# TNF-induced astrocyte reactivity and augmented β3 integrin levels are regulated by Rab endocytic pathways

P-03.1-19

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The pro-inflammatory environment generated by brain injury induces “Gliosis”, a process where astrocytes undergo hypertrophy, migration and increased expression of proteins including glial fibrillar acidic protein (GFAP), syndecan-4, and ß3 integrin. Increased levels of αVβ3 integrin in reactive astrocytes promote cell migration through association with the neuronal protein Thy-1. The mechanisms implicated in the up regulation of αVβ3 integrin in astrocytes under pro-inflammatory conditions remain unclear. We performed a comparative meta-analysis of gene expression profiles from four different datasets (GSEs) of non-reactive versus reactive astrocytes. The hierarchical clustering comparisons were performed using the MultiExperiment Viewer program, and affected signaling pathways were examined with the Kyoto Encyclopedia of Genes and Genomes. DITNC1 astrocytes were treated with the proinflammatory cytokine TNF for 48 h to induce reactivity in vitro. β3 integrin levels were evaluated by western blotting. GTPase activities were measured using pull-down assays. Thy-1-induced astrocyte migration was evaluated in transwell assays. The bioinformatic analysis revealed alterations in Rab endocytic pathways in reactive astrocytes. TNF treatment changed the expression of proteins associated with the appearance of reactivity markers, such as GFAP, and increased surface levels of β3 integrin in the DITNC1 cells. This same treatment altered Rab and Rac1 GTP loading. Thy-1-induced astrocyte migration was also associated with the changes observed in Rab protein levels. These studies aid in understanding the molecular mechanisms involving Rab endocytic pathways in astrocyte reactivity, an important process observed in pathological conditions, such as neurodegenerative diseases like Amyotrophic Lateral Sclerosis and following brain injury.

Study financed by FONDECYT 1200836 (LL), 1170925, 1210644 (AFGQ) and FONDAP 15130011 (AFGQ). Postdoctoral FONDECYT 3170169 (JD), and 3170645 (RDP)