

# Novel molecular imaging methods for cancer detection

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# Summary

Treatment of cancer and monitoring the disease post treatment depends on the complex molecular information obtained both before and after treatment. Molecular imaging for the detection of cancer reflects overall heterogeneity in the tumor quantitatively, longitudinally and non-invasively unlike invasive biopsy. This requires that the imaging tracer/technique should be able to detect the biological process pre and post treatment accurately. Research in medical imaging has been progressing in four main categories 1) improvement of medical equipment (hardware), 2) standardization of protocols worldwide, 3) developing new tracers/imaging biomarkers and 4) advancements in image analysis (software). In this thesis, we focused on the later two innovations. New molecular imaging tracers were evaluated and discussed in the first part of the thesis. In the second part of the thesis we evaluated molecular patterns and tumor heterogeneities on CT imaging features using Radiomics, an advanced image analysis software for the extraction of imaging features.

### **Part 1: Evaluation of new antibody based imaging tracers for detecting invasive phenotype**

Antibody-based imaging has a great potential in the area of theranostics because of its high specificity towards the target. Furthermore, theranostics approach where diagnosis is performed with therapeutic compounds, using antibodies aid in faster validation of the antibodies in clinic for both imaging and drug development. Tumors expressing high levels of Epidermal Growth Factor Receptor (EGFR) are associated with a more aggressive phenotype and resistance to treatment. Cetuximab, a monoclonal antibody targeting EGFR has been a standard treatment for colorectal and head and neck cancer patients. However, not all patients screened for EGFR expression has shown to benefit from the treatment. Labeling the antibody with Zirconium-89 ( $^{89}\text{Zr}$ ) allows for the visualization of the reachability of the therapeutic antibody within the tumor both longitudinally and non-invasively using Positron Emission Tomography (PET). It was shown earlier by our group that [ $^{89}\text{Zr}$ ]-Cetuximab uptake in tumors did not correlate with EGFR expression. In **chapter 3**, we evaluated if the antibody uptake is influenced by tumor microenvironmental parameters, such as hypoxia and microvessel density, in three tumor models (SCCNij202, SCCNij3 and SCCNij82), which differ in EGFR expression. We have observed that the uptake of the antibody was independent of EGFR expression and was associated with hypoxia and microvessel density in the tumors. Altogether, the results

suggest that the tumor uptake of [<sup>89</sup>Zr]-Cetuximab was dependent on multiple factors, since both hypoxia and vessel density had significant influence on the uptake of the antibody.

Since, intact antibodies have low penetration capabilities into tumors due to their larger size, small antibody molecules or chimeric antibodies are highly preferred. Small antibodies possess the antigen specificity similar to intact antibody while the smaller size allows for larger reachability into tumor. Matrix metalloproteinase-2 (MMP2) is associated with invasive cancer phenotype and therefore could serve as imaging biomarker for the invasive phenotype. In **chapter 4**, we evaluated if the small immuno protein antibody of MMP2 (aMMP2-SIP) could be a potential imaging biomarker for imaging MMP2 in three tumor models (HT1080, U373 and U87) and their corresponding MMP2 knock down (MMP2KD) models. These models demonstrated intermediate (U373 and HT1080) to high (U87) MMP2 expression levels. The results clearly demonstrated that the uptake of aMMP2-SIP was dependent on MMP2 activity rather than MMP2 expression. In addition, we have shown that tumor microenvironmental parameters, such as hypoxia, perfusion and vessel density had no influence on the antibody uptake. Since aMMP2-SIP specifically detects MMP2 activity in the tumors without the need for activation unlike other MMP probes, it can be a potential imaging biomarker for imaging MMP2 activity.

## **Part 2: CT derived radiomics and underlying tumor heterogeneities: proof-of-concept studies**

Computed Tomography imaging is widely used in the clinic mainly for anatomical information. However, it is hypothesized that CT images have hidden information about tumor heterogeneities and molecular patterns that can be unveiled by using advanced image analysis tools such as Radiomics. Radiomics extracts a large number of features based on pixel intensities, distribution of gray level intensities (texture), shape and size. It was indeed clinically proven that the Radiomics signatures derived from different modalities including CT have shown to be prognostic in various cancers and associated with genetic patterns. However, the direct relationship between imaging features and underlying genetic or molecular patterns is not clear. Therefore, in order to establish a direct relationship between genetic patterns or tumor heterogeneities and the imaging features, we performed preclinical studies as proof-of-concept in the second part of the thesis. Firstly, in **chapter 5**, we evaluated if a gene

change can be detected on CT images using Radiomics. To address this, we used doxycycline inducible GADD34 overexpression in HCT116 tumors were grown as xenografts in mice. Radiomics analysis was performed on CT images (40kVp and 80kVp) acquired before radiation treatment at tumor volume of 200mm<sup>3</sup>, at 4-day post RT and at tumor volume of 500mm<sup>3</sup> post RT. Consistent features were observed only in gene-induced tumors combined with RT both early and at later time points post. Our results were similar at both energy levels indicating that gene-change could influence image features and a direct relationship exists between gene-change leading to phenotypic changes and imaging features.

We further assessed if tumor heterogeneities such as hypoxia can influence CT imaging features in **chapter 6**. We first evaluated if temporary changes in oxygen, such as 7% oxygen breathing leading to increased hypoxia, can impact the imaging features. Radiomics analysis was performed on CT images acquired before and after oxygen modification. We found 15 features that were significantly different between pre and post 7% oxygen breathing, common in two independent datasets. We observed a positive correlation with hypoxia, assessed using HX4 PET/CT imaging, for 13 features, while 2 features correlated negatively. We furthermore investigated if imaging features are influenced by hypoxia-targeted treatment, such as the hypoxia-activated prodrug (rats) and nitroglycerin (patients). 6 common features were significantly influenced by these hypoxia directed treatment in rats and patients. All the features correlated with HX4 uptake measured as SUVmean or TBR. One wavelet feature strongly correlated with both hypoxic fraction and hypoxic volume in both datasets. These results suggest that features acquired from CT imaging are influenced by changes in the levels of hypoxia in the tumor.

In conclusion, this thesis evaluates new imaging tracers and techniques and the influence of tumor microenvironment altogether with a focus to improve cancer detection with higher accuracy and for better treatment monitoring.