Statistical classification of potential radial glia cells based on nuclear shape measures after mechanical induction from astrocyte cultures

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Radial glia cells (RG) are the principal embryonic neural stem cell (NSC) that generate different types of progenitors, neurons and glia, also serving as substrate for neuronal migration. At the end of neurogenesis, most RG directly transforms into astrocytes. RG are bipolar cells that form a radial palisade spanning the entire neuroepithelia from the ventricular (apical) to the pial (basal) surfaces. This apical-basal anchorage generates mechanical tensions that are crucial for RG integrity, differentiation potential, and function. This work is part of a research project focused on the contribution of niche mechanobiology in determining RG lineage progression and cell fate.

Primary astrocytes cultures from newborn mice cerebral cortex were grown for 3 days *in vitro* in two class of substrates: polymethyl methacrylate with 2µm linear topographies (ln2PMMA) and borosilicate glass coverslips (control). We have described that the micro3D substrate ln2PMMA mimic surface properties and topology of the RG embryonic niche, signals that are sufficient to induce astrocytes to RG transformation.

We propose a combination of biological, image processing and statistical analysis procedures to unravel the contribution of nuclear deformation induced by ln2PMMA in the mechanical/topological modulation of RG lineage progression.

For that purpose, a MATLAB algorithm has been developed to high-throughput image analysis able to crosslink biological data with nuclear shape measures at a single-cell level. By immunofluorescence and confocal microscopy, we obtained nuclear shape measures (area and eccentricity) of cells identified with specific, cell-lineage markers for astrocytes (GFAP), for RG/NSC (nestin, Pax6 and Sox2), for oligodendrocyte progenitors (NG2) and for tripotential intermediate progenitors (GSX2). Nuclei were stained with Hoechst.

We classified cells/nuclei in the resulting database attending to the lineage markers expressed and according to the literature. Then we define a binary variable that identifies 'promising' cells, i.e., cells that would successfully evolve into RG. Similarly, we defined a binary variable for cells that are 'non-promising' to be RG. Note that some cells have value 0 in both variables. For each binary variable, we estimated logistic regression models, depending on the substrate, nuclear shape parameters and the cell density. Statistical models estimate the effect of substrate and were interpreted in order to understand the effect of covariates.

Keywords: Neural stem cells, logistic regression, image analysis.