CHAPTER SEVEN

Summary

– 论文摘要 –

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Summary

Optical imaging has shown its appeal as a sensitive, cost-effective and time-efficient preclinical research technique for both *in vitro* assays and *in vivo* whole body imaging. Especially, the development of injectable tumor-specific near-infrared fluorescent (NIRF) probes has made fluorescence imaging (FLI) an effective tool for real-time detection of tumors and metastases in various experimental models. These NIRF probes also have the potential to be used clinically.

In this thesis, we explored diverse applications of using NIRF probes, with specific properties, to visualize and characterize the development of cancer and cell death. In **Chapter 2**, we addressed the questions whether it is possible to detect tumor progression in a luciferase-expressing 4T1-luc2 mouse breast cancer model by using combinations of activatable and targeting NIRF probes; and if there is a correlation between FLI and bioluminescence imaging (BLI) measurements. To answer these questions, sensitivity and specificity studies were performed. Also, a correlation study between the tumor FLI and BLI signals was carried out. In addition, we explored the possibilities to simultaneously detect multiple tumor characteristics by dual-wavelength FLI (~700 and ~800 nm) combined with spectral unmixing.

In vitro, we showed that the activatable NIRF probes (ProSense680 and MMPSense680) and the targeting NIRF probes (IRDye 800CW 2-DG and IRDye 800CW EGF) were either activated by or bound to 4T1-luc2 cells. In our bioluminescent mouse breast cancer model, we orthotopically implanted 4T1-luc2 cells in nude mice. This enables us to follow tumor progression longitudinally both by BLI and dual-wavelength FLI, when using combinations of NIRF probes. An enhanced tumor-to-background ratio and a strong positive linear correlation between FLI and BLI measurements were observed for all probes tested. Moreover, immunohistology on sections revealed that each probe exhibited a specific tissue distribution pattern. For this, the *in vivo* use of probe combinations might provide a more comprehensive image of tumor characteristics. These studies suggest that dual-wavelength FLI is a feasible approach to simultaneously detect different features of one tumor and to follow tumor progression *in vivo*. This approach opens up new perspectives for the detection of tumors and metastases in various experimental models and could also be translated into the clinic, for example, image-guided surgery.

Different tumors are characterized by the expression of specific cell surface- or intracellular markers or by the expression of particular marker profiles. These biomarkers can be used to localize and identify tumors or as target of anti-tumor therapy. This implies that the identification and treatment of different tumor types requires specific probes or sets of probes and treatments. However, apart

from these heterogeneities, most types of cancer, especially those aggressive and fast growing ones, always display a common feature, namely cell death. Cell death and in particular necrosis at the tumor center is believed to be the result of chronic ischemia caused by vascular collapse. This occurs when the rate of tumor cell growth exceeds that of angiogenesis, which is associated with poor prognosis. In order to locate and/or treat tumors, these necrotic areas, present in most tumor types, can be used for optical imaging of cancer as a universal target.

To this end, we analyzed two representative cell death NIRF probes, targeting either an extracellular or an intracellular biomarker, in a cryolesion mouse model of traumatic brain injury. Synthetic zinc(II)-dipicolylamine PSS-794 binds to phosphatidylserine that is expressed on the plasma membrane of early and late apoptotic as well as necrotic cells (**Chapter 3**); and fluorescently labelled 4-(N-(S-glutathionylacetyl) amino) phenylarsonous acid (GSAO-AF750) binds to the cytosolic heat shock protein-90 (HSP-90) of apoptotic and necrotic cells, coincident with loss of plasma membrane integrity (**Chapter 4**).

In our cryolesion model, an area of local tissue death is initiated by shortly applying a liquid N_2 pre-cooled metal cylinder to the parietal region of a mouse head. Studies with PSS-794 and GSAO-AF750 showed that both NIRF probes selectively accumulate in the brain cryolesion. Local cell death and probe accumulation was confirmed with histology. Moreover, we developed a new *in vitro* dry-ice cell death assay. Luciferase-expressing 4T1-luc2 cells were grown to confluence in a culture plate. A focal area of cell death was induced in the center of the culture well by applying a bar of dry ice to the underside of the culture plate. Subsequently, cells were incubated with different cell death targeting NIRF probes, washed and subjected to FLI and BLI measurements. The presence of dead cells in the center was confirmed by FLI signals and living (luciferase active) cells in the periphery area were revealed by BLI signals. This rapid and robust assay can be used to screen fluorescently labelled compounds with potential cell death targeting properties. Combined, these data suggested the strong specificity of the two NIRF probes in detecting cell death both *in vitro* and *in vivo*.

Next, we investigated the potential of using a HSP-90 alkylator GSAO-AF750 to non-invasively detect cell death as a biomarker of tumor response to chemotherapy (**Chapter 5**). Because the HSP-90 chaperone is an important element in oncogene addiction and tumor cell survival, its expression is enhanced by chemotherapy. As a fluorescently labelled HSP-90 alkylator, GSAO-AF750 demonstrates a favorable biodistribution. It is cleared quickly from the circulation via kidneys and does not accumulate in healthy organs or tissues. In this study, the cell death targeting property of GSAO-AF750 was examined *in vitro* by flow cytometry using Jurkat T cells treated with staurosporine. In our murine mammary tumor model, we non-invasively imaged cyclophosphamide-induced cell death using GSAO-AF750, which further validated its targeting specificity in

labelling apoptotic and necrotic tissues in solid tumors in mice. Thus, this study offers a novel strategy for dynamic monitoring of tumor treatment outcome. Furthermore, optical imaging of cell death, in general, could be clinically applied to diagnose certain disease where cell death is involved at an early stage, monitor therapeutic efficacy to anti-tumor strategies, or evaluate drug toxicity.

Chapter 6 gives a general discussion about the optical imaging, both at microscopic and macroscopic level. For the future perspective, this chapter outlooks the possibility to embrace new optical (or multimodal) imaging technologies to address important needs in clinical translation. In terms of imaging cancer and cell death, the current challenges and emerging strategies are highlighted. Moreover, the pros and cons of various available tumor cell death imaging agents are discussed.

Taken together, the preclinical studies in this thesis showed that non-invasive optical imaging of cancer and cell death is of great significance and can satisfy an unmet need for complimentary information that would support other diagnostic imaging modalities such as MRI, SPECT/PET and CT. It could also be a fast and low-cost alternative in cases where the other diagnostic imaging modalities cannot be utilized. For this to happen, *in vivo* optical imaging needs to undergo another step in terms of refining its imaging modality.