RhoA mediated transcriptional pathways in tumor cell growth

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G-protein coupled receptors (GPCRs) are well-accepted targets in drug therapy. GPCRs that couple to G α 12 and G α 13 proteins regulate guanine nucleotide exchange factors (GEFs) for RhoA activation. The G α 12 and G α 13 proteins, as well as the Rho GEFs, are oncogenic and there is considerable evidence implicating RhoA signaling in aberrant cell growth. Our earlier studies demonstrated that thrombin and sphingosine 1-phosphate (S1P) elicit mitogenic responses in a human glioblastoma cell line (1321N1) through PAR1 and SIP2/3 coupling to RhoA activation. Two transcriptional co-activators recently linked to RhoA signaling, YAP and MRTF-A were found to be concomitantly activated in response to S1P and thrombin, and required for expression of a range of target genes (Yu et al, MCB, 2017). Further studies used shRNA knockdown GSC-23 glioma stem cells from patient-derived xenografts (PDX). Knockdown of YAP or MRTF-A significantly attenuated in vitro self-renewal capacity as assessed by limiting dilution, stem cell gene expression and neurosphere formation. Orthotopic xenografts of the MRTF-A and YAP knockdown PDX cells formed significantly smaller tumors than control cells. In vitro studies using GSC-23 shRNA or 1321N1 CRISPR-Cas9 knockout cells demonstrated that YAP signaling was required for S1P to increase cell migration and invasion, MRTF-A was required for cell adhesion, and both co-activators were required for proliferation. RNA-sequencing of S1P-treated MRTF-A or YAP knockout 1321N1 cells identified 44 genes induced through RhoA and highly dependent on YAP, MRTF-A, or both. Tissue factor (F3), a target gene regulated selectively through YAP, was found to be required for S1P stimulated invasion and migration, whereas HBEGF (Heparin binding EGF-like growth factor), a target gene selectively induced through MRTF-A, was required for cell adhesion. TF and HBEGF expression were also selectively decreased in the GSC-23 derived tumors lacking YAP or MRTF-A respectively. Our findings suggest that YAP and MRTF-A and their target genes play critical roles in functional responses to RhoA signaling, that in vivo GBM tumor growth requires these pathways, and that their blockade could provide a new modality for treatment of GBM.