

Fibroblast cell line with inducible cassette exchange system- tool to study gain and loss of gene function

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INTRODUCTION:

To understand many biological mechanism gain-and loss-of gene function are necessary approach. However, gene manipulations of this type are confounded by expression variation and type of cell line. Technical challenge is to generate cell line in which the inducible locus is expressed robustly, it is not silenced and it is not leaky.

METHODS:

We generated fibroblast cell line with single copy of targeting Cre/Lox cassette exchange. The cassette is targeted with circular plasmid P2lox/cre caring the gene of interest. Successful lox/cre recombination provides reconstitution of NEO gene within the cassette that allows positive selection with neomycin. The whole cassette is under doxycycline inducible (TRE) promoter.

RESULTS:

To evaluate the effectiveness of our inducible fibroblast cell line (i3T3) we recombined the exchange locus with luciferase. The generated luciferase inducible cell line (i3T3-Luciferase) was inducible in time and dose dependent manner to doxycycline treatment. Positive luciferase signal was detected as early as 30 min post induction. Levels of luciferase can be titrated with dilutions of doxycycline from 0.5 to 500 ng/ml. For functionally evaluation of the cell line we recombined the inducible locus with MyoD and Myf5, two main myogenic transcription factors. MyoD and Myf5 initiated myogenic differentiation in fibroblast. Myogenesis was evident by induction of myogenic pathway and formation of terminally differentiated myotubes. Our inducible cell line allowed us to study direct targets of MyoD and Myf5 and to dissect the process of myogenic reprogramming of fibroblast.

DISCUSSION & CONCLUSIONS:

We engendered fibroblast cell line with doxycycline inducible cassette exchange system. The cassette can be efficiently targeted with any gene of

interest. We demonstrated the efficiency of recombination and the inducibility of the system. Finally we used our cell line for exploring the process of reprogramming of fibroblasts to myoblasts.

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