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INFRARED MICRO-SPECTROSCOPY TO DISTINGUISH GLIOMA AND GLIOMA-STEM CELLS

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Abstract — In the last years the development of microfluidic devices enabled infrared micro-spectroscopy (IRMS) to be applicable to biological samples. In the present study this method was used to find differences between glioma cells and glioma stem cells. The ability of glioma stem cells to initiate new tumor formation and their resistance to therapies makes these cells one of the main reasons why the most malignant form of glioma – glioblastoma is at present incurable. Several classic markers of glioma stem cells have been reported but none of them is their exclusive feature. Here we present preliminary results showing that by applying principal component analysis to IRMS data we can separate glioma stem cells and non-stem cells into two groups proving the potential of this technique. Further experiments are ongoing.

Index Terms — GLIOMA, stem cells, glioblastoma, infrared microspectroscopy, differentiation

1 BACKGROUND

Glioblastoma, the most common and aggressive type of brain tumors, remains incurable despite the recent progress in diagnostics and treatment of cancer. In the last ten years it has become evident that one of the main reasons for unsuccessful treatment and tumor recurrence after surgical resection lies in the ability of tumor stem cells – a small subpopulation of tumor cells – to give rise to different cell types that then form a tumor bulk.
It has been recently suggested that differentiation therapy might be a suitable approach to eradicate these cells. In this approach, a drug (therapeutic) would be used to induce differentiation of stem cells and such differentiated cell, although not dead, would not have the potential to induce new tumor formation.

2 OBJECTIVES

The main objective of the GLIOMA project is to find new markers of glioma stem cells. To be able to direct the therapy to these cells and at the same time preserve normal brain function, new methods to distinguish glioma stem cells, glioma non-stem cells or non-tumoral cells are needed.
The aim of the present study was to use infrared microspectroscopy (IRMS) to search for possible spectral differences between glioma stem and non-stem cells. We also suggest IRMS could be used to monitor the differentiating capacity of different compounds.

3 APPROACH & METHODS

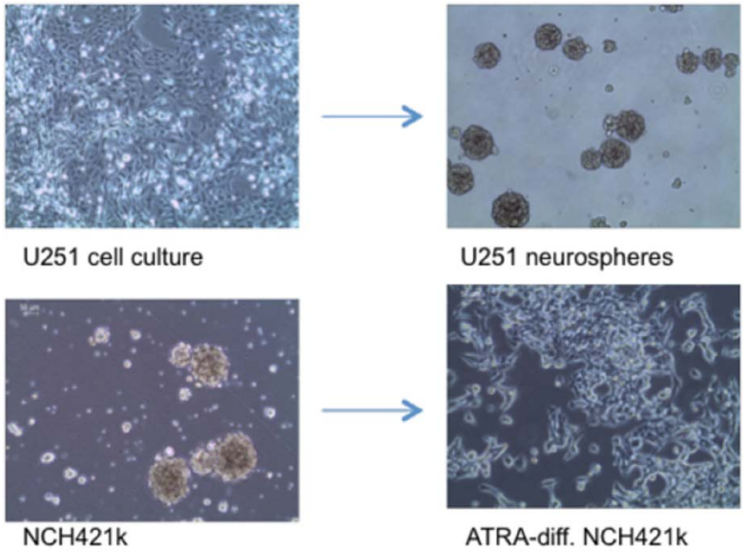
To be able to find differences between glioma cells and glioma stem cells, we have used two model cell lines. U251 is a glioblastoma cell line that can be enriched for the stem-cell population by culturing in a specific media. NCH421k are glioma stem cell-like cells which by treatment with all-trans retinoic acid (ATRA) get differentiated, losing their stem cell properties.
Each sample was split to two parts and analyzed simultaneously by QPCR and IRMS. Q-PCR was used to control the differentiation by measuring expression levels of stem cells markers nestin, CD133 and glioma marker GFAP. IRMS was used to compare spectral characteristics of stem cells versus differentiated cells. All IRMS experiments were done in the collaboration with SISSI-beamline in Elettra-Sincrotrone Trieste.

Microfluidic device using CaF_2 optical windows

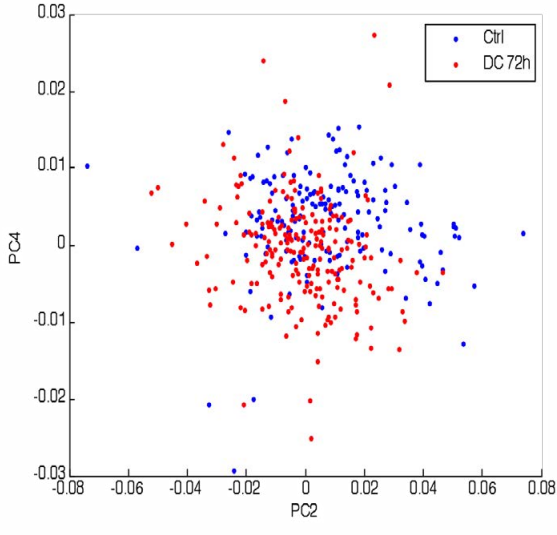
IRMS gives information on chemical composition of the cell that is based on detecting vibrations of different chemical bonds at corresponding wavelengths. The advantage of IRMS is that cells can be analyzed without prior labeling or fixation. A microfluidic system is used to be able to acquire infrared spectra of live cells. The device is designed to avoid the saturation of signal to the water contribution and to supply fresh buffer to support cell viability.
Diagnostic and characterization capabilities of IRMS have been largely documented and they are based on the multi-screening ability of the technique that gives comprehensive information on polysaccharides, lipids, nucleic acids and proteins in a single spectrum. In a recent publication we reported on the possibility to determine cell cycle phase of live unlabelled cell by IRMS (Bedolla et. al, 2013).

4 RESULTS

NCH421k and U251 were grown either as neurospheres, where they exert their stem cell properties or as monolayer, where they are differentiated.



In order to analyze the data, we used two different mathematical approaches – principal component analysis (PCA) and hierarchical cluster analysis (HCA); both of them gave interesting preliminary data that prove the potential of this experimental technique. When using the data acquired for the control (blue) and the differentiated NCH421k cells after 72h of exposure to ATRA (red), PCA show a grouping of the data in two groups.



5 POTENTIAL NEW PRODUCTS & SERVICES

A final goal of the study is to be able to detect and calculate the proportion of stem cells in a primary sample, which is important, since prognosis in glioblastoma correlates with the percentage of stem cells. Also, IRMS could be used to test if other agents used to differentiate stem cells result in the same spectral changes.

6 CURRENT COLLABORATIONS

Within the GLIOMA project we collaborate with National Institute of Biology, Ljubljana, Slovenia and "Santa Maria della Misericordia" Health-University Institute, Udine, Italy.

7 COLLABROATIONS NEEDED

Collaboration with an institution that has access to primary samples is required.

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