Characterization of epidermal growth factor (EGF) receptor targeting Nanobodies and ABIN for the treatment of EGF receptor-dependent tumours

The aim of our research was the generation and characterization of epidermal growth factor receptor (EGFR)-specific single domain antibody fragments derived from llama heavy chain antibodies (Nanobodies). Various tumours of epithelial origin frequently express high levels of EGFR. By activating numerous signal transduction pathways this receptor is able to enhance cell proliferation, survival and angiogenesis. Studies with monoclonal antibodies have demonstrated that binding to EGFR can lead to inhibition of ligand binding, signal attenuation and even apoptosis. Monoclonal antibodies however have relatively large dimensions which complicates their penetration into the tumour tissue. The use of a scFv, an antibody fragment, was shown to overcome this problem. ScFv's however are prone to aggregation and proteolysis, and display reduced affinity compared to the parental antibody. In contrast, Nanobodies display a superior stability and are considered to be the smallest antibody fragments with intact antigen binding capacity. We were able to generate Nanobodies that could specifically recognize EGFR on tumour cells overexpressing EGFR. Multivalent Nanobodies were able to inhibit the EGF-induced EGFR activation and could affect tumour growth in mice bearing tumour xenografts. In vivo biodistribution studies with monovalent Nanobodies indicated that these entities were specifically taken up in tumours overexpressing EGFR. Interestingly, the uptake was in correlation with the level of EGFR expression, indicating that Nanobodies could be used as diagnostic probes to monitor EGFR levels in course of therapy. Uptake in healthy tissues or organs, except for the kidneys, was minimal. These results also suggest that monovalent Nanobodies could be used to deliver anti-proliferative molecules specifically to tumour cells. In this context we evaluated the ability of ABIN molecules to block EGFR-induced NF-□B activation. This pathway is believed to be exploited by tumour cells to support their uncontrolled growth and resistance to therapy. We were able to demonstrate that ABIN could reduce the NF-□B activity in tumour cells and at the same time reduce their proliferation. Fusion of ABIN to EGFR-specific Nanobodies may therefore be an interesting strategy to treat EGFR-dependent tumours.

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