

Targeted Therapy

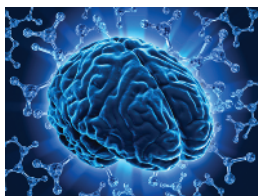
Major finding: Stem cell-delivered anti-EGFR nanobodies localize to and inhibit the growth of glioblastoma.

Approach: Anti-EGFR nanobodies were conjugated to imaging modalities or the proapoptotic protein TRAIL.

Impact: Nanobodies may improve the treatment of patients with EGFR-positive brain tumors.

EGFR-TARGETING NANOBODIES RELEASED BY STEM CELLS REDUCE TUMOR GROWTH

EGF receptor (EGFR) is amplified or mutated in a large percentage of glioblastoma, but treatment with anti-EGFR monoclonal antibodies has been largely unsuccessful, in part due to the blood-brain barrier. An alternative approach is the use of EGFR-specific nanobodies, which are smaller antibody fragments that consist of only the antigen-targeting domain, provide increased tissue penetration, and have been shown to block EGF binding to EGFR. To further investigate nanobodies as a treatment for glioblastoma, van de Water and colleagues engineered secretable, bivalent versions of EGFR-targeting nanobodies (ENb) and expressed them in neural stem cells (NSC), which specifically migrate to brain tumors and may therefore improve therapeutic delivery. ENbs were efficiently secreted by NSCs and prevented ligand stimulation of EGFR-expressing glioblastoma cells *in vitro*, thereby inhibiting the activation of downstream signaling and reducing glioblastoma cell viability. ENbs fused to fluorescent or bioluminescent imaging modalities retained the ability to prevent EGFR activation and showed the sustained localization of NSC-secreted ENbs to glioblastoma tumors, in contrast with systemically delivered ENbs that also localized to the liver and



kidney, indicating that these fusions may be useful for tumor imaging. Furthermore, NSC-secreted ENbs immunoconjugated to the proapoptotic protein TRAIL diminished cell viability and induced caspase-dependent apoptosis in glioblastoma cell lines with varying resistance to TRAIL, suggesting that ENb-TRAIL fusions may tar-

get a broad spectrum of tumor cells. Importantly, intratumoral implantation of NSCs expressing ENbs significantly reduced the growth of established intracranial glioblastomas and suppressed the invasion of primary glioblastoma xenograft tumors; expression of ENb-TRAIL augmented these inhibitory effects and further prolonged the survival of tumor-bearing mice. These results support clinical testing of tumor-specific delivery of anti-EGFR nanobodies as a therapeutic approach for the treatment of glioblastoma. ■

van de Water JA, Bagci-Onder T, Agarwal AS, Wakimoto H, Roovers RC, Zhu Y, et al. Therapeutic stem cells expressing variants of EGFR-specific nanobodies have antitumor effects. Proc Natl Acad Sci U S A 2012;109:16642-7.

Surveillance

Major finding: The growth of polyploid cancer cells is suppressed by T-cell-dependent immunosurveillance.

Mechanism: Hyperploidy renders cancer cells immunogenic via ER stress and membrane calreticulin exposure.

Impact: Cancer cells must overcome this extrinsic immunosurveillance to survive and proliferate.

IMMUNOSELECTION PROTECTS AGAINST HYPERPLOIDY IN CANCER CELLS

Oncogene activation stimulates growth-suppressive control mechanisms, such as the endoplasmic reticulum (ER) stress response, which triggers increased membrane exposure of calreticulin to enhance immune-mediated elimination of malignant cells. Senovilla and colleagues investigated whether polyploidization of cancer cells, a common and early event in many tumors, induces a similar immunosurveillance mechanism to constrain the growth of cells with aberrant DNA content. Treatment of mouse and human cancer cells with agents that promote hyperploidy, including cytochalasin D and nocodazole, promoted initiation of the ER stress response and relocalization of calreticulin to the cell surface, suggesting that these cells may be targeted by immunosurveillance. In support of this idea, nocodazole-treated hyperploidy cells exhibited increased T-cell priming in response to tumor antigens *in vitro* and formed tumors less efficiently in immunocompetent mice compared with immunodeficient animals; this inhibition of tumor growth was dependent on calreticulin expression and the presence of CD4+ and CD8+ T cells

and IFN activation. Tumors that did form in immunocompetent mice underwent immunoselection, resulting in decreased nuclear size and total DNA content and reduced membrane exposure of calreticulin. Interestingly, a correlation between an antitumor immune response and diminished nuclear size was observed in carcinogen- and oncogene-driven tumor models as well as in human breast cancer samples. Furthermore, ectopic expression of a membrane-targeted calreticulin protein was sufficient to limit tumor growth in immunocompetent mice in the absence of hyperploidy, indicating that surface expression of calreticulin renders cells immunogenic and triggers tumor immunosurveillance. These results identify a cell-extrinsic pathway that selects against polyploidy in tumors and suggest that malignant cells acquire mechanisms to escape this growth restraint. ■

Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, et al. An immunosurveillance mechanism controls cancer cell ploidy. Science 2012;337:1678-84.