrations. Although the parameter, which induces accelerated cell growth, was not found with the research, I showed the trends of how cells react with different activation kinetics. For future research, I wish to find out about the mechanism of how ERK activation gives the specific cellular outcomes by the precise investigation of ERK activation and growth induction.

References

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