Overexpression of *Eyes Absent Homology 4* (Eya 4) in Malignant Peripheral Nerve Sheath Tumor Cells Alters the Retinal Determination Transcription Complex and Promotes Tumorigenesis

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NF1 is an autosomal dominant disorder affecting approximately 1 in 3500 individuals worldwide. NF1 patients are at risk for malignant peripheral nerve sheath tumor (MPNST), a life threatening sarcoma. The NF1 gene product, neurofibromin, is one of a family of GTPase activating proteins (GAPs) that accelerates the hydrolysis of active Ras-GTP to inactive Ras-GDP. MPNST cells have high Ras-GTP and elevated EGFR. EGFR and HRas inhibitors slow growth of MPNST cell lines, supporting the idea that dysregulation of these signaling pathways may contribute to tumorigenesis. In order to dissect the mechanism of transformation, microarray analysis was carried out between MPNST vs. normal human Schwann cells. EYA4, a transcription factor with phosphatase activity, was upregulated 37 fold in MPNST (Miller et al., 2008).

To test the hypothesis that EYA4 overexpression is related to tumorigenesis via Ras or EGFR signaling, we knocked down EYA4 in MPNST cells using shRNA and studied expression of EYA4 and its binding partners DACH and SIX1 upon Ras and EGFR activation or suppression.EYA4 knockdown slowed cell growth, reduced cell proliferation and caused cell death. Cell migration was reduced and turmorigenesis profoundly reduced when EYA4 knockdown stable cells were injected into athymic nude mice. An EGFR selective inhibitor and dominant negative HRas reduced EYA4 and SIX1 expression and induced DACH expression, implicating the EYA4-DACH-SIX 1 complex in NF1 tumorigenesis.