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Mouse Bibroblast are reprogrammed to *Oct-4* and *Rex-1* gene expression and alkaline phosphatise activity by embryonic stem cell extract

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Several strategies have been investigated to induce reprogramming of somatic nuclei or cells. Although it is unknown which are the mechanisms and molecules involved in the reprogramming process, these experiments demonstrate that the molecular marks that define the epigenetic identity of a cell may be removed and the genome of a terminally differentiated somatic cell may acquire a totipotent or pluripotent state.In the last decade crucial experiments have shown that cell extracts isolated from different types of differentiated somatic cells were capable to reprogram gene expression in other somatic cell/isolated nuclei types. Cell fusion experiments have demonstrated that embryonic stem cells (ESCs) and embryonic germ cells show cellular reprogramming activity, indicating that these cells contain factors that can endow somatic cells with pluripotency. We entered this field of investigation with the aim of producing de-differentiated cells using cells extracts obtained from ES cells [1,2]. We first derived seven ES cell lines and chose two of them for the production of whole cell extracts. We used these extracts to reprogram three different populations of fibroblasts: STO, NIH-3T3 and fetal fibroblasts. After extract treatment the reprogramming process was observed through the analysis of the expression of a panel of stemness markers, including *Oct-4*, *Nanog* and *Rex-1* genes, OCT-4, Forssman antigen and SSEA-1 proteins and alkaline phosphatase (AP) activity.
The results[3] showed an enduring reprogramming activity of the ESC extract, although on a small number of cells that varies from ~0.003 to 0.04% of the total population of fibroblasts and with an effect limited to the induction of *Oct-4* and *Rex-1* gene expression and alkaline phosphatase activity. We never detected the expression of OCT-4, SSEA-1 and Forssman antigen proteins; also, we clearly demonstrated that ESCs may survive the procedure of extract preparation, may be source of contamination that is expanded in culture and give false positive results.
Future work will include the isolation of the small population of reprogrammed cells: these cells could be then further analysed or cultured in an environment that, without the presence of fibroblasts that have not been reprogrammed, would perhaps give them better chances of surviving and expanding. Once the reprogramming procedure is firmly established and somatic cells are proved to have permanently acquired full pluripotency characteristics, it will be possible to try to break down the components of the extract and identify the protagonists of the reprogramming process.

Publications

1. Redi CA, Monti M, Merico V, **Neri T**, Zanoni M, Zuccotti M, Garagna S. “Stem cells”. Endocr Dev. (2007) 11,145-151.

2. Rebuzzini P, **Neri T**, Zuccotti M, Garagna S, Redi CA “The karyotype analysis of the euploid cell population of a mouse embryonic stem cell line revealed high incidence of chromosome abnormalities that varied during culture”, Cytogenetic and Genome Research, **in press**.

3. **Neri T**, Rebuzzini P, Monti M, Merico V, Garagna S, Redi CA, Zuccotti M, "Mouse fibroblasts are reprogrammed to Oct-4, Rex-1 gene expression and alkaline phosphatase activity by embryonic stem cell extracts", Cloning and Stem Cells, (2007) 9, 394-406.