Modeling Treatment Resistance and Invasion in Glioblastoma using Biomaterials
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Glioblastoma (GBM) is the most lethal, yet common, cancer originating in the brain with a median survival of 12-15 months. Poor clinical outcomes are attributed to aggressive treatment resistance and invasion throughout the brain. While there is an obvious need to develop better GBM therapies, a lack of humanized models that capture the complex microenvironment present in and around brain tumors has limited the clinical relevance of in vitro studies. Thus, we have developed biomaterial-based, 3D culture platforms providing a controlled extracellular environment that supports formation of patient-derived tumor spheroids that can be used to model the disease and screen treatments in a scientifically rigorous manner. Biomaterials are based on hyaluronic acid (HA), a major component of the brain extracellular matrix (ECM) that interacts with CD44. Increased expression of HA and CD44 correlate with GBM progression.

Multiple patient-derived GBM cell lines were cultured in HA-based hydrogels. A mixture of cell phenotypes, expressing Sox2, GFAP, βIII-tubulin and HIF1-α, all of which are found in clinical tumors, were observed in hydrogel cultures. Mechanical modulus, HA content and presence of the integrin-binding RGD peptide where varied in scaffolds, GBM cells responded to treatment with either targeted EGFR inhibition (e.g., erlotinib) and alkylating chemotherapies (e.g., temezolomide) when cultured as gliomaspheres, while resistance was observed with patient-matched xenografts in mice. In contrast to gliomasphere cultures, patient-matched cells in 3D culture in soft hydrogels (~200 Pa storage modulus) displayed robust resistance when relatively high concentrations of HA (0.5 wt%) or RGD peptides were included. When both high HA content and RGD peptides were included, significantly fewer cells responded to drugs in either category. Similarly, drug response was restored with shRNA knockdown of either CD44 or integrin-αv with a double knockdown resulting in very few surviving cells (Fig. 1, top). Further investigation revealed co-activation of Src downstream of CD44 and/or integrin-αv engagement. Pharmacological inhibition of Src with dasatinib restored drug responses to similar levels as with concurrent knockdown of CD44 and integrin-αv (Fig. 1, bottom left). Finally, results indicate that activated Src promotes drug resistance through inhibition of the pro-apoptotic factors Bak, Bax and Puma. Given rapid infiltration through the brain, preventing tumor cell invasion is a desired goal of new therapies. We show that, in 3D hydrogels cultures, migration of patient-derived GBM cells requires engagement of CD44 and integrin-αv as well as downstream Src phosphorylation (Fig. 1, bottom right). Together results indicate that mechanisms of drug resistance and invasion in GBM are intimately coupled. Overall, biomaterial platforms provide a controlled experimental context in which to characterize how the GBM microenvironment facilitates tumor aggression and provides an ex vivo test bed for new therapies.

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