Lighting up cancer aggressiveness: targeting the urokinase plasminogen activator receptor for intraoperative optical imaging
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Part V
Summary and general discussion
Chapter 8

Summary

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Summary

Part I. Introduction
Cancers accounts for approximately one-in-six deaths worldwide. Despite the advances in treatment options over the past couple decades surgery remains the cornerstone for almost all solid tumors. Radical excision is crucial for curative treatment, however, intraoperative tumor identification is hampered by (1) fibrosis, inflammation and necrosis due to neoadjuvant therapy, and (2) an altered surgical field due to the adoption of laparoscopic and robotic procedures. In response, various imaging techniques have been introduced to the operating theater to aid surgeons in tumor discrimination of which fluorescence guided surgery (FGS) is one. FGS utilizes near-infrared light to visualize, an often intravenously administered, tumor-targeting tracer in real-time. In order to create an optimal contrast between malignant and non-malignant tissue such a tracer needs to target a tumor-specific protein that is highly expressed on tumor tissue but absent on the surrounding tissue.

Part II. uPAR as a tumor target in various tumor types
The urokinase plasminogen activator receptor (uPAR) is such a tracer that is highly expressed on malignant tumor cells and tumor-associated stromal cells, while being practically absent on non-malignant (normal, benign or reactive) cells. In addition, in various malignancies uPAR expression is a prognostic factor. In Chapter 2 the prognostic value of uPAR expression for patients with pancreatic adenocarcinoma was studied. 66% of cases expressed uPAR on malignant cells while 82% expressed uPAR on stromal cells. uPAR expression on malignant and stromal cells is inversely related with overall survival and disease-free survival. These results further strengthen the case that uPAR is strong prognostic marker for aggressive diseases and could potentially be used for treatment stratification. Furthermore, the expression of uPAR on malignant and tumor-associated stromal cells render it as a possible target for FGS.

Another case where FGS could revolutionize treatment as tumor borders are difficult if not impossible to determine is high-risk squamous cell carcinoma of the head-and-neck region. Using a practical approach, Chapter 3 determined to identify possible tumor targets for FGS of these challenging cases. The expression patterns of seven targets with fluorescent tracers undergoing development were evaluated on tumor cells, tumor-associated stromal cells and normal epithelium. The epidermal growth factor receptor, integrin $\alpha_v\beta_6$, and uPAR were identified as possible targets with the former two having high expression on tumor cells, no expression on stromal cells, and moderate to high expression on normal epithelium.
In contrast normal epithelium was consistently negative for uPAR while tumor and tumor-associated stromal cells were moderately positive.

**Part III. uPAR as target: beyond cancer imaging**

uPAR overexpression is not exclusive to cancer but also occurs in a range of other diseases where extracellular matrix remodeling plays an important role. Therefore, **Chapter 4** takes a closer look at the role of uPAR in atherosclerosis, rheumatoid arthritis (RA), Alzheimer’s disease (AD), multiple sclerosis (MS) and inflammatory bowel disease (IBD). In addition, avenues are identified where uPAR targeted molecular imaging could offer insights for new directions in diagnosis, surveillance or treatment options. These range from utilizing molecular imaging to increase our understanding of MS or AD to identification atherosclerotic plaques that are at risk for rupture to predicting disease aggravation in RA or IBD.

**Part IV. Development of uPAR targeted tracers**

After identifying a possible target, a fluorescent tracer that specifically targets this receptor needs to be developed. There are various pharmacological and practical that need to be considered when designing a fluorescent tracer. This discussion is introduced in **Chapter 5** where the results of a fluorescent uPAR targeting monoclonal antibody and peptide are compared in light of two recently published articles where both tracers were evaluated in preclinical head-and-neck cancer models. The long half-life of the antibody results in delayed but prolonged imaging. More rapid imaging can be achieved with the peptide, however, it suffers from urokinase competition for uPAR binding.

In **Chapter 6** a novel humanized fluorescent antibody, based on the mouse monoclonal antibody targeting domain 2-3 of uPAR of the previous chapter, is introduced and evaluated preclinically. In preclinical subcutaneous and orthotopic human urothelial cell carcinoma models tumors can be specifically delineated from background tissues with significantly higher tumor-to-background ratios than multiple isotype controls. In addition, the multimodal functionality of the tracer is demonstrated by photoacoustic 3D in depth imaging.

As mentioned earlier, antibodies have a prolonged half-life which delays the optimal imaging window. Decreasing the molecular weight of the antibody alters the biodistribution of the tracer, resulting in earlier tumor penetration and background clearance and ultimately allowing for earlier imaging. In **Chapter 7** F(ab’)2 and Fab fragments are created from the humanized monoclonal antibody introduced in the previous chapter and extensively compared in multiple preclinical mouse models. The ultimate imaging window is reduced from 72 hours to 24 hours post administration with the fragments. While the tumor-to-background ratios do not differ
between the full-sized antibody and the two smaller fragments, the peak tumor signal decreases with size of the tracer. The earlier tumor visualization achieved by antibody fragments comes at the expense of peak fluorescence intensity which could potential influence sensitivity of the tracer.