General Introduction

DNA damage

Human cells are continuously exposed to numerous exogenous and endogenous agents that damage the DNA. DNA damage alters replication and transcription, causes cell death, and can lead to mutations and oncogenic transformations. Ionizing Radiation (IR) is considered an exogenous DNA damaging factor which cells are exposed to either environmentally or as anthropogenic genotoxic agent. The DNA damage inflicted by IR is considered harmful, leading to mutations or oncogenic transformations, or helpful as therapy to induce cell death in cancer cells. DNA Double Stranded Breaks (DSBs) are inflicted by IR and are the most dangerous since they can cause permanent DNA damage such as deletions and translocations eventually leading to cell death or oncogenic transformations [1]. To counteract these deleterious actions, cells have evolved a number of DNA repair systems that each can repair a subset of these lesions. In this thesis, we focus on the analysis of induction and the repair of DBSs.

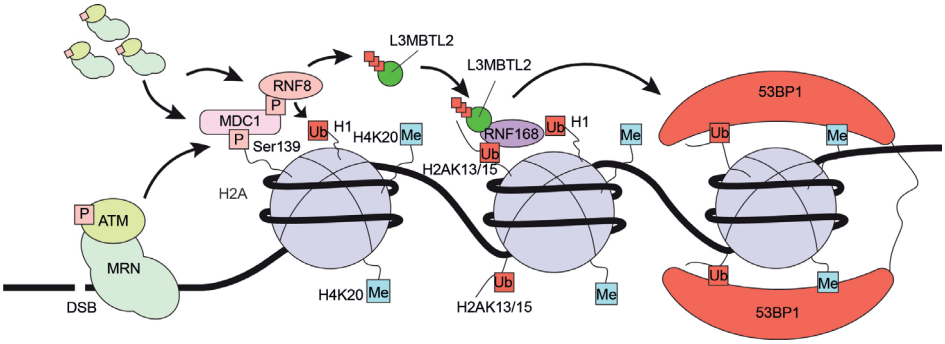

Figure 1. Schematic overview of chromatin signaling following DNA damage. *DSBs sensing by MRN leads to ATM mediated phosphorylation of the histone variant H2A (γH2AX) resulting in a positive feedback loop, recruiting more MRN/ATM complexes. ATM mediated phosphorylation of MDC1 initiates the recruitment of RNF8, which is the start of an ubiquitination cascade starting with the polyubiquitination of L3MBTL2 and histone H1. Both ubiquitinated L3MBTL2 and Histone H1 are recognized by RNF168, resulting in the ubiquitination of H2A at positions K13 and/or K15 (H2AK13/15). The ubiquitination of H2A together with the methylation of H4 (H4K20) serve as a scaffold that is recognized by 53BP1. The accumulation of 53BP1 protects DNA-ends from resection, thereby facilitating DSB repair pathway choice.*

Recognition of DSBs is the first step after IR hits cells (Figure 1). One of the first players in the process is the MRN-complex, a ring shaped complex consisting of three different proteins. The complex consists of the monomeric Nijmegen Breakage Syndrome 1 (NBS1) protein, which functions as a scaffold for two RAD50, and two Meiotic Recombination 11 (Mre11) proteins. The two RAD50 proteins form the ring that slides over the DNA break [2-4]. When the MRN-complex is bound to the DSB the serine/threonine kinase Ataxia Telangiectasia Mutated (ATM) is recruited and binds to the C-terminus of NBS1 [5]. ATM is then activated by auto-phosphorylation which separates the dimer into two active monomers [6]. Once activated, ATM phosphorylates proteins downstream of the DNA repair pathway. One important regulating

step is phosphorylation of histone H2AX at the serine-139 (γH2AX) residue by ATM [7]. γH2AX recruits proteins necessary for the next step in DNA repair. Mediator of DNA damage checkpoint 1 (MDC1) is directly recruited by γH2AX where its Ser-Asp-Thr (SDT) domain is phosphorylated by casein kinase 2 (CK2) [8, 9]. MDC1 amplifies the DNA damage signal by creating a positive feedback loop to concentrate MRN-ATM complexes at the DSB site, which will phosphorylate additional H2AX histones [10]. Recruited MRN-ATM complexes will phosphorylate the T-Q-X-F domain of MDC1 [11]. This phosphorylated MDC1 recruits the E3 ubiquitin-protein ligase RNF8 to the damage site, starting an ubiquitination cascade [12]. RNF8 ubiquinates both histone H1 and L3MBTL2, this attracts RNF168, a second E3 ubiquitin protein ligase [13,14]. RNF168 mono-ubiquitinates lysine (K) 13-15 residues of histones of the H2A family, forming K63 ubiquitin chains [14]. This ubiquitinated histone can be recognized by a dimer of p53-binding protein 1 (53BP1) [15]. This also requires interaction of 53BP1 with histone H4 methylated on lysine 20 (H4K20me) [16]. These alterations are recognized by the ubiquitination-dependent recruitment (UDR) motif and Tudor motif, respectively [15, 17]. 53BP1 then accumulates at the damaged site [18]. With no demonstrated enzymatic activity, 53BP1 is encoded by the TP53BP1 gene and has 1972 amino acids [19, 20]. 53BP1 has several important structural elements which include two BRCA1 carboxy-terminal (BRCT) domains, one glycine/arginine-rich region (GAR), multiple tandem Tudor domains and two dynein 8 kD light chain (LC8) binding sites. In addition, 53BP1 includes 32 PIK kinases and 41 cyclin-dependent kinase (CDK) phosphorylation sites [19]. The majority of these sites have been linked to roles in signaling after ionizing radiation (IR), protein interactions and cell cycle checkpoints [21-23]. The purpose of 53BP1 accumulation is to protect DNA ends from resection. Therefore, it is thought that 53BP1 recruitment, retention, and exclusion determines which DSB repair pathway is activated. There are four pathways by which the DSB can be repaired; the two major pathways Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR), and two less common pathways Alternative End Joining (A-EJ) and Single Strand Annealing (SSA).

Non-homologous End Joining

The most direct way to repair a DSB is to ligate the break ends together, by NHEJ which is considered the most available pathway during cell cycle, albeit error-prone [24] (Figure 2). DNA ends at the damaged site are recognized by the heterodimer of Ku70 and Ku80, which forms a ring fitting directly on the broken DNA and thereby protecting the DNA ends from resection [25, 26]. Unlike any other DSB repair pathway, NHEJ requires no sequence homology [27]. The Ku70/Ku80 heterodimer recruits the DNA-PK catalytic subunit (DNA-PKcs) via a flexible linker its C-terminal region, composing the DNA-PK complex and stabilizing the DNA-ends. Recruitment of Artemis induces the assembly of the Artemis:DNA-PK complex, which opens up an variety of nuclease activities, being able to trim many kinds of DNA damaged ends for end-joining [27]. The addition of Artemis complements the whole DNA-PK complex, replacing the complex upstream for DNA-PKcs to be positioned at the DSB [28]. To finalize the DSB repair, the DNA

ends are stabilized and positioned by XLF and PAXX. Then, the factor XRCC4 activates DNA ligase IV (LIGIV), which ligates the ends [29].

Homologous recombination

A second pathway to repair DSB is Homologous Recombination (HR). Whilst only possible when a sister chromatid is available (S/G2 phase), this pathway is error-free and operational when DNA breaks do not consist of polished ends but are missing nucleotides (Figure 2) [30]. The start of the HR is initialized by the aforementioned MRN complex, which initializes the resection of the broken ends. Hereafter, the recruitment of CtIP, which is in complex with BRCA1, completes resection up to a couple of hundred nucleotides [31, 32]. Exonuclease 1 (EXO1), DNA replication helicase/nuclease 2 (DNA2) or the Bloom syndrome (BLM) complexes then process this early resection intermediate to generate longer 3' single stranded DNA (ssDNA) overhangs [33, 34]. These large 3' overhangs are highly susceptible for degradation by nucleases. Replication protein A (RPA) has high affinity to bind to single stranded DNA and will bind to 3' overhangs, which stabilizes ssDNA and protects it from nucleases [35]. Effective repair of HR relies on homology (>100 bp) with the daughter strand [36]. First, RPA has to be replaced by Rad51. This is done via the mediator protein BRCA2, which is recruited by BRCA1 through PALB2 [37]. Rad51 is transported to the resection by BRCA2, allowing Rad51 to bind ssDNA and displace RPA, wrapped around the ssDNA in a helical way [38]. This Rad51-ssDNA filament then facilitates the search for a homologous sister chromatid. Rad54 facilitates this search and has been described to have many functions all involved in the binding to a homologous chromatid, called synapsis, extensively reviewed in [39]. Pre-synapsis, Rad54 is described to stabilize and assist Rad51 filaments on the ssDNA, not requiring any ATP activity by RAD54 [40]. During synapsis, Rad54 shows ATP-dependent facilitation of Rad51 filaments in translocating along the DNA and clearing of nucleosomes from the synapsis site through chromatin remodeling activity. Lastly, post-synapsis Rad54 enhances D-loop formation with Rad51, assisted by PALB2, and is necessary for the dissociation of Rad51 from the DNA [41-43]. Once Rad51 is dissociated from the ssDNA, proliferating cell nuclear antigen (PCNA) can be loaded onto the D-loop. PCNA can stimulate the activity of Pol δ and pol η which carry out post invasion DNA synthesis, leading to the repair and resolving of the intertwined DNA molecule [44].

Alternative End Joining

Alternative end-joing (A-EJ) mainly occurs in the absence of key components of the NHEJ pathways, such as Ku70/80 or LIGIV. Repair of DSBs via A-EJ is based on joining the two DNA ends together, very similar as NHEJ [45]. However, most A-EJ repair requires limited 5' to 3' DNA end resection (up to 20 nucleotides) with 3-8 bp of homology within the resected ssDNA tails [46]. The limited resection is initiated by the MRE11 nuclease, leaving short ssDNA overhangs [47]. In these overhangs, minimal annealing takes place, generating micro homology, as short as 3-8 bp, promoted by DNA polymerase θ (Polθ) and its unique helicase-like domain at its N-

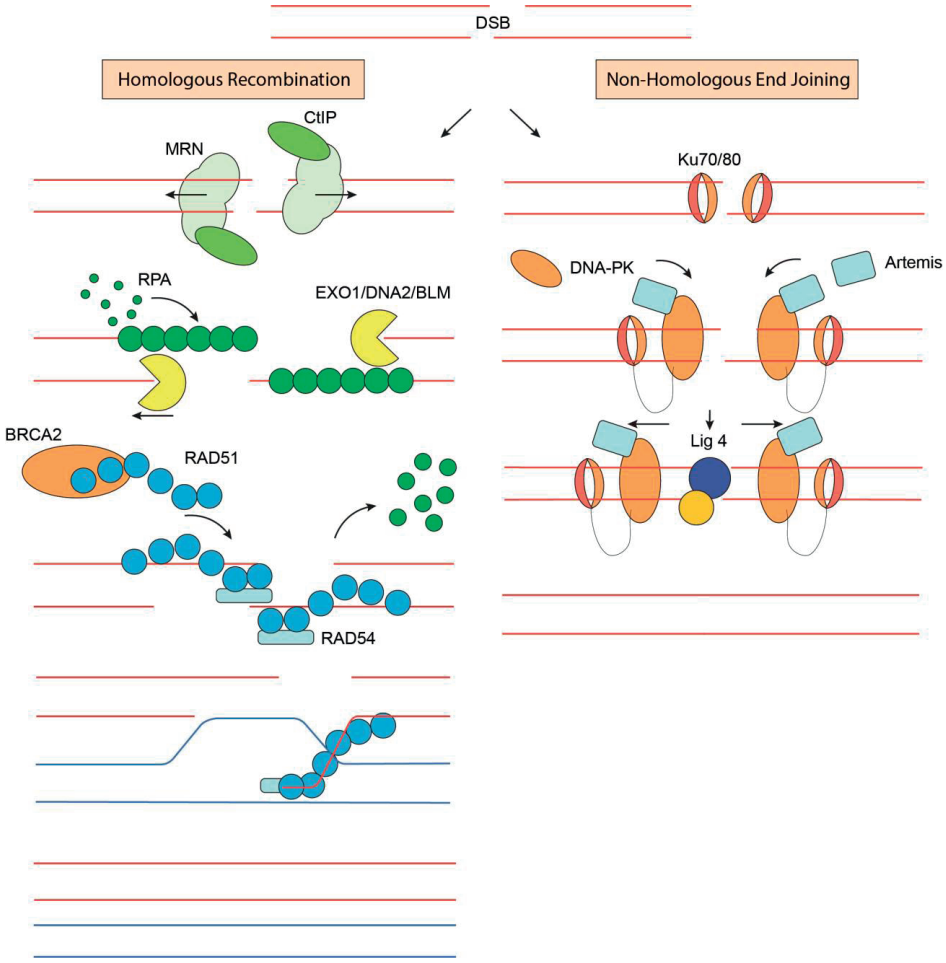

Figure 2. A schematic overview of DSB repair. *The initial step of Homologous Recombination (HR, left) the DNA is resected by MRN and CtIP, leading to long-range resection by EXO1, DNA2 or BLM. Resection of double stranded DNA results in single stranded DNA, which is protected from degradation by RPA. HR processing requires the recombinase protein RAD51 that is recruited via BRCA2 and replaces RPA on the single stranded DNA. Recruitment of RAD54 stimulates the strand invasion of the homologous daughter strand (blue) resulting in DSB repair. During Non-Homologous End Joining (NHEJ, right), DNA ends are recognized by the dimer Ku70/80 resulting in the protection of DNA ends. The DNA-PK complex in combination with Artemis processes DNA ends that are not resected. In the end, processed DNA ends are ligated by LIGIV, which is facilitated by XRCC4.*

terminus. Such annealing of micro homologies generates overhanging bases that are removed by nucleases generating gaps, which are in turn filled by Polθ. The double function of Polθ leads to the unique possibility to stabilize the annealing of two ssDNA tails with as little as 3 bp of homology [48, 49].

Single Strand Annealing

Similar as to HR, repair via the single strand annealing (SSA) pathway is limited to the late S and G2 phase and requires resection. The difference between HR and SSA is the extend of homology. HR requires a daughter strand to have more than 100 bp homology, whereas SSA typically requires homology of 50 bp [50]. Thus, when extensive resection takes place, but does not meet the requirements of HR, annealing of homologous bp takes place, facilitated by RAD52 [51]. Just like in A-EJ, annealing of homologous bp within a DNA strand generates overhanging bases, which have no homology. These non-complementary overhangs are removed by the endonuclease XPF-ERCC1. Finally, the remaining nicks are sealed by LIG1. Due to the removal of non-complementary sequences, SSA is a highly deleterious pathway [52].

DSB repair pathway choice

Almost all DSBs that are inflicted outside the S and G2 phase are repaired by NHEJ in human cells and even within the G2 phase, up to 80% of IR induced DSBs are repaired by NHEJ [53]. However, the other pathways still play a crucial part in DSB repair. The choice between these DSB repair pathways is dictated by the extent of homology. There is an increasing requirement for homology from NHEJ (0-4 bp), A-EJ (3-8 bp), SSA (>50 bp) and HR (>100 bp) (Figure 3) [54]. The extent of homology is a direct consequence of the resection length, which is in turn tightly regulated by by end resection factors, such as CtIP/BRCA1 or the MRN complex and DNA end protection protein, such as 53BP1, RAP1-interacting factor 1 (RIF1) and the shieldin complex [55-58].

CtIP and MRN, generating 3’ ssDNA, mediate initial DNA end resection. This short ssDNA serves as scaffold for long-ranged resection by EXO1, DNA2 and BLM [59]. Processes such as HR, degradation of faulty replicated DNA, and DSB repair choice rely on effective DNA end resection. Therefore, initiation, extension, and termination of DNA end resection is strictly regulated and involves many mechanisms. The accumulation of 53BP1 protects DNA end from resection which involves interactions with replication timing regulatory factor 1 (RIF1) [60]. In turn, RIF1 recruits the Shieldin complex consisting of SHLD1, 2, 3 and REV7 [58, 61, 62]. Like 53BP1, the shieldin complex has no known enzymatic activity. Furthermore, the presence of BRCA1 and CtIP is thought to antagonize the accumulation of 53BP1 in late S and G2 phases, thereby limiting DNA end protection and promoting HR over NHEJ [60, 63]. Together, these observations suggest that pathway choice, which is based on the extent of homology, is regulated by opposing roles of DNA end resection factors in combination with factors, which protect DNA ends from (extended) resection.

Ionizing radiation induced foci

The recruitment of repair factors to damaged chromatin sites requires complex spatial and temporal coordination among the proteins and within the chromatin. This assembly and modifications of proteins in reaction to DSBs can be visualized microscopically and are called ionizing

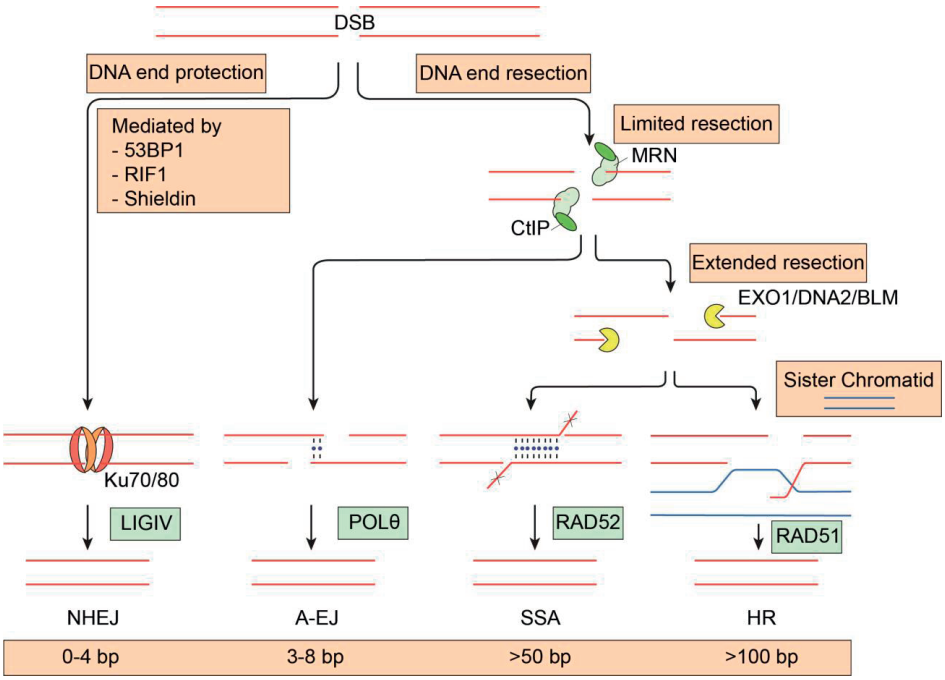

Figure 3. The influence of resection extent on DSB repair pathway choice. *The choice between Non-Homologous End Joining (NHEJ), Alternative End Joining (A-EJ), Single Strand Annealing (SSA) and Homologous Recombination (HR) is mostly a consequence of the extend of homology between the DNA ends of the DSB. The homology between the two DNA ends is regulated by the range of DNA end resection, depending on how well DNA ends are protected from nucleolytic resection. Factors such as p53 binding protein 1 (53BP1), RAP1-interacting factor 1 (RIF1) and the shielding complex (Shieldin) protect DNA ends, limiting resection and thereby allowing recruitment of the Ku70/80 dimer. This leads to the repair via the NHEJ pathway in which LIGIV plays a crucial role in ligating DNA ends together. On the other hand, if DNA end protection is limited, DNA end resection is initiated by CtBP-interacting protein (CtIP) and the MRE11-RAD50-NBS1 (MRN) endonuclease complex allowing limited homology, leading to A-EJ in which Polymersase θ (POLθ) is involved. In the case of absent DNA end protection, long-range resection is mediated by nucleases Bloom syndrome protein (BLM), DNA replication ATP-dependent helicase/nuclease (DNA2) and exonuclease 1 (EXO1). Long ranged homology leads to SSA in which the annealing of the homologous base pairs is mediated by RAD52 and where non-homologous sequences are cut off (indicated by the crosses). In the case of the presence of a homologous sister chromatid in which the extend of homology is even larger; HR takes in which RAD51 plays an essential role in strand invasion. The range of homology is indicated below each pathway in the highlighted box. Figure is adapted from [54].*

radiation-induced foci (IRIF) [64]. With the use of indirect immunofluorescence or by tagging protein of interest with fluorescent tags, like GFP the understanding of proteins residing in IRIF and their retention in these structures has broadened and has led to the quantitative observation of an increase of the number of IRIF per cell in a dose-dependent manner [65]. In addition, IRIF number and size change over time following IR, which could be used as surrogate marker for DNA damage processing [66, 67]. Two of the most commonly known DSB markers are γH2AX and 53BP1. As these two markers do not discriminate between possible DSB repair pathways,

functional protein in the HR pathway are often used to quantify HR specific repair [68]. Markers such as RAD51 and BRCA2 are the most used protein to investigate HR repair. In addition, RPA is used to investigate resection, which is essential for HR.

Radiotherapy

Inducing DNA damage in tumor cells to halt their division and growth is an important basis for several cancer treatments. Radiotherapy (RT) is an important tool to treat cancer and is estimated to be used in two-third of all cancer patients, as unique or combined treatment [69]. RT is based on IR, which is radiation with sufficient energy to change the material through which it passes and therefore inflicting several types of DNA damage. IR deposits energy to the material, which it is passing through. A measure of how much energy IR transfers to material is characterized as the linear energy transfer (LET). By definition, LET is the amount of energy transferred to the material the IR traverses per unit distance [70]. A high LET means that a particle generates an abundance of dense ionizations in the matter it traverses, depositing a large amount of energy. The amount of deposited energy has a direct effect on the penetration depth in the material and is different between types of IR (Figure 4A). Dosimetry is used to understand this difference by evaluating radiation-weighting factors, such as the absorbed dose. Mostly, these differences are dependent on the type of radiation and the energy that they carry [71]. Deposition of similar energy by two different IR types could lead to other biological effect, such as increased cell death. This increased effect can be calculated as a ratio, leading to the relative biological effectiveness (RBE), which if generally higher using high-LET irradiation.

In this thesis, we focus on two different applications of radiotherapy: External beam radiotherapy (EBRT) and Radiopharmaceutical therapy (RPT). The basis of EBRT is treatment from outside, thereby irradiating a specific part of the body, which contains the cancer. For example, to treat lung cancer, a large part of the chest is irradiated. Unlike EBRT, with RPT the radioactivity is administered in the bloodstream, delivering the cytotoxic radiation directly to cancer cells. Important to note: an external beam delivers the irradiation per cell regardless of the number of cells. However, in RPT, the number of cells that are clustered together influences the absorbed irradiation per cell and the number of cells that have been targeted (Figure 4B).

External Beam Radiotherapy

External beam radiotherapy (EBRT) can be performed using photons, protons, or electrons. The most used radiation type in EBRT are photons (X-rays), which can penetrate the body and reach tumors located deep in the body. X-rays are a form of electromagnetic radiation, which consists of waves propagating through space that carry electromagnetic radiant energy. The wavelength of X-rays is in the range of 0.1 to 10 nm, giving them a corresponding energy of 100 eV to 100 keV [72]. Important to note: X-rays are not the same as gamma rays (γ-rays). The X-rays are artificially generated by accelerating electrons, which collide with a metal target, whereas γ-rays originate from atoms during radioactive decay. In addition, the maximum energy of the

produced X-ray spectrum can be controlled, whereas the energy of an emitted γ-ray from a certain atom is always the same. However, despite these differences, X-rays generated with a certain energy have the same potential in causing DNA damage as that of γ-rays harboring that same energy naturally.

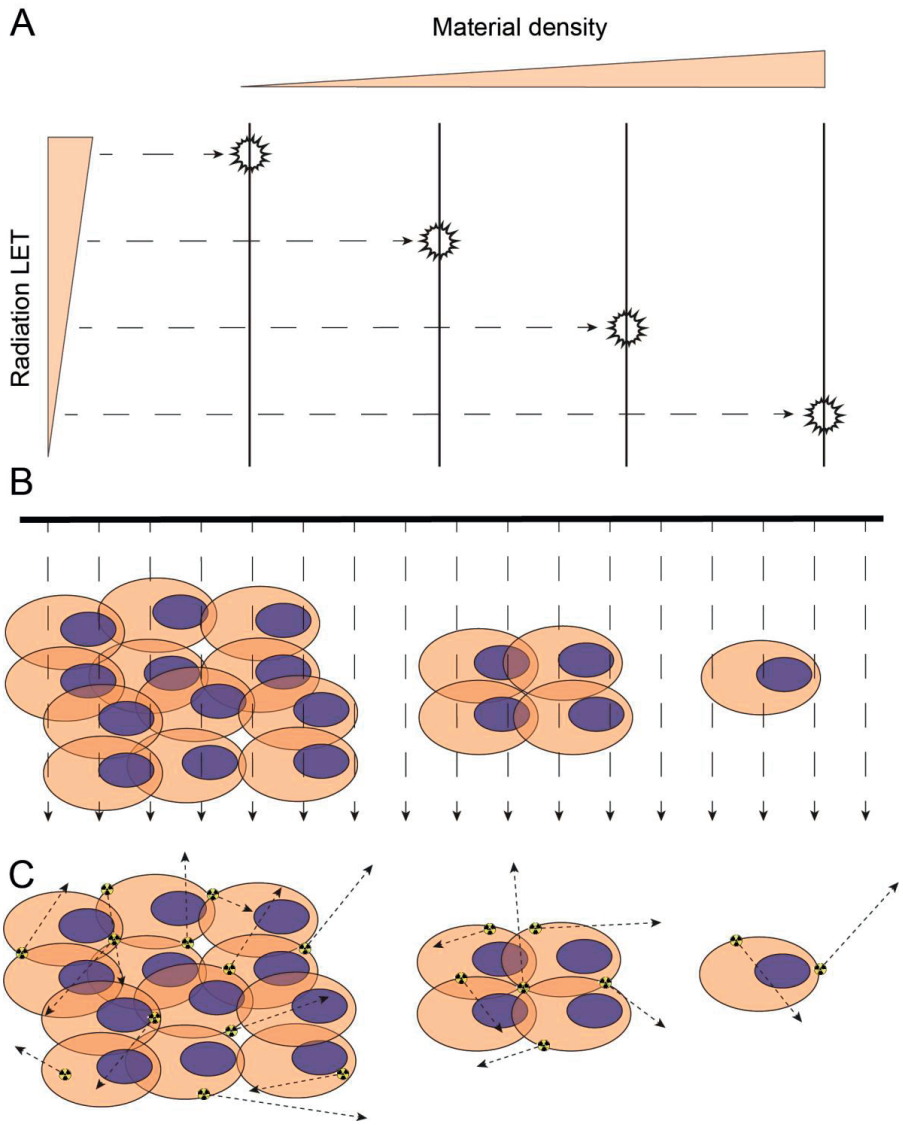

Figure 4. Penetration depth of IR and radiation delivery of EBRT or RPT. *(A) Schematic overview of the increasing penetration depth of IR in dense material with decreasing LET. (B) External Beam Radiation Therapy (EBRT) will deliver the same dose to cells, regardless of how large the targeted tumor is. However, in Radiopharmaceutical Therapy (RPT) (C), the delivered dose is dependent on the range of radioactive emission and the number of cells in which the radioactivity successfully is delivered.*

X-rays are generated with relatively low energy and have a low-LET, producing dispersed DNA damage throughout cell nuclei (Figure 5A). In addition, the types DNA damage induced by X-ray irradiation (mainly DNA single stranded breaks) are comparable with endogenous DNA damage in which 75% of the DNA damage is comprised of single stranded breaks [73]. Hence, the DNA damage repair pathways are highly efficient in repairing such damage. Therefore, the RBE values of X-rays are low, compared to other types of IR, which inflict more complex DNA damage for which repair is more difficult. Therefore, EBRT requires high X-ray doses during treatment, causing situations involving unacceptable toxicities toward healthy tissues in the patient. For example, metastasized cancer or tumors which are located in the vicinity of crucial or sensitive organs will lead to off target irradiation exposure [74]. A more targeted approach such as RPT offers the possibility to treat tumor cells specifically, reducing possible side effects.

Radiopharmaceutical Therapy

The use of RPT is defined by delivering radionuclides to tumor cells using specific tumor-associated (molecular) targets. Unlike EBRT, when RPT is administered, the cytotoxic radiation is delivered systemically in the body, much like chemotherapy. Targeting the radionuclide to specific tumor cells or their microenvironment is done using delivery carriers that either recognize specific endogenous targets of tumor cells or accumulate in microenvironments surrounding the tumor. Due to the systemic approach, development of RPT has been a highly multidisciplinary field, including expertise in oncology, radiobiology, pharmacology, radiochemistry, dosimetry, medical physics, and radionuclide imaging. This wide array of practitioners has led to a lack of focus in development. However, the shown efficacy with minimal toxicity in combination with a remarkable potential of RPT has led the attention of pharmaceutical companies and thereby a large financial benefit [75, 76].

Although the base knowledge of killing tumor cells by radiation is similar between RPT and EBRT, delivering radionuclides specifically to tumor cells, as is done in RPT, has unique properties which need to be understood to employ the full potential of RPT [77]. For example, the delivered dose using PRT is cumulative over several weeks, while in radiotherapy that same dose is delivered in short fragments, creating a large difference in the administered dose rate [78]. Moreover, the efficiency of RPT is largely based on targeting of carriers, which could be sub-optimal, leading to asymmetrical delivery of IR [79]. Thus, the efficacy of RPT relies on the efficiency of local energy deposition.

Alpha particle therapy

The aspect of internal irradiation in PRT provided the possibility to use radionuclides that emit high energy radiation that are not suitable for external irradiation, since such radioactivity does not penetrate the skin. In this thesis, we focus on the type of radiation, which is currently investigated intensively and shows preliminary therapeutic potential: alpha particle (α-particle) irradiation [80].

An α-particle consist of two protons and two neutrons, similar as the helium nucleus, and are emitted from certain radionuclides during their radioactive decay. Unlike photons or electrons, α-particles traverse only up to 100 µm in tissue, largely dependent on their emission energy

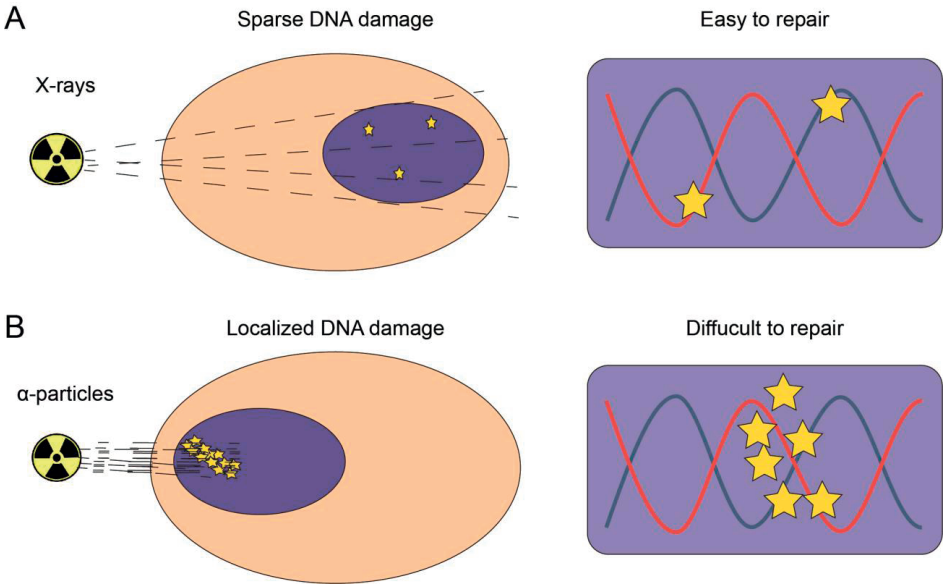

Figure 5. Schematic visualization of DNA damage induction by X-ray or α-particle irradiation. *(A) Low-LET X-ray irradiation inflicts few ionizations to the material it passes and therefore only sparse DNA damage. Generally, the inflicted DNA damage is easy to repair by several DNA damage repair pathways. (B) High-LET α-particle irradiation has a very dense ionization pattern, which inflicts highly condensed DNA damage. Such clustered DNA damage is considered highly complex and requires more specialized DNA repair pathways making this type of DNA damage more difficult to repair.*

(Figure 5B) [81]. Such short range is beneficial in a therapeutic setting as α-particles will less likely reach healthy tissue surrounding the target tumor. However, off-target binding and clearance still cause toxicity to other health organs. For example, most RPT molecules are extruded via the kidneys causing irradiation deposition and thereby renal toxicity.

One of the reasons of the characteristic short path length of α-particles is the high-LET, which is estimated to be 50 - 200 times higher compared to X-rays [82]. Radiation with such high-energy deposition is thought to inflict much more complex DNA damage compared to, for example, X-rays. In addition, the energy deposition of α-particles is highly localized and is thought to induce clustered DNA damage, which requires more complex DNA damage repair pathways [81, 83]. These observations show that the biological effect of α-particle irradiation is much higher compared to X-rays, resulting in a high RBE (up to 20 times) and greater treatment value.

Encapsulating α-particle emitting radionuclides

Although α-particle based PRT has large therapeutic potential, the use of α-particle irradiation comes with an additional challenge: recoiling daughter radionuclides [84]. During recoiling events, high-energy radionuclides break apart from the delivery vehicle and are free to roam in the body, possibly inflicting harm to surrounding tissue (Figure 6). Retaining the recoiling daughter radionuclides has seen much interest in the chemistry field, searching for solutions to this problem [85]. Liposomes have shown some potential in retaining mother radionuclides (up to 98%) but not recoiled daughter radionuclides, which was less than 20% [86]. In this thesis we focus on nano-carriers composed of polymers, which are more robust and therefore have more potential in retaining daughter radionuclides [87, 88].

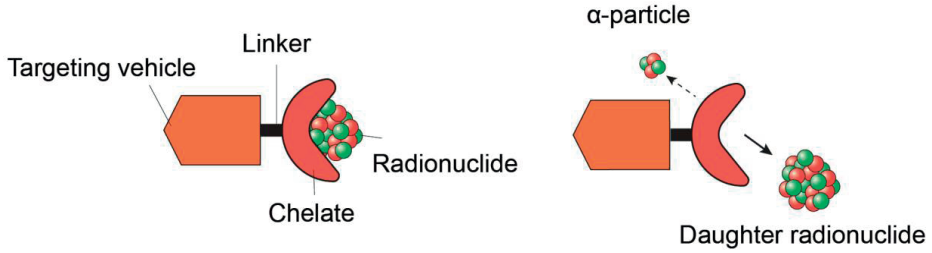

Figure 6. Recoiling daughter nuclides. *During radioactive decay, the remaining nuclide is often referred to as the daughter nuclide. In Radiopharmaceutical Therapy, the radionuclide is attached to the targeted vehicle using a chelator, which ensures efficient and strong binding of the radionuclide. However, during alpha decay, the daughter radionuclide experiences high recoiling energy, which ruptures the binding to the chelator. Therefore, with the use of long-lived α-particle emitting radionuclides comes a higher chance of setting their daughter radionuclides free from their carrier [85].*

Polymersomes

Polymersomes (PMs) are nano-carriers formed from amphiphilic block copolymers. Block copolymers undergo self-assembly when the concentration exceeds the critical aggregate concentration [89]. Most PMs are mode from block copolymers that consist of a hydrophobic part (mainly poly(styrene) and poly (ethyl ethylene)) and a hydrophilic part which mainly consist of poly(ethylene glycol) also known as PEG [90]. The consequence of using both hydrophilic and hydrophobic parts to prepare PMs is an aqueous core that is surrounded by a hydrophobic bilayer (Figure 7A).

By using different assembly techniques or extruding the PMs through polycarbonate filters, the size of PMs can be altered. Adjustments to PM sizes can be dependent on the application. For example, circulation times of PMs below 200 nm in diameter are drastically longer compared to larger PMs due to mechanical filtration in the spleen [90]. In addition, the lenght of the PEG-chain, is reported to affect circulation time, tumor uptake and clearance pathways.

For PMs to be of use in the field of PRT, radionuclides have to be encapsulated or labeled to the surface of PMs. Pentetic acid (DTPA) is often used to chelate radionuclides for application in bio distributions. By attaching DTPA to the hydrophilic outer layer, surface labeling of PMs can be achieved. However, this approach showed to be not very effective for PRT, as labeling efficiency was high but unstable [91]. In contrast, encapsulating radionuclides within PMs showed more promising results.

By encapsulating DTPA in the process of PM preparation, radionuclides can be trapped in the aqueous core, retaining more than 95% of the radionuclide after 24h (Figure 7B) [92]. The first reports of encapsulation of radionuclides in PMs were mainly performed using indium-111 [88, 91-93]. Further research explored the encapsulation of therapeutic radionuclides, mainly focusing on a-particle emitters actinium-225 and bismuth-213 [94-96].

Encapsulating high-energy α-particle emitting radionuclides in PMs has shown great potential of retaining the recoiling daughter radionuclides. Adding $InPO_4$ or $LaPO_4$ nanoparticles to PMs increased the recoil retention up to 20% and 28%, respectively [95]. In addition, Monte Carlo simulations show that adding high atomic number material to the PMs design, such as iron (Fe), improves recoil retention drastically (59.3%) [97].

These characteristics and improvements of PMs seem very promising for therapeutic use. However, aspects such as, circulation time, toxicity, and uptake have been lacking or are completely absent regarding PMs. Before PMs could be therapeutically viable, not only the chemical advantages should be investigated, but also the biological consequence of injecting PMs systemically.

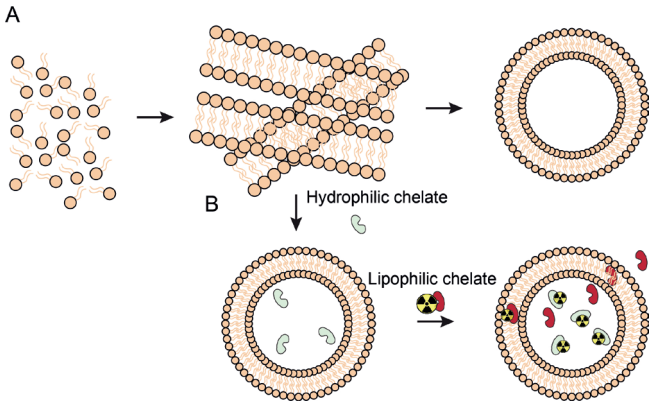

Figure 7. Preparation and radioactive labeling of Polymersomes. *(A) Schematic visualization of the self-assembly of block-copolymers, eventually forming bi-layered Polymersomes (PMs). The self-assembled PMs consist of an aqueous core surrounded by a hydrophobic bi-layer. (B) During the self-assembly is it possible to add hydrophilic chelates to the mixture, leading to the addition of a chelate in the core and therefore the ability to label the PMs with radionuclides. PMs are radiolabeled by adding radionuclides bound to lipophilic chelates, which can pass the hydrophobic bilayer, transporting the radionuclides to the core. In the PM core, the radionuclides are transported to the hydrophilic chelate, which is unable to escape through the bilayer resulting in a radiolabeled PM. The leftover lipophilic chelate is washed out ensuring only encapsulated radionuclides in the mixture.*

Scope of this thesis

Although EBRT has been applied for many decades as anti-tumor therapy, it has not been effective against metastasized cancer. RPT has shown to be a successful addition to the treatment options, targeting specific molecular targets and low energy radionuclides. However, complete cure of metastasized disease is seldom reached, due to insensitivity of tumor tissue and the induction of adverse side effects after therapy. These observations call for treatment optimization, searching for viable combination therapies and other irradiation strategies, inflicting more DNA damage whilst limiting side effects.

In this thesis project we therefore analyzed; (1) the effect of eliminating NHEJ, HR or both on IR protection, (2) a methodology for external α-particle irradiation, (3) DSB processing after high- or low-LET irradiation and (4) intracellular uptake processing of PMs used for local delivery of α-particles.

Chapter 1 describes the mechanisms of DNA damage repair together with how radioactivity is used in the clinic and which nano-carries would be suited best, to carry high-energy radionuclides.

In **Chapter 2**, we aim to understand how HR and NHEJ cooperate in IR protection. By exposing mutant adult mice and their embryonic stem cells or fibroblasts to IR we investigate the consequence of HR or NHEJ deficiency. Using mRNA expression profiling, effects of the introduced mutations were analyzed in endogenous conditions. Finally, the cell biological consequence of eliminating NHEJ or HR was examined by confocal and super resolution microscopy using 53BP1 kinetics.

In **Chapter 3**, we describe a method to address the lack of affordable and easily accessible external α-particle irradiation systems. This chapter presents a detailed approach on the development of an easy to use novel irradiation set-up for cell biological experiments to study the impact of α-particles.

We applied the developed irradiation system in **Chapter 4** to compare the DSB processing in living cells after high- or low-LET irradiation. This chapter describes a detailed analysis method to track DSB formation and processing in living cells. By comparing high- and low-LET irradiation, we aimed to quantify differences in DSB processing using live-cell, confocal and super-resolution imaging.

Polymersomes are nano-cariers, which have a high potential to be applied in targeted radionuclidetherapy using α-particle emitters. In **Chapter 5**, we investigated the effect of PM size on uptake in different cell types. In addition, we analyze geometrical distribution and post-uptake processing of PM using co-localization studies. Furthermore, we show DNA damage induction of radiolabeled PMs.

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