ISOLATION AND CHARACTERIZATION OF STEM CELLS FROM MOUSE COLON

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INTRODUCTION.

Stem cell (SC) biology is a one of the fastest growing fields of modern biomedical science, since utilization of stem cells shows significant promise for curing disease and rehabilitating patients. However, the understanding of basic biology for many organ and tissue specific stem cells is still at its preliminary stage. Before stem cells can be applied in clinical settings, we need to be able to develop *in vitro* techniques that can be routinely used to identify, isolate and characterize these cells. Reliable *in vitro* methods for propagating and growing SCs are also in great demand. Several recently published reports describe isolation and propagation of SC from mouse and human colon *in vitro* using culture mediums supplemented with various growth factors.

In our experiments, we used Rat Mammary Tumor cell line as a supporting feeder cell layer and the media supplemented with several grows factors for mouse colon SC growing and expansion. The goal of our experiments was to identify potential markers that can be used for simple isolation of the SC from mouse colon using flow cytometry techniques.

In order to characterize marker profile of the SC from mouse colon, we performed a flow cytometry analysis using 11 different antibodies including: CD49f, CD29, CD166, CD117, CD90/Thy1, CD9, CD44, CD54, CD13, CD133, CD26. In our experiments we used established long-term mouse colon SC cultures from a wild type mouse (passages ranging from 7-9). Primary cells extracted from mouse colon on the day of experiment and served as a control. We found that two markers showed a significantly higher enrichment in established cell lines compared to the primary cells. These markers were CD133 and CD26. In case of CD133, 98% of cells from established mouse SC lines possessed this marker compared to 4% of primary cells. In case of CD26, average of 66% of mouse colon SC from established cell lines possessed this marker, compared to 0.11% of cells from primary culture.

MATERIALS AND METHODS.

Processing of Mouse Tissues to obtain a cellular suspension made by using Trypsin and Collagenase II. The colon stem cells were grown on mammary tumor cells of the female rat (LA7) functioning as feeder cells. Cells were frozen using DMSO and for cell culture we used DMEM/F12+FBS+I.T.S+Gentamicine mixture solution as a growth medium. For stem cell differentiation a Matrigel analysis was performed. Differentiation state of stem cells was assessed using immunohistochemical analysis by staining cells with antibodies against EpCam protein (epithelial cell marker), Mucin 2 (mucus cell marker) and Villin (enterocytes).

RESULTS.

We found that CD133 and GD26 markers showed a significantly higher enrichment in established cell lines compared to the primary cells. In case of CD133, 98% of cells from established mouse SC lines possessed this marker compared to 4% of primary cells. In case of CD26, average of 66% of mouse colon SC from established cell lines possessed this marker, compared to 0.11% of cells from primary culture. These data may indicate that CD133 and CD26 are potential markers that can be used for SC isolation from the mouse colon. One of the critical future experiments that needs to be done is to isolate C0133 positive, CD26 positive and CD133/CD26 double positive cells and compare the grows rate and colony forming ability between these cells and the total cells fraction of established culture.

CONCLUSIONS.

1. Stem cells were successfully isolated from mouse colon and grown using feeder cell technology
2. Stem cells were differentiated into at least one cell type normally found in the colon indicating the presence of true stem cells
3. Cultured stem cells were analyzed for the presence of 11 cell surface markers
4. Two cell surface markers, CD133 and CD26 showed elevated level of expression in comparison between primary cells and cultured stem cells
5. This surface markers have a potential to be used for stem cell isolation in the future

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