Development of novel anti-cancer strategies utilizing the zebrafish xenograft model

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Chapter 6

Summary

Cancer is still a leading cause of death worldwide. Chemotherapy is often the treatment of choice to treat cancer, although side effects alter normal cell physiology and may affect the patient's quality of life. Progress in biomedical research has shown that pharmacological targeting of cancer cells is not the only therapeutic option. Interactions between tumour cells and their surrounding stroma may support cancer cell survival and spreading, and offer a potential new treatment strategy. In addition, the application of new inducible photosensitizers and specific drug delivery carriers can improve selectivity of anti-cancer treatments. Zebrafish models have increasingly been applied to cancer research and drug discovery. In this thesis, a zebrafish embryonic cancer model is presented as an innovative model organism to study the role of macrophages in tumour angiogenesis. In addition, we successfully demonstrated that zebrafish provide a fast-vertebrate cancer model to test the administration regimen of drugs, conditions of light irradiation, host toxicity and anti-cancer efficacy of photodynamic therapy and photoactivated therapy drugs. Finally, we observed that light-triggered, cell-specific delivery of liposome-encapsulated doxorubicin reduced cancer cell burden without enhanced cytotoxicity in live zebrafish embryos.

In **chapter 2**, we explored the function of zebrafish macrophages in tumour xenografts. He et al had shown that transient depletion of macrophages by Pu.1 morpholino treatment blocked tumour angiogenesis at the primary site of metastatic onset [1]. Here, we used metronidazole to chemically deplete macrophages in *Tg* zebrafish *(Mpeg:GAL4:UAS:NTR:mCherry)* engrafted with cutaneous melanoma cells and observed that after macrophages depletion, tumour angiogenesis was impaired. The macrophages were attracted into tumour sites and promoted tumour vessel formation. Emerging evidence suggests that lactic acid as a product of glycolysis can attract macrophages and induce angiogenesis targeting highly glycolytic cancer cells [2]. To test this, a macrophage attraction assay was performed by injecting lactic acid into the zebrafish hindbrain. By counting the number of macrophages, we observed that lactic acid indeed attracted zebrafish macrophages. Chemical inhibition of tumour cell glycolysis by 2-Deoxyglucose (2DG) blocked the lactate secretion. Engraftment of these cells into zebrafish embryos reduced attraction of macrophages and impaired tumour angiogenesis suggesting that macrophages provided specific cytokines to support angiogenesis.

In **chapter 3**, we validated a photodynamic therapy (PDT) compound, TLD1433, in zebrafish ectopic and orthotopic models. Importantly, this ruthenium-based photosensitizer has passed a phase I clinical trial

for PDT treatment of bladder cancer. In our study we investigated a possible repurposing of this drug for treatment of conjunctival melanoma (CM). Firstly, the therapeutic potential of light activated TLD1433 was tested on several cell lines derived from conjunctival melanoma (CRMM1, CRMM2 and CM2005), uveal melanoma (OMM1, OMM2.5, MEL270), epidermoid carcinoma (A431) and cutaneous melanoma (A375). The best responding cell lines, CRMM1 and CRMM2 were selected for *in vivo* testing of this PDT compound. The maximally-tolerated dose of TLD1433 was determined in wild type embryos and embryos engrafted with CM cells by applying three drug-administration routes (water administration, intravenous and retro-orbital administration). The zebrafish embryos engrafted with CM cells tolerated less TLD1433 compared with wild type embryos. Using the maximally-tolerated dose of TLD1433, we observed that TLD1433 sensitizer inhibited tumour growth in the CM ectopic model after intravenous (IV) and retro-orbital (RO) administrations, and in the CM orthotopic model after RO administration. These results clearly illustrate that the zebrafish embryonic cancer models can be utilized to optimise the route of administration and the dose for photoactivated chemotherapy compounds.

In **chapter 4**, the light-activated anticancer properties of a novel trisheteroleptic ruthenium complex [**2**]$(PF_6)_2$ were validated *in vitro* and in an embryonic zebrafish CM model. The metal complex $[\mathbf{2}]^{2+}$ was designed based on previous work in order to increase cellular uptake, its photoinduced anticancer activity with a low dark toxicity. This photoactivated chemotherapy (PACT) compound can be light-activated via an oxygen-independent photosubstitution reaction. It is for the first time that the toxicity and efficacy of such a ruthenium-based complex was tested in the zebrafish embryonic cancer model. Different concentrations and administration methods of this drug were examined to find an optimal balance between toxicity and a therapeutic effect. Our results revealed a higher efficacy of this ruthenium compound in the *in vivo* orthotopic CM model than in the ectopic CM model, indicating that this novel compound should be further explored in the local treatment of conjunctival melanoma in more advance preclinical models.

In **chapter 5**, we demonstrated that light-triggered and cell-specific targeting of doxorubicin-filled liposomes diminished growth of xenografted breast cancer cells in a zebrafish embryonic model. Light-induced dePEGylation was used to shield the E_4/K_4 peptide interaction. The liposome-cell interactions depend on the recognition and binding of two coiled-coil forming peptides – peptide E on liposomes and peptide K on cancer cells. Light-triggered dePEGylation improved cancer cell-liposome fusion and allowed specific delivery of liposomal doxorubicin to target cancer cells. In addition, folate-decorated liposomes (F-liposomes) targeted the overexpressed folate receptor on xenograft MDA-MB-231 cells and also promoted direct fusion of liposome and cell membranes. The experimental component fusion systems (peptides E and K) and tumour-cell specific receptor folate-decorated liposomes both delivered doxorubicin to tumour cells to induce tumour cell death. As the liposomes and tumour cells expressed fluorescence, they were easily detected in the zebrafish embryos, allowing measurements of

fluorescence areas and intensity. This work illustrates that the zebrafish embryonic cancer model can serve as an efficient platform for optimization of nanomedicine toxicity, biodistribution, stability and anti-cancer efficiency.

In conclusion, we started out to use the zebrafish embryonic tumour model to investigate the interaction between host macrophages with their tumour microenvironment. The lactic acid secreted by tumour cells could attract macrophages and then induced tumour angiogenesis. Secondly, we used zebrafish embryonic cancer models for PDT and PACT compound testing. The PDT compound TLD1433, and PACT compound [**2**]$(PF_6)_2$ showed a selective anti-cancer efficacy. Finally, in order to increase the cellular uptake of compounds, cell-specific targeting of liposomes was introduced. Liposome-encapsulated doxorubicin was delivered into tumour cells upon light-activation and reduced tumour burden.

Collectively, in this thesis we demonstrated that zebrafish embryonic cancer models are excellent for the discovery of new drugs and their use has the potential to speed up development of novel anti-cancer treatments with translational potential.

References

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