Interventional molecular imaging, a hybrid approach

Summary

Chapter 15

In this thesis the use of (hybrid) imaging agents in combined pre- and intraoperative detection of the sentinel lymph node (SLN) and during evaluation of the chemokine receptor 4 (CXCR4) expression levels were discussed. A summary is provided below.

Part I: Hybrid guidance for sentinel lymph node biopsy

SLN biopsy presents physicians with the opportunity to detect early metastatic disease. Radiocolloids such as ^{99m}Tc-nanocolloid are currently used to preoperatively identify SLNs and to provide acoustic gamma probe guidance during their resection. To enable optical guidance, additional dyes are commonly injected during surgery. In **Chapter 2 to 7** new surgical guidance methods that integrate preoperative SLN identification with intraoperative optical detection were discussed.

Chapter 2

Non-targeted hybrid nanoparticles represent a relatively unexploited area of chemical/nanotechnological development. In the field of nanotechnology the SLN biopsy procedure is appealing, as diagnostics of the SLN provide a clear clinical precedent where nano-sized molecules (10 – 100 nm) are preferred. Nano-sized particles induce sufficient retention in the SLN, after which the fluorescent dyes and radiolabels attached to these particles can be detected in the lymph node. A good example is the hybrid radiocolloid ICG-^{99m}Tc-nanocolloid, which is formed after the mixing the clinically applied components ^{99m}Tc-nanocolloid and the fluorescent dye indocyanine green (ICG).

Chapter 3

In an orthotopic mouse model for metastatic breast cancer the dynamics of ^{99m}Tc-nanocolloid, ICG and ICG-^{99m}Tc-nanocolloid were studied. It was shown that in mice the drainage and retention pattern of both the parental and the hybrid radiocolloids were highly similar, while free ICG was rapidly cleared.

Chapter 4

The potential of combined pre- and intraoperative imaging using ICG-^{99m}Tc-nanocolloid was further evaluated in a spontaneous mouse prostate tumor model. To provide a more translational tracer formulation, in this study a cocktail-solution of imaging agents was used. The stability of the hybrid radiocolloid was found to be higher than that

of the non-colloidal ICG-^{99m}Tc-human serum albumin complex. Furthermore, in this Chapter the first (ex vivo) patient data were presented.

Results from the two preclinical studies (**Chapter 3 and 4**) revealed that in mice the hybrid approach helped improve fluorescence based guidance and enabled both accurate surgical planning and intraoperative detection, based on a single injection.

Chapter 5

In a reproducibility study in 25 patients with melanoma in the head and neck region, the trunk, or with penile carcinoma, a clinical comparison between lymphoscintigraphic drainage patterns of the European gold standard ^{99m}Tc-nanocolloid and the hybrid imaging agent ICG-^{99m}Tc-nanocolloid was made. Similar to the preclinical situation, this study revealed identical drainage patterns.

Chapter 6

The added value of ICG-^{99m}Tc-nanocolloid during laparoscopic procedures was shown in eleven patients who underwent a robot-assisted laparoscopic prostatectomy and lymph node dissection. Preoperatively, SLNs could be identified with lymphoscintigraphy and with single photon emission computed tomography combined with computed tomography (SPECT/CT). The hybrid nature of the imaging agent enabled intraoperative SLN identification via fluorescence imaging. During surgery, radioguidance and fluorescence detection proved to be complementary; Fluorescence particularly improved surgical guidance in areas with a high radioactive background signal such as the injection site, whereas radioguidance was found to supplement the limiting tissue penetration of the fluorescence signal.

Chapter 7

An additional benefit of ICG-^{99m}Tc-nanoccolloid is that the optical component can also be exploited in ex vivo applications. Ex vivo fluorescence imaging was used to determine the location of ICG-^{99m}Tc-nanocolloid injection deposits in embedded prostate samples. It was found that the location of the deposits of the imaging agent correlates with the lymphatic drainage pattern as seen preoperatively on lymphoscintigraphy. From these results it could be concluded that the location of intraprostatic deposition of the imaging agent influenced the lymphatic drainage pattern, and as such, the SLN procedure as a whole.

Part II: CXCR4 targeting applications

For specific visualization of tumor cells, imaging agents that target a biomarker expressed on a tumor cell are of value. The biomarker CXCR4 is overexpressed in many types of cancer and is an emerging target in the field of molecular imaging and therapeutics. Preclinical evaluation of differently labeled AC-TZ14011 peptide based CXCR4 targeting imaging agents, including hybrid derivatives, were discussed in **Chapter 8 to 13**.

Chapter 8

During evaluation of a targeting imaging agent, both the preclinical set-up and the receptor affinity of the imaging agent are of influence on the obtained results. Transfected cell lines with (very) high levels of CXCR4 expression can best be used for the initial evaluation of an imaging agent. When binding to the receptor has been verified, models with a level of overexpression that is more comparable to the five-fold overexpression found in the clinical situation should be used for further evaluation. The CXCR4 receptor affinity of Ac-TZ14011 peptide derivatives can be influenced by the addition of an imaging label. While these charge and/or size related alterations slightly decrease the affinity for the receptor, it still remains in the nanomolar range and thus sufficient for in vivo use.

Chapter 9

Functionalization of Ac-TZ14011 with the fluorescent dye FITC enabled evaluation of CXCR4 expression levels and receptor localization in vitro in breast cancer cells. CXCR4 staining was predominantly found on the membrane of the cell and/or in vesicles formed after endocytosis. Ac-TZ14011-FITC enabled discrimination between cells with only a four-fold difference in CXCR4 expression. Furthermore, the efficacy of Ac-TZ14011-FITC was comparable to commercially available anti-CXCR4-antibodies.

Chapter 10

An indium labeled version of the Ac-TZ14011 peptide (^{111}In-DTPA-Ac-TZ14011) could be used to evaluate CXCR4 expression levels in a MIN-O mouse tumor model that resembles human ductal carcinoma in situ. Longitudinal assessment of CXCR4 expression during lesions development enabled discrimination between early preinvasive tumor lesions, intermediate and late stage invasive MIN-O tumor lesions. In the MIN-O lesions membranous CXCR4 expression was heterogeneous and receptor expression levels increased during lesion progression.

Chapter 11

Addition of a multifunctional single attachment point reagent (MSAP) that contains both a DTPA chelate and a fluorescent dye resulted in a hybrid version of Ac-TZ14011 (MSAP-Ac-TZ14011). Functionalization of Ac-TZ14011 with the MSAP label resulted in a lower affinity for the CXCR4 receptor compared to the unlabeled peptide. However, the affinity was similar tot that found after functionalization with either the FITC or DTPA label, and allowed for visualization of CXCR4 in both the pre- and intraoperative setting.

Chapter 12

Multimerization could be used to minimize the negative influence of the MSAP label on the binding affinity of Ac-TZ14011. Unlabeled, the CXCR4 affinity of the dimer and tetramer was somewhat lower than the affinity of the monomer. When labeled with the hybrid label the CXCR4 affinity of the dimer and tetramer increased. Distribution studies revealed that the presence of additional peptides reduced non-specific uptake.

Chapter 13

The hybrid imaging concept was further extended by using the fluorescent beacon on MSAP-Ac-TZ14011 to study the presence of the CXCR4 receptor in fresh tumor tissue biopsy specimens. Flow cytometric analysis of these tumor specimens revealed different CXCR4 positive populations and could be used to accurately stage the progression of MIN-O lesions. These findings were shown to accurately predict visualization of tumor tissue using imaging methods such as SPECT/CT and fluorescence imaging. Furthermore, the fluorescent signature could be used to validate the CXCR4 staining in tumor samples ex vivo. As such, the hybrid nature of MSAP-Ac-TZ14011 enabled integration of biomarker screening, in vivo and ex vivo validation of tumor tissue using a single agent.

Chapter 14 (Future perspectives)

Further development of fluorescence camera's and development and clinical implementation of new imaging agents are essential for improvement of surgical accuracy. Current efforts have been focused on fine-tuning of the utility of the hybrid surgical guidance concept described in this thesis.

The biomarker specific hybrid approach can also be applied for other biomarkers, for instance αvβ3 integrin. ^{111}In-MSAP-RGD, which binds to αvβ3 integrin can be used to visualize tumor margins and metastases.

(Breast) tumors can be marked with marker seeds to improve localization during surgery. Hybrid marker seeds can be used to expand the currently applied marking approach. The fluorescent features can be used for estimation of surgical safety margins.

Even when fluorescence is combined with preoperative imaging and intraoperative detection of gamma rays, surgical identification of deeply situated SLNs can still be difficult. Pilot experiments have shown that navigation of a fluorescence laparoscope based on 3D SPECT/CT images is feasible and can possibly be used to further optimize surgical guidance.

Conclusion

The results described in this thesis demonstrate the potential of hybrid imaging agents in the translation of preoperative findings to the operating room. The hybrid concept was shown to be of additional value during clinical SLN biopsy procedures and during the visualization of tumor cells in a preclinical setting. Current expansions of this exciting research field are expected to further increase the applicability of hybrid imaging agents.