Ionising Radiation Quality and Dose Effects on DNA Double Strand Break Repair

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Ionising radiation (IR) can induce a wide array of different types of DNA damage and, in the context of cancer therapy, is used to eradicate tumour cells. The underlying success of DNA damage-inducing radiation treatment is the rationale that tumour cells coordinately respond to DNA damage, thereby inducing a variety of responses that induce cell death or inhibit cellular proliferation. However, cells have evolved tightly controlled DNA damage repair mechanisms that can counteract the DNA damaging effects, possibly leading to radiation resistance. The most harmful type of DNA damage are DNA double-stranded breaks (DSBs) that are repaired in two fundamentally distinct manners, Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR), depending on whether a DNA template is used during the process. More understanding how HR and NHEJ function together in DSBs repair, could assist in the search for possibilities to improve cancer therapy based on IR or counter IR resistance.

In cancer therapy, tumours are mostly treated from outside the body, using external beam radiation therapy (EBRT). However, irradiation of tumours deep within the body that reside next to healthy tissue can lead to toxicity. The development of radiopharmaceutical therapy (RPT) has improved treatment of cancers that are located deep in the body and of metastasised disease. In RPT, radionuclides are attached to delivery vehicles and systemically injected, delivering the radiation directly to the tumour. Internal irradiation provides the possibility to use radiation with a high linear energy transfer (LET), which is not an option for external irradiation due to a low penetration depth. High-LET radiation has a large probability to eradicate tumour cells, due to its potential to inflict a high amount of DSBs to cells in close proximity. However, efficient delivery of high-LET radiation is difficult and what types of DNA damage are inflicted is not yet fully understood.

This thesis describes; (1) The cooperation of NHEJ and HR in IR protection in mice and cells; (2) the development of a novel high-LET external irradiation device; (3) the differences in DSB processing after high- and low-LET irradiation and (4) polymersome processing after cellular uptake to assess efficient and safe delivery of high-LET radionuclides.

In mammals, the two major DNA double-strand break repair pathways, HR and NHEJ, have overlapping as well as specialised roles. The relative contribution of these two DSB repair pathways can differ depending on mammalian developmental stage (i.e. cell type) and on the specific type of DNA damage. This implicates that the combination of NHEJ and HR regulates the grade of IR protection and further understanding how that combination is balanced could lead to more precise radiation treatment. We generated mice deficient for HR, NHEJ and both to investigate how NHEJ and HR cooperate in IR induced DNA damage repair. We found that mice lacking NHEJ, but not HR, have elevated and sustained p21 expression after IR, particularly in the gut. In addition, NHEJ appeared to play a role in 53BP1 foci dissolution in both mouse embryonic stem (mES) cells and mouse embryonic fibroblasts (MEFs). Furthermore, HR only contributed to IR protection in NHEJ deficient adult mice or in mES cells.

The notion of a balanced role between HR and NHEJ after low-LET IR implicates a similar scenario for high-LET irradiation. Indeed, high-LET irradiation is thought to induce complex DNA damage in which HR, having the requirement of a DNA template, plays a larger role than NHEJ. To investigate whether HR or NHEJ have different impact in DSB repair after high-LET irradiation we first addressed a technical problem: commercially available α-particle sources are often smaller in

diameter than culture dishes, thereby precluding standardised biological experiments, such as clonogenic survival assays. We report a novel procedure using large field external α-particle irradiation for standard radiobiological experiments. With the use of this setup, we showed clonogenic survival assays which are reproducible and can be compared with other types of external irradiation. Furthermore, by optimising the set-up we add super-resolution microscopy imaging to our toolbox to investigate DNA damage inflicted by α-particles.

We used the developed irradiation setup to compare DSB processing after α-particle and X-ray irradiation using live-cell imaging. We found that, in contrast to the already initially larger α-particle-induced 53BP1 foci, X-ray-induced foci increased in 53BP1 protein content and size over time. Moreover, α-particle irradiation induced 53BP1 foci co-localised with multiple individual RPA foci, indicative for multiple resection events at a single damaged site, which was not observed after X-ray irradiation. In conclusion, our results indicate that the condensed energy deposition pattern of high-LET α-particles induces closely interspaced DSBs. The abundance of multiple DSBs in close vicinity throughout the cell nucleus leads to 53BP1 protein insufficiency and ineffective DNA end protection.

In practice, irradiation using α-particles would be internal and not external. Therefore, delivery vehicles (DVs) are designed to specifically deliver the α-particle emitting radionuclides to the target tumour cells. However, with the high energy of α-particle emitting radionuclides comes a challenge: recoiling daughter radionuclides break free from their DV and can distribute freely in the body, potentially causing harm to healthy tissue. Robust polymersomes (PMs) could provide support, being highly effective in retaining recoiling daughter radionuclides. We set out to characterise the cellular uptake of PMs and found that PM uptake is cell type dependent and mitotic cells have increased uptake. In addition, PM uptake is mediated via endocytosis where after post-uptake transportation went via microtubules, eventually leading to lysosomal aggregates. Furthermore, we show that PMs, which carry α-particle emitting radionuclides, only induce DNA damage to the cell in which they are taken up, as seen in 2-D cell culture. These findings suggest that PM uptake and processing can vastly differ between cell lines, which could possibly influence DNA damage inducing capabilities.

Overall, the studies presented in this thesis show fruitful collaborations between physics, radiology, and biology disciplines in which the basis encompasses: gaining fundamental knowledge of biological processes with the use of technological advances. The novel insights and assays presented in this thesis could be useful for advancements in clinical treatment or drug development.

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