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Reinvigorating antitumor immunity in brain tumors with short peptides and siRNA-nanocarriers targeting the tumor microenvironment

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Background/Aim. Tumor cells stimulate molecular, cellular and physical changes within their host tissues creating a tumor microenvironment (TME), a complex and continuously evolving structure which supports tumor growth and progression. Its composition varies between tumor types, but hallmark features include accumulation of myeloid cells, stromal cells, blood vessels, and extracellular matrix. Antitumor immunity is inhibited or eluded by tumor-secreted factors that reprogram infiltrating myeloid cells and create the immunosuppressive TME. Advancements in single-cell techniques allowed us to systemically profile the multiple-omic status of the TME at a single-cell resolution, revealing the phenotypes and functionalities of disease-specific cell populations. **Methods.** Using single-cell RNA and protein sequencing and spatial transcriptomics we identified the functional diversity and localization of immune cells in the TME of experimental malignant brain tumors. **Results.** Microglia and peripheral monocytes massively infiltrate brain tumors and become polarized to promote glioma invasion, immunosuppression and angiogenesis. Computational analyses revealed interactions between tumor, myeloid cells and lymphocytes and pointed to some factors responsible for tumor-induced reprogramming of immune cells. The emerging pathways have been blocked with innovative peptides or siRNA and the effects of therapeutic interventions on TME reprograming and antitumor immunity have been assessed. Genetic manipulation of microglia in diseases using small interfering RNA (siRNA) was however hampered by the lack of safe and efficient siRNA delivery methods. We assessed the amphiphilic dendrimers (AD) for functional siRNA delivery and gene knockdown in primary microglia cultures. AD protected the siRNA from degradation and facilitated its cellular uptake. AD effectively delivered *Id1*-targeting siRNA to primary microglia and decreased target gene and protein expression. Id1 is a negative regulator of myeloid cell differentiation and is upregulated in glioma-stimulated microglia and *Id1* knockdown led to attenuation of microglia reprograming stimulated by glioma cells. AD complexes were also effective as *in vivo* siRNA-nanocarriers in orthotopic glioma model in mice. **Conclusion.** The results open up new perspectives in functional genomic studies and therapeutic targeting of microglia in brain tumors and other CNS diseases.
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