CB-15. NKCC1 REGULATES MIGRATION CAPACITY OF GLIOBLASTOMA TUMOR INITIATING CELLS BY MODULATING ACTIN CYTOSKELETON THROUGH SMALL RHO GTPASES
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Glioblastoma (GBM) is a highly invasive and lethal brain tumor due to its high invasion and recurrence. The mechanisms that confer GBM cells their invasive behavior and their regulatory system have not been fully elucidated. We have shown that pharmacological inhibition and decreased expression of the electroneutral Na⁺+K⁺+Cl⁻ cotransporter-1 (NKCC1) in GBM leads to a decreased cell migration and invasion capacity, in vitro and in vivo. We have previously reported that NKCC1 knockdown cells (NKCC1 KD) show significantly larger focal adhesions, suggesting a potential role of NKCC1 in cell adhesion. Here, we focused on the role of NKCC1 on the cytoskeleton dynamics of GBM cells. We found that glioma cells display a significant decrease on their cell spreading capacity upon NKCC1 knockdown or pharmacologic inhibition by Bumetanide (BMT) (p < 0.0001). F-actin staining showed a change in the organization of fibrillary actin of the cells, where NKCC1 KD cells displayed a ring shape actin morphology that was absent in control cells. In addition the bundled actin content was decreased by NKCC1 inhibition (GBM 612 p < 0.05; GBM 965 p < 0.001). The molecular regulation of actin dynamics can be due to the activity of RhoA acting through Cofilin, which acts as an initiator of actin polymerization. We observed that the small GTPase RhoA and Rac1 active levels decreased 40-50% in the NKCC1 KD cells. This result suggests that NKCC1 regulates cytoskeleton dynamics through multiple targets that include filament actin regulation and RhoA and Rac1 activity. Based on our results, NKCC1 modulates migration of GBM cells by at least two different mechanisms: cell volume regulation (through ion transport) and regulation of the actin cytoskeleton. Due to its essential role in cell migration and high expression in GBM, NKCC1 may serve as a specific therapeutic target to decrease GBM cell invasion.