

Signaling

Major finding: GNAQ activates multiple oncogenic signaling pathways via ARF6 activation in uveal melanoma.

Mechanism: ARF6 is activated by the GEP100 GEF and promotes GNAQ localization to cytoplasmic vesicles.

Impact: ARF6 may be a therapeutic target in uveal melanoma and other GNAQ-driven tumors.

ONCOGENIC GNAQ ACTS THROUGH A SINGLE NODE IN UVEAL MELANOMA

Oncogenic activating mutations in the G-protein-coupled receptor (GPCR) Gαq subunits GNAQ or GNA11 occur in the majority of uveal melanomas. These mutations drive tumorigenesis through activation of downstream signaling pathways including PLC/PKC, Rho/Rac, and YAP; however, the mechanism by which Gαq mutations activate these pathways remains unclear. The small GTPase ADP-ribosylation factor 6 (ARF6) has been implicated as a potential effector of Gαq signaling, given that Gαq proteins associate with ARF guanine nucleotide exchange factors (GEF) that activate ARF6, and has been linked to tumorigenesis, leading Yoo, Shi, Grossmann, and colleagues to investigate the role of ARF6 in uveal melanoma cells with an activating GNAQ mutation. Mutant GNAQ enhanced cell proliferation in an ARF6-dependent manner, elevating levels of active ARF (ARF-GTP). GNAQ acted through ARF6 to activate known downstream PLC/PKC, Rho/Rac, and YAP signaling. Further, GNAQ-induced ARF6 activation promoted β-catenin signaling and localization to the nucleus. Mechanistically, the ARF-GEF GEP100 formed a complex with GNAQ and activated ARF6, and ARF6 in turn promoted mutant GNAQ

localization to cytoplasmic vesicles, thereby increasing downstream oncogenic signaling. Collectively, these results suggest that ARF6 may be a critical oncogenic GNAQ signaling node and a therapeutic vulnerability in uveal melanoma. A high-throughput screen to find allosteric ARF6 inhibitors identified NAV-2729 as a potential candidate, and modeling predicted binding with the ARF6 GEF-binding area. NAV-2729 blocked ARF6 activation, promoted GNAQ mislocalization from cytoplasmic vesicles to the plasma membrane, reduced PLC/PKC, Rho/Rac, YAP, and β-catenin signaling, and suppressed anchorage-independent growth of uveal melanoma cells. Moreover, NAV-2729 reduced tumor establishment and growth in an orthotopic xenograft model of uveal melanoma. Altogether, these findings indicate that GNAQ activates multiple oncogenic pathways via ARF6, and implicate ARF6 as a candidate therapeutic target in uveal melanoma. ■

Yoo JH, Shi DS, Grossmann AH, Sorensen LK, Tong ZZ, Mleynek TM, et al. ARF6 is an actionable node that orchestrates oncogenic GNAQ signaling in uveal melanoma. *Cancer Cell* 2016;29:889–904.

Immunotherapy

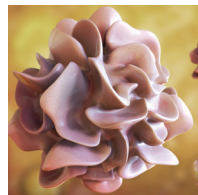
Major finding: Negatively charged ligand-free RNA liposomes effectively trigger an antitumor immune response.

Mechanism: RNA liposomes deliver antigens to dendritic cells and induce dendritic cell-mediated IFNα release.

Impact: Systemic delivery of RNA liposomes may be a broadly potent cancer immunotherapeutic approach.

SYSTEMIC RNA DRIVES INNATE AND ADAPTIVE ANTITUMOR IMMUNE RESPONSES

Strategies to develop cancer vaccines using dendritic cells (DC), which are very proficient antigen-presenting cells that trigger the immune response in lymphoid tissue upon early detection of pathogens, include the systemic delivery of antigen-encoding nucleic acids complexed with cationic liposomes. However, the delivery of positively charged RNA liposomes (RNA-LPX), which exhibited greater *in vitro* transfection efficiency than negatively charged RNA-LPXes, failed to induce significant antitumor responses. Using the three most common lipid formulations, Kranz, Diken, and colleagues established a panel of luciferase (*Luc*) RNA-LPXes of different net charges by altering lipid:RNA ratios to ascertain the effects of RNA-LPX net charge on targeting DCs and evoking antitumor immunity. Intravenous delivery of *Luc* RNA-LPXes revealed that a shift from a net positive to a net negative charge resulted in a shift from preferential targeting of the lungs to preferential targeting of the spleen. Because *in vivo* transfection efficiency decreased with increasingly negative net charges, RNA-LPXes with a moderately negative net charge, which effectively targeted antigen-presenting cells (APC) such as DCs, were used for the remaining studies. Systemic deliv-



ery of RNA-LPXes encoding a pathogenic antigen or ovalbumin induced APC maturation, increased toll-like receptor 7 (TLR7)-mediated production of IFNα by DCs, and enhanced the expansion of APCs and activated antigen-specific T cells. Consistent with these findings, immunization with ovalbumin RNA-LPX encoding various types of tumor antigens cleared colon cancer and melanoma metastases, caused tumor regression, and prevented tumor regrowth in tumor-bearing mice. Similarly, in a phase I trial, three patients with melanoma who were treated with RNA-LPX vaccines encoding four tumor antigens exhibited increased IFNα and an enhanced activated T-cell response in a dose-dependent manner. Together, these findings identify an antiviral-like mechanism by which RNA liposomes concomitantly promote potent adaptive and innate immune responses, and provide evidence for the clinical activity of a systemically delivered nucleic acid-liposomal vaccine. ■

Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* 2016;534:396–401.