Strategies for gastrin releasing peptide receptor targeted imaging in prostate cancer
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Chapter 6:

Summary
Prostate cancer (PCa) is most commonly diagnosed in men over the age of 50, but the genetic history of their family are already an indication of a possible predisposition. When diagnosed at an early stage, the 5-year survival rate of PCa is almost 100%. A series of rapid tests can be helpful for the diagnosis, such as the prostate specific antigen (PSA) blood test and the digital rectal examination (DRE). The gold standard diagnosis method is transrectal ultrasound guided biopsy which has also drawbacks as sampling error. PSA discovery certainly improved the early detection of PCa over the last decades and became indispensable for diagnosis and follow-up of PCa patients, but also increased overdiagnosis and overtreatment of PCa by generating false positives. Therefore, a noninvasive specific imaging tool is urgently needed. Positron emission tomography (PET) and single photon computed tomography (SPECT) with high sensitivity, specificity and resolution may help to investigating the staging and restaging of prostate cancer and obtaining molecular tumor information. Gastrin releasing peptide receptor (GRPR) may be a valid target for nuclear imaging of Prostate Cancer. GRPR is highly expressed in several human cancer cell lines (breast cancer, colon cancer, prostate cancer) and could be used as a potential target for cancer detection. Radiolabeled bombesin analogues could be potential probes for GRPR targeted nuclear imaging. Unfortunately, only a few bombesin probes have entered the clinical phase and the results obtained are hardly comparable because the different probes are synthesized in heterogeneous conditions, with different protocols, different cell lines and animal models. This means that it is problematic to choose the best performing BN candidate for clinical validation. Although developments of radiotracers for PET imaging are mostly studied and newly designed every day, it is also very important to developing validated tracers for SPECT imaging because SPECT imaging is still very common and used. $^{99m}$Tc and $^{111}$In are often used radionuclides for nuclear imaging with SPECT because are easily obtained and cheap. Besides these reasons, these two radionuclides have a long half-life ($^{99m}$Tc has $t_{1/2} = 6$ h and $^{111}$In has $t_{1/2} = 2.8$ days) and easy coordination chemistry and easy labeling protocols.

Two newly synthesized SPECT tracers have been evaluated in chapter 3 and 4. In these two chapters, a novel BN-homodimeric sequence, made by two Aca-BN(7-14) amino acid
chains tethered together, has been synthesized and radio-labelled by $^{99m}$Tc (chapter 3) and $^{111}$In (chapter 4). The rationale behind these two tracers was that multimers, such as dimers, might achieve higher peptide targeting ability by increasing the local ligand concentrations via multimerization strategy. Moreover, bombesin homodimeric analogues were radiolabeled with longer-lived SPECT-radionuclides because these large molecules are characterized by slow tissue penetration and slow wash-out with respect to smaller amino acid sequences. Firstly, $^{99m}$Tc-Hynic-Aca-[BN(7-14)]$_2$ was proposed as molecular imaging agent with improved tumor affinity because it showed a higher IC$_{50}$ value in comparison to the homologous $^{99m}$Tc-Hynic-Aca-BN monomer. This difference in IC$_{50}$ value was demonstrated by in vitro cellular uptake kinetics studies. It was further demonstrated that the higher local bombesin ligand concentration at the GRPR improved the targeting ability of the bombesin peptide. Unfortunately, in vivo studies showed that $^{99m}$Tc-HABN$_2$ exhibited comparable tumor uptake in comparison to $^{99m}$Tc-HABN, high radioactivity accumulation also in the non-GRPR tissues and long kidney and intestines retention. Considering the in vitro targeting characteristics and the in vivo potential of Aca-BN(7-14)NH$_2$ homodimer, we further decided to optimize the in vivo kinetics of bombesin homodimer by changing chelators, labeling methods, and radionuclide. $^{111}$In was employed because it might fit the properties of this peptide and could help to monitor the kinetics of this probe on a long time frame. This probe showed sufficient in vivo stability in combination with high tumor uptake. Our experiments indicated that this GRPR-analogue may also be tailored for clinical translation and eventually for peptide receptor radionuclide therapy (PRRT). However, even if $^{111}$In-DOTA-[Aca-BN(7-14)]$_2$ showed higher tumor uptake than $^{99m}$Tc-HABN$_2$, a high pancreatic retention and slow renal wash-out was observed. Further studies should be considered to improve in vivo kinetics of bombesin homodimers to achieve a fast wash-out. Considering the pitfalls of these two SPECT-labeled dimeric probes, we decided to evaluate BN monomeric sequences with improved pharmacokinetics after chemical stabilization in chapter 5. We compared two stabilized full length BN monomers with a chemical modification introduced in their sequences. The stabilization provided high in vivo stability to the peptides. These two monomers were labeled with $^{18}$F via aluminum chelate strategy and evaluated for their
targeting ability with PET. Preclinical studies demonstrated the high potential of these $^{18}$F-BN (C5 and C6) probes for a possible clinical translation. $^{18}$F-NOTA-BNCS/C6 were obtained with high radiochemical yield (up to 70%) and high purity (>99%). These radiopharmaceuticals can be prepared within a short synthesis time, differently from other $^{18}$F strategies which require prosthetic groups (i.e. SFB or FBA) and multistep reactions. *In vitro* and *in vivo* evaluations demonstrated the high targeting abilities of these two new designed probes.

In the future, bombesin probes could be used for prostate cancer imaging, diagnosis of lymph node or bone metastases and follow up of bone metastases after chemo/hormonal/radio-therapy. In this way, the use of more invasive techniques (like trans rectal ultrasound and PSA blood tests) could be reduced. For clinical translation of the probes studied in this dissertation additional studies should be conducted.