Notch Signaling: a New Potential Target in the Treatment of Uveal Melanoma

Laura Asnaghi¹, Michael L. Coonfield¹, Karisa C. Schreck¹, Eli E. Bar¹, James Handa², Shannath Merbs², Katy Ebrahimi², J. William Harbour³, Charles G. Eberhart¹²

¹Department of Pathology, Johns Hopkins University, School of Medicine, 400 N. Broadway Ave, Baltimore, MD 21287 USA  ²Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University, School of Medicine, Baltimore, MD 21287 USA  ³Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St Louis, MO 63110 USA.

Abstract

Uveal melanoma is the most common primary intra-ocular malignancy in adults, and causes significant mortality due to its propensity to metastasize. Our aim is to investigate the role of Notch signaling in promoting uveal melanoma proliferation and invasion. We examined five established uveal melanoma cell lines, and found using qPCR that the Notch 1-2-3 receptors, Jag1-2 ligands and the pathway target Hes1 were expressed to varying degrees in all lines. We then blocked Notch signaling using the gamma-secretase inhibitor (GSI) MRK003, and found that only three of the lines (OCM1, OCM3, OCM8) had their growth inhibited by the drug. Interestingly, these three lines had significantly higher levels of Hes1 mRNA as compared to the two lines resistant to GSI treatment (Mel 285, Mel 290). GSI treatment induced a dose-dependent reduction of anchorage-independent clonogenic growth, with 50% inhibition in OCM1, 70% in OCM3, and 40% in OCM8, as well as a significant reduction in Hes1 mRNA and protein levels. Apoptosis, as measured by cleavage and activation of caspase-9, was also induced. Finally, we observed inhibition of cellular invasion in GSI treated cultures using transwell migration and scratch assays. Preliminary studies of snap-frozen primary tumors indicate that Notch pathway components are expressed in primary uveal melanoma in vivo at levels as high or higher than those in our cell line models. In addition, oligonucleotide microarray analysis showed a significant increase in Jag2 and Notch3 expression levels in primary uveal melanomas that metastasized as compared to those that did not. Our findings suggest that the Notch pathway plays an important role in inducing cellular proliferation and invasion in uveal melanoma, and that inhibiting this pathway using pharmacological agents may be effective in preventing tumor growth and metastasis.