Structural insights into regulation of Trio by Gaq, a driver oncoprotein in uveal melanoma

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Uveal melanoma (UM) is the most common eye cancer in adults, with 80% of cases caused by mutations in either Gaq or Ga11 that decreases GTPase activity and thereby renders these proteins constitutively active. Although tumors can be controlled through eye surgery or radiation therapy, in up to 50% of UM patients the tumor undergoes metastasis to other parts of the body at which point it becomes extremely difficult to treat. Mitogenic signaling by Gaq/11 in UM is mainly exerted through its activation of Trio, a widely expressed and highly conserved Rho guanine nucleotide exchange factor (RhoGEF). Trio contains two different tandem Dbl and pleckstrin homology domains, termed TrioN and TrioC, that act as GEFs for Rac and RhoA GTPases respectively. Gaq directly activates TrioC. Targeting the Gaq-Trio C interaction may be a suitable therapeutic strategy for UM as well as for other cancers in which Trio gene is amplified. Our current studies are focused on determining the structure of TrioC bound to Gaq using cryo-electron microscopy (cryo-EM) single particle reconstruction. Purified TrioC-Gaq complexes were applied to cryo-EM grids, vitrified, and then imaged by transmission electron microscopy. The resulting 2D cryo EM projections were averaged and aligned to generate 2D classes which revealed Gaq bound to Trio. Currently, the resolution of refined cryo-EM 3D reconstructions is not high enough to provide details of the interactions which may be due to dynamic protein. We are pursuing to stabilize the complex for high quality structural details.