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Characterisation and *in vitro* and *in vivo* evaluation of a breast cancer cell- and neuroblastoma cell binding peptide (p160), identified through phage display technology.

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Specific targeting of chemotherapeutical drugs to tissues of interest with limited uptake by healthy tissues is of high importance for the development of cancer treatment and therapy. Major drawbacks of current cancer therapies are side effects and low specificity for tumour cells. A very important method for the identification of new specific binding ligands is the technology of phage display libraries. Random peptide phage display libraries find wide application for the isolation of peptides with high affinity for cancer cells. Those peptides can be further investigated and play a significant role for the development of peptide-based radiopharmaceuticals against cancer. Therefore, the aim of this thesis was the evaluation and characterisation of the properties of a peptide (p160) with affinity for human breast cancer and human neuroblastoma cells, which has been selected through phage display.
The peptide p160 (VPWMEPAYQRFL) was prepared by solid phase peptide synthesis and radiolabelled with ^{125}I or ^{131}I. The bioactivity of p160 was evaluated *ex vivo*, *in vitro* and *in vivo*. *Ex vivo* binding experiments were carried out on cell membrane proteins isolated from the human breast cancer cell line MDA-MB 435 and the human neuroblastoma cell line Wac-2, revealing that the radiolabelled p160 peptide can bind to the cell membranes; however this binding can not be competitively abolished by the unlabelled compound. *In vitro* binding experiments were carried out using the human breast cancer cell lines MDA-MB 435 and MCF 7 and the human neuroblastoma cell line Wac-2 as targets. The specific binding of p160 to the target cell lines was confirmed in competition experiments using the unlabelled p160 as competitor for radioligand binding and various peptides as negative control competitors. Those experiments showed that the binding of ^{125}I-labelled p160 to the Wac-2, MDA-MB 435 and MCF 7 cells was inhibited up to 95 % by the unlabelled p160, while the negative control

competitors D-p160 and octreotide did not inhibit the uptake of the radioligand. Performing the binding experiments on human umbilical vein endothelial cells (HUVEC), the binding capacity was found to be lower, supporting the hypothesis of a specific uptake of p160. FACS studies with FITC labelled p160 in Wac-2 cells, revealed an increased fluorescence signal, which was up to 80 % decreased in the presence of the unlabelled p160 peptide.

Internalisation of p160 was investigated with confocal microscopy, demonstrating a concentration of the fluorescence in irregular clusters at the periphery of the cells. Moreover, internalisation of ^{125}I-p160 was measured after incubation of the radioligand with the target cells and removal of the surface bound activity through incubation with an acidic buffer. Those experiments revealed an internalisation of about 40 % of the total bound activity in the MDA-MB 435 cells and 50 % in the Wac-2 cells. Kinetic analysis of ^{125}I-p160 in Wac-2, MCF 7 and MDA-MB 435 cells was investigated through incubation of the peptide with the tumour cells for different time periods, showing an initial time dependent increase and subsequently a time dependent decrease of the uptake.

The biodistribution of ^{131}I-labelled p160 was performed in tumour bearing mice. The organ distribution studies without and after perfusion of the animals revealed a higher accumulation in the tumour than in most of the organs. A comparison of ^{131}I-p160 with a ^{131}I-labelled Arg-Gly-Asp peptide in mice bearing MDA-MB 435 tumours, which are known to express $\alpha_v\beta_3$ integrins on their surface, showed a higher tumour to organ ratio for ^{131}I-p160. Furthermore, the *in vivo* kinetic properties of p160 were investigated, revealing a time dependent decrease of the uptake in tumour and other tissues, which might be explained by the metabolic instability of the peptide in serum.

The metabolic stability of p160 was investigated *in vitro* and *in vivo*. The products of the fast metabolic degradation of the peptide in serum were isolated by HPLC and characterised by mass spectrometry.

Aiming on the optimisation of the binding and metabolic properties of p160, a various number of fragments and derivatives of the peptide were synthesised and investigated for cell binding and serum stability. The results of those studies indicated that the sequence EPAYQR might be of significance for the binding of p160. Among those peptides, βAla-p160-8-2 showed a more than 2-fold increased binding capacity to Wac-2 cells when compared with native p160. Furthermore, time kinetic biodistribution of βAla-p160-8-2 showed a decrease in uptake over time in healthy tissues but not in the tumour, where the uptake remained almost constant.

Finally, in order to evaluate the influence of radioiodination on the binding properties of p160, the peptide was labelled with ^{99m}Tc- and tested for binding. The ^{99m}Tc-labelled p160

showed a higher binding capacity than the ^{125}I-labelled compound. Moreover, ^{99m}Tc-p160 demonstrated a time dependent increase of the uptake in Wac-2 cells, indicating that deiodination might influence the binding and kinetic properties of the peptide.

In conclusion, the peptide p160 has properties that make it an attractive carrier for tumour imaging and the intracellular delivery of isotopes or chemotherapeutic drugs. The uptake experiments *in vitro* revealed that the binding of p160 to human breast cancer and neuroblastoma cells might be mediated through a specific receptor, whereas the organ distribution in tumour bearing mice showed a higher binding to the tumour than to the other organs, which is favourable for cancer treatment. However, further investigation is necessary in order to study the cellular handling and optimise the properties of p160 for tumour imaging and drug targeting purposes.