Targeted Molecular Imaging of Brain Cancer

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Brain cancer remains amongst the most lethal diseases. The urgent necessity for more effective clinical management paradigms, coupled with the recent advancements in understanding the molecular biology underlying brain cancer tumorigenesis, has galvanized the emergence of novel, patient-tailored, diagnostic and therapeutic strategies based on targeting tumor-specific antigens. Within this context, molecular imaging has become an invaluable tool for visualizing, characterizing, and quantifying biological processes occurring at the molecular level, including expression of tumor specific targets, tumor metabolism and proliferation rates, and changes in tumor physiology such as tumor perfusion and oxygenation status. This dissertation focused on imaging the expression of several tumor-associated targets implicated in the proliferation, angiogenesis, tumor invasion, metastasis, and cancer stem cells traits of aggressive brain cancers using positron emission tomography (PET), and to a lesser extent fluorescence imaging.

Chapters 2 and 3 describe the design and generation of novel RGD peptide-based radiotracers for PET imaging of integrin $\alpha_v\beta_3$ expression, a well-known marker of tumor angiogenesis, in a mouse model of glioblastoma. These tracers were engineered to feature enhanced radionuclidic, targeting, and pharmacokinetic properties for *in vivo* imaging applications. The elevated accumulation of the tracer in tumorous tissue together with a rapid clearance from circulation and non-target tissues, allowed for the accurate delineation of subcutaneous glioblastomas. *In vitro*, *in vivo*, and *ex vivo* studies confirmed the specificity of the radiotracer towards integrin $\alpha_v\beta_3$. We also concluded that compared to ^{64}Cu, the positron emitting radiometal ^{44}Sc possesses properties (e.g., half-live, β^+ branching ratio, simple coordination chemistry) that are better suited for peptide-based imaging.

CD146 expression has been linked to increased cancer aggressiveness, metastasis, and decreased patient survival in a plethora of cancer, including brain cancer. In Chapters 4 and 5, we set out to produce YY146, an anti-CD146 monoclonal antibody, which was radiolabeled with ^{64}Cu and ^{89}Zr for noninvasive PET imaging of glioblastoma. The radiotracers ^{64}Cu-NOTA-YY146 and ^{89}Zr-Df-YY146 showed preferential uptake in the tumors of mice bearing subcutaneous or orthotopic U87MG xenografts, allowing for an accurate delineation of small brain nodules in the PET images. Tumor uptake correlated with expression levels of CD146 in a highly specific manner. Additionally, we also showed the potential therapeutic effects of YY146 on the cancer stem cell traits of U87MG cells, demonstrating that YY146 can mitigate those aggressive phenotypes. More importantly, histological analysis of human brain cancer tissue uncovered the clinical relevance of CD146 as a target of glioblastoma.

Given the heterogeneous and multifactorial character of brain cancer, combinatorial therapies are increasingly becoming more popular. Given the coexpression of epidermal growth factor receptor (EGFR) and CD105 in glioblastomas, we generated a bispecific immunoconjugate to target EGFR and CD105 simultaneously. Two antibody Fab fragments were conjugated using a bioorthogonal “click” chemistry ligation and labeled with ^{64}Cu for PET imaging. *In vivo* PET imaging revealed significantly higher uptake of the bispecific construct in U87MG tumors, compared to each monovalent Fab fragment. This synergistic enhancement in tumor affinity allowed for impressive tumor-to-background ratios that facilitated detection of malignancies at an early stage, while maintaining excellent specificity.