Investigation of Adhesion and Mechanical Properties of Human Glioma Cells by Atomic Force Microscopy

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Abstract

Glioma is one of the most common brain tumor characterized by active cell migration and invasion that make this tumor able to rapidly infiltrate into the surrounding brain tissue.
By analyzing human tumor explants a novel class of glioma-associated-stem cells (defined as GASC for high-grade glioma -HG- and *gasc* for low-grade glioma -LG-) has been identified [1]. They are not tumorigenic cells and act supporting the biological aggressiveness of glioma-initiating stem cells (defined as GSC for HG and *gsc* for LG) facilitating also their motility.
To get a deeper understanding about the role of the glioma-associated-stem cells in tumor progression, we have investigated the interaction forces between glioma-initiating stem cells and glioma-associated-stem cells by single cell force spectroscopy and the cell deformability by indentation with atomic force microscopy (AFM). A first finding is that the adhesion strength of GSC on GASC appears to be significantly lower than that observed for *gsc* on *gasc*. On the contrary, GSC spread and firmly adhere on *gasc* with an adhesion strength increased as compared to the one obtained on GASC (Fig.1). These findings demonstrate that the grade of glioma-associated-stem cells plays a relevant role in modulating cancer cell adhesion, which in turn affects glioma cell migration and thus cancer aggressiveness [2].
Our findings show also that, both HG and LG glioma, cancer-initiating-stem cells are softer than non tumorigenic glioma-associated-stem, in agreement with their neoplastic features [3]. The different cell stiffness might thus provide a way to detect them in the tumor tissue. Taking into account such observation, we have explored the possibility to identify glioma initiating stem cells on the basis of their mechanical properties. Therefore we have measured the cellular stiffness of all cells deriving from patients' explants with HG gliomas. In order to distinguish the stem cells, the indentation measurements are coupled with a fluorescent test based on the detection of aldehyde dehydrogenase (ALDH) activity, which has been demonstrated to be successful in the isolation of stem cells within several tumors [4]. The same measurements are performed on astrocytes, an immortalized non-tumor glial cell line, and on U87, an immortalized glioblastoma cell line. The stiffness of these cells is plotted as function of fluorescent intensity (Fig.2). The comparison of these data displays that in the glioma cells derived from HG glioma explants, a fraction of soft highly fluorescent cells can be detected. This fraction could represent the cancer initiating stem cells, being also their stiffness value comparable to that found in the mechanical analysis of the single sub-population derived from human glioma explants..
Such findings suggest that the analysis of cell stiffness can be exploited to detect cancer initiating stem cells within a mixed cell population of glioma. This could represent a new diagnostic or prognostic parameter to be exploited in clinics.

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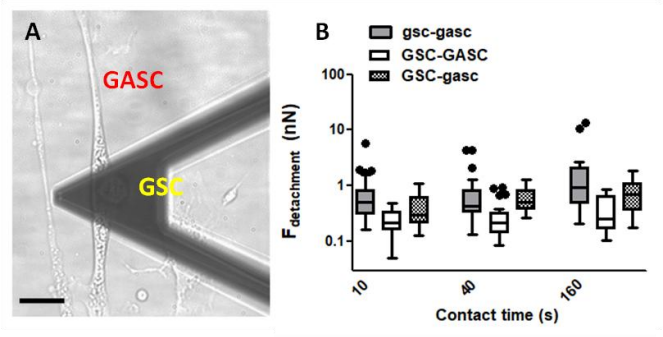

Figure 1: Bright field microscopy image of a GSC immobilized on a tipless cantilever in contact with a GASC cultured on glass coverslip (scale bar 20 μm) (A); Comparison of time dependent detachment force evaluated for *gsc-gasc*, GSC-GASC and GSC-*gasc*, For a better visualization and comparison of the data, Y scale is reported as logarithmic scale.

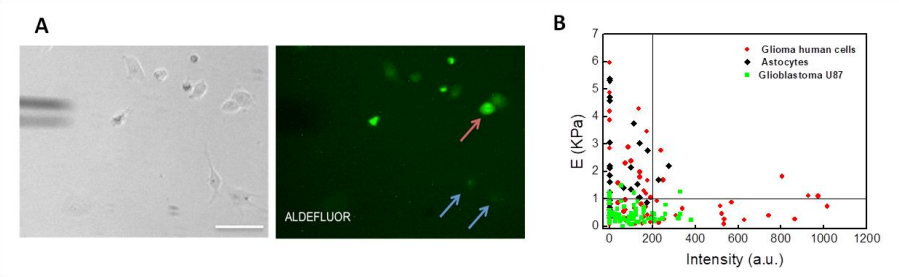

Figure 2: Bright field and epifluorescence images of glioma cells derived from human explants (A); Cellular stiffness plotted versus fluorescence intensity (B).