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Authors

Lyubasyuk, Vera
Ouyang, Hong
Yu, Fa-Xing
et al.

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YAP inhibition blocks uveal melanogenesis driven by GNAQ or GNA11 mutations

Vera Lyubasyuk[1], Hong Ouyang[1], Fa-Xing Yu[2], Kun-Liang Guan[2], and Kang Zhang[1,*]

[1]Department of Ophthalmology and Shiley Eye Center and Institute for Genomic Medicine; University of California; San Diego; La Jolla, CA USA;
[2]Department of Pharmacology and Moores Cancer Center; University of California; San Diego; La Jolla, CA USA

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Uveal melanoma (UM) is the most common adult intraocular tumor. UM often involves activating mutations in guanine nucleotide binding protein (G protein), q polypeptide (GQ), or G Protein, α 11 (G11). We show that the Yes-associated protein (Yap) inhibitor verteporfin blocks tumor growth of Gq/11-mutated UM cells.

Uveal melanoma (UM) arises from melanocytes residing in the choroid, ciliary body, and iris.[1] Although rare, it is the most common adult eye cancer and has a poor long-term prognosis. Once it has metastasized there is no effective therapy and average survival is 2 to 8 months.[2] The mutational spectrum of UM is very different from that of cutaneous melanoma. Instead of carrying mutations in v-raf murine sarcoma viral oncogene homolog B1 (BRAF) or neuroblastoma RAS viral (v-ras) oncogene homolog (NRAS), approximately 80% of cases of UM have activating mutations in either guanine nucleotide binding protein (G protein) q polypeptide (GNAQ), or G protein α 11 (GNA11).[3,4] GNAQ and GNA11 are the α subunits of heterotrimeric G proteins that are activated by many G protein-coupled receptors (GPCRs) following ligand stimulation. However, mutations at arginine 183 (R183) or glutamine 209 (Q209) of GQ/11 convert the G protein into a constitutively active and oncogenic form.[4]

G protein-coupled receptor (GPCR) signaling has recently been linked to the Hippo tumor suppressor pathway.[5] The major effectors of the Hippo pathway are Yes-associated protein (Yap) and transcriptional coactivator with PDZ-binding motif (Taz), 2 homologous oncoproteins that function as transcription co-activators and are repressed upon phosphorylation by upstream kinases Lats1/2.[6] The report that GPCRs coupled to Gq/11 are able to induce the oncogenic activity of Yap and Taz[5] led us to investigate whether the Hippo pathway could be a mediator in active Gq/11-induced tumorigenesis, and particularly in UM development. A similar study has been conducted by Gutkind's group, and is not covered in detail here.[7]

To explore this hypothesis, we tested whether YAP can be activated by the cancer-associated mutant form of GQ/11. We found that ectopic expression of mutant protein (GQ^{R183Q}, GQ^{Q209L}, or $G11^{Q209L}$) in human embryonic kidney 293A cells caused dramatic dephosphorylation, nuclear localization, and activation of YAP. Next, we investigated YAP activation status in a panel of 13 cell lines established from primary or metastatic UM. Our results demonstrated that YAP is activated in GQ/11-mutant UM cells but inactivated in BRAF-mutant UM cells. In a collection of formalin-fixed, paraffin-embedded sections of enucleated tumors, we observed a strong correlation between mutated GQ/11 and YAP nuclear localization. Moreover, in UM cell lines carrying the GQ mutation, knockdown of GQ by short hairpin RNA (shRNA) led to increased phosphorylation and decreased nuclear localization of YAP. These findings indicate that YAP is activated by mutant GQ/11 widely present in UM, and also suggest a role of YAP oncoprotein in mutant GQ/11-induced tumorigenesis.

In a subcutaneous xenograft mouse model, UM cells (92.1, Gq^{Q209L}) were able to form solid tumors in immunocompromised mice. However, when Gq was knocked down by shRNA, 92.1 cells failed to develop tumors. In contrast, melan-a cells (immortalized melanocytes) were unable to form tumors subcutaneously, whereas melan-a cells with Gq^{Q209L} expression were tumorigenic. These results proved that mutant Gq plays an important role in driving tumor formation. To test the effect of Yap in mutant Gq/11-induced tumorigenesis, we knocked down Yap in 92.1 cells and melan-a (Gq^{Q209L}) cells. Tumors formed by Yap-deficient cells were significantly smaller than those formed by control cells (**Fig 1A**). Therefore, Yap appears to be essential in mediating the oncogenic effect of mutant Gq/11.

Tead-family transcription factors (Tead1-4) can directly interact with Yap and mediate most of the transcriptional output of Yap.[7] Small molecules such as verteporfin can disrupt the interaction between Yap and Tead1-4, and thus inhibit Yap biological function.[8] We

*Correspondence to: Kang Zhang; Email: kang.zhang@gmail.com
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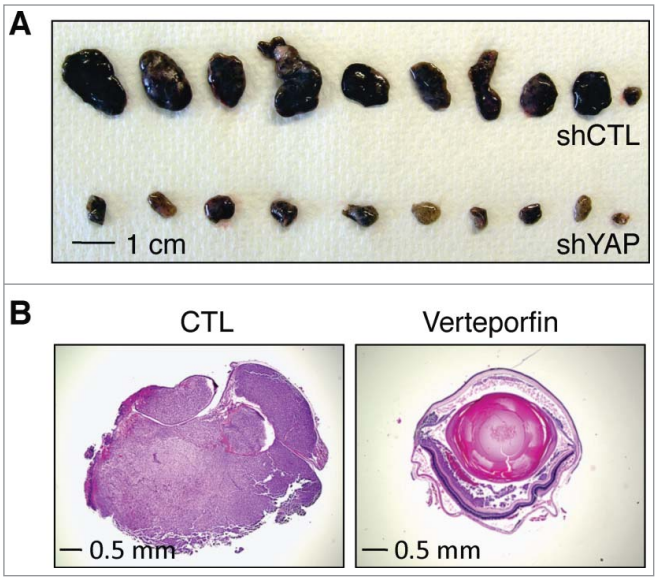

Figure 1. YAP inhibition suppresses the growth of uveal melanoma cells bearing GNAQ or GNA11 mutations. (**A**) 92.1 cells (2×10^6) transfected with control shRNA (shCTL) or shRNA targeting Yes-associated protein (Yap) were injected subcutaneously into nude mice. After 1 month, tumors that formed were harvested and photographed. (**B**) 92.1 cells (5×10^4) were mixed with nanoparticles containing verteporfin or buffer (CTL) and injected into the suprachoroidal space in the right eye of severe combined immunodeficient (SCID) mice. Verteporfin was administered by intraperitoneal route at a dose of 100 mg/kg every other day over a period of 14 d. After 6 weeks, very large masses of tumors developed in the control mice, but not in the verteporfin-treated mice. Eyes were harvested, sectioned, stained with hematoxylin and eosin (H&E), and analyzed microscopically for the presence of tumors.

showed that 92.1 cells could form a tumor when injected into the suprachoroidal space of mouse eyes; however, when verteporfin was co-administered the tumor formation capability of 92.1 cells was largely abolished (**Fig. 1B**).[9] These data suggest that inhibiting Yap activity may serve as a novel approach to the treatment of UM lesions driven by Gq/11 mutation.

One interesting discovery of this study is that Yap activation is required for UM lesions by caused Gq/11 mutation but not for those associated with another, less frequent, mutation in Braf. YAP was hyperphosphorylated (inactivated) in Braf mutated cells, and YAP knockdown in these cells failed to reduce their tumorigenicity significantly. Moreover, Braf mutant UM cells were less sensitive to Yap inhibition, as a much higher dose of verteporfin was required to effectively kill these cells. Therefore, the mutation background of UM lesions must be taken into consideration when Yap inhibitors are used for therapeutic interventions, and Yap inhibition may only applicable to the category of UM harboring Gq/11 mutation.

UM is the most common eye tumor in adults and a systemic treatment for metastatic UM is urgently needed. GQ/11 mutation functions as a cancer driver and is widely present in UM lesions, but a drug that targets constitutively active GQ/11 is currently not available. However, YAP activation resulting from Gq/11 mutation in UM makes YAP a potential drug target. The ability of verteporfin to inhibit YAP and suppress tumorigenesis demonstrates an impressive opportunity for treatment of the metastatic form of UM. Moreover, YAP activation plays a broad role in cancers driven by altered GPCR signaling and the potential therapeutic value of YAP inhibitors may be extended to other types of malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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