A TOOL FOR DETECTING THE RATIO AND LOCALIZATION OF CANCER STEM CELLS IN HETEROGENOUS CANCER SAMPLES

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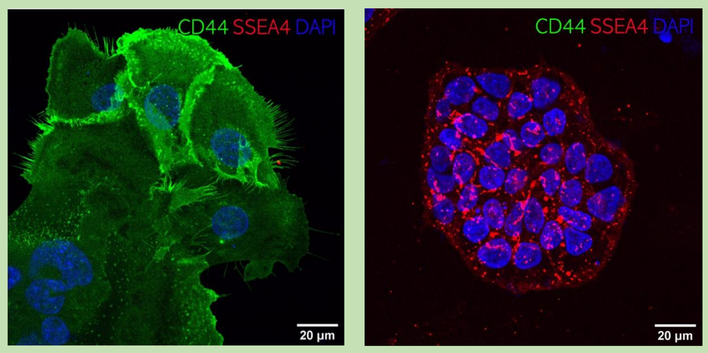
MOLECULAR BIOSCIENCES, CELL BIOLOGY

INTRODUCTION

Cancer stem cells (CSCs) are stem cell-like cancer cells that have harnessed developmental signaling pathways for their own gain. CSCs are believed to mediate the major hurdles of the treatment of cancer – metastasis, relapse, and resistance to treatments. The challenge that remains is that CSCs are extremely difficult to detect and to reliably identify from normal stem cells. Mass cytometry and imaging mass cytometry are mass spectrometry-based methods that facilitate the simultaneous studying of over 40 different biomarkers in single cell level from a single sample. The methods employ antibodies labeled with metal isotopes that are detected with mass spectrometry.

AIMS

- To create a panel of several biomarkers aiming to detect cancer stem cells in heterogenous cell samples with mass and imaging mass cytometry
- To test the created panel with Helios mass cytometer and Hyperion imaging mass cytometer



Head and neck squamous cell carsinoma metastasis cells

Embryonic stem cell line H9 cells

MATERIALS AND METHODS

SELECTION OF MARKERS

- 16 biomarkers of interest are selected from markers expression of which is known to differ from differentiated cells in stem cells and CSCs.
- For 6 of these markers (in bold) there are no commercial metal labeled antibodies and thus the antibodies are self-labeled with a kit from supplier.
- The antibodies for the rest 10 markers are purchased labeled from the supplier.

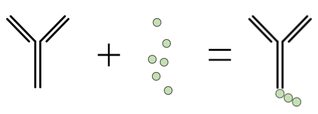
THE SELECTED MARKERS

EXTRACELLULAR	INTRACELLULAR
TRA-1-60	NANOG
SSEA-1	KLF4
SSEA-4	Oct3/4
SSEA-5	SOX2
CD44	DNMT3B
CD133	ALDH
Lgr5	**LIN28A**
	LIN28B
	L1TD1

VALIDATION OF ANTIBODIES

- Before metal labeling, the function of the 6 antibodies with the used protocols is tested in cell differentiation experiments performed with cells from embryonic stem cell line H7 and embryonal carcinoma cell line NTERA2.
- The expression of biomarkers in differentiated and undifferentiated cells is analyzed with flow cytometry and confocal microscopy.

ANTIBODY LABELING

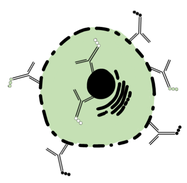


Labeling done with a labeling kit according to the kit's protocol

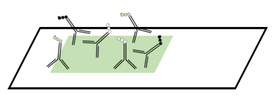
SAMPLES

- Embryonic stem cell line H9
- Embryonal carsinoma cell line 2102ep
- Normal fibroblast cell line

SAMPLE STAINING



Cell suspension samples for mass cytometry



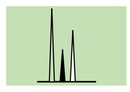
Sample slides for imaging mass cytometry

MASS CYTOMETRY

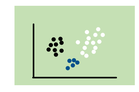
Separation of cells with a cytometer

Ionization with argon plasma

Detection of metals with mass spectrometry

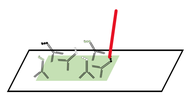


Data analysis with viSNE



IMAGING MASS CYTOMETRY

Laser ablation µm² at a time



Visual presentation of metals detected with mass spectrometry

Data analysis with HistoCAT

RESULTS AND CONCLUSIONS

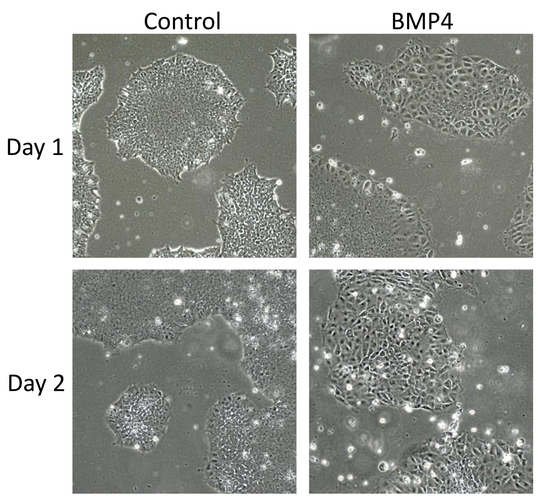


Fig. 1. Comparison of H7 cells at 1 and 2 days of the BMP4 differentation protocol against undifferentiated control H7 cells.

VALIDATION OF ANTIBODIES

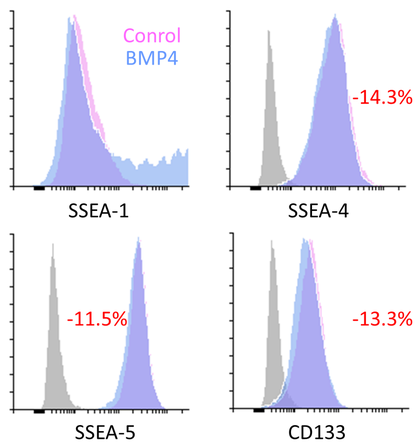


Fig. 2. Flow cytometry data of H7 cells after 2 days of BMP4 differentiation against control cells with markers SSEA-1, SSEA-4, SSEA-5 and CD133.

The initial results (fig. 2) show that after exposing H7 cells to BMP4 for 2 days the expressions of the markers connected with stemness, SSEA-4, SSEA-5 and CD133, are downregulated and the marker attributed to differentiated cells, SSEA-1, is upregulated when compared to control cells. This suggests that the tested antibodies are functioning with the selected protocol.

Experiments and result processing are currently still ongoing.