In Vitro Characterization of the Published Glypican-3-Targeting Peptide TJ12P2 Reveals a Lack of Specificity and Potency

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Abstract

Background/Objectives: The cell surface proteoglycan glypican-3 (GPC3) is reportedly overexpressed in hepatocellular carcinoma (HCC) tissues, but not in benign liver tissues, rendering this protein a potential target for radionuclide theranostic approaches. Peptides are generally a promising class of targeting molecules for the development of radioligands because they combine straightforward synthetic access with favorable pharmacokinetics. Among the published peptides with disclosed structures, one of the most promising radioligands is [18F]AlF-NOTA-TJ12P2, which has a reported comparably high binding affinity to GPC3 and a high hydrophilicity. In this study, we aimed to design novel GPC3-targeting radioligands based on the TJ12P2 peptidic scaffold. Methods: Peptides were synthesized on solid phase using an Fmoc protecting group strategy. For comparative investigations, the reference nanobody HN3 was expressed in E. coli, isolated and subsequently modified with NODA-GA or SulfoCy3. The binding of native peptides, scrambled variants and reference nanobodies to GPC3 was investigated by surface plasmon resonance (SPR) interaction analysis, and fluorescently labeled versions of peptides and nanobodies were used for fluorescence microscopy in HepG2 (GPC3+) or SK Hep1 (GPC3-) cells. The chelator-bearing peptides were radiolabeled with gallium-67 and their stability towards radiolysis and in human serum was investigated. The binding of radiolabeled peptides and nanobodies to HepG2 cells was assessed in real-time ligand binding experiments. Results: The synthesized native peptides did not exhibit binding towards GPC3 in SPR interaction analyses, and the observed response was comparable to that of the scrambled variants at equal concentrations. Additionally, no binding to or uptake of the fluorescent constructs into cells was observed with fluorescence microscopy regardless of cellular GPC3 expression level. In real-time radioligand binding experiments, very fast association and dissociation of the gallium-67 labeled peptides to GPC3 positive HepG2 cells was observed, suggesting either extremely fast binding kinetics or unspecific binding of the peptides. Conclusions: Taken together, these findings suggest that the peptide TJ12P2 lacks specific binding to GPC3 in vitro and might not serve as a basis for the development of radioligands targeting GPC3.