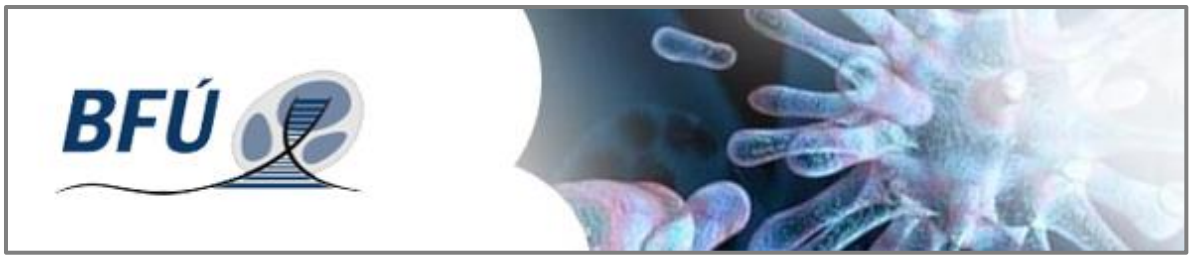

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Habilitation Thesis - Summary

DNA Damage and Repair upon Cell Exposure to Different Types of Ionizing Radiation – the Importance of Chromatin Context and New Perspectives of Cancer Radiotherapy

Brno, 1st October 2019 RNDr. Martin Falk, Ph.D.

SUMMARY

The collection of papers presented in the current habilitation thesis summarizes the contribution of the author and his team to the development of radiobiology. Specifically, the present thesis creates a better understanding of the biological effects of different types of IR, including γ-rays, protons, and various accelerated ions with a high LET. The included papers introduce step by step the team's contribution, leading from recognition of the principles of the higher-order chromatin organization in the cell nucleus to suggesting a new model that describes the relationship between the physical properties of IR, higher-order chromatin structure (or other cell-type-specific intracellular factors), and DNA damage induction, repair, and misrepair. Consequently, the impact of our findings on the (tumor) cell radioresistance and therapeutic possibilities of how it could be therapeutically manipulated or overcome is considered. The current thesis provides discussion on the published results, which are supplemented with explaining and summarizing comments, putting the findings in a broader context. Figures and pieces of text from the original papers are also enclosed in a modified form to provide the reader with a deeper understanding of the problems without asking the reader to go over the original works. The results are organized into three chapters: The first chapter (Chapter 2.1) explores the principles of the higher-order chromatin organization (nuclear architecture) and its role in fundamental physiological processes in normal cells and pathological processes in tumor cells, respectively. The second chapter (Chapter 2.2) then focuses on the question of how the higher-order chromatin structure participates in the mechanisms of radiation damage induction, repair, and formation of chromosomal aberrations. Finally, the third chapter (Chapter **Chyba! Nenalezen zdroj odkazů.**) addresses the causes of tumor cell radioresistance and possible methods for therapeutic lowering. The achieved results are briefly summarized below.

In Chapter 2.1, we propose the principles of the higher-order chromatin organization in the nuclei of normal cells[1–5] and point to their alterations in cancer cells.[6–9] Together with others[1] we showed—which contrasts the previous opinion of the biological community—that the cell nucleus is a highly organized organelle with nonrandom higher-order chromatin structure that, importantly, has functional aspects.[1–5]

In the interphase nucleus, chromosomes appear as so-called chromosomal domains (or territories), showing their internal structure and distribution in the cell nucleus that follows some rules, though of a statistical character. In spherical human cells, for instance, lymphocytes, the territories of gene-dense chromosomes tend to occupy a more central space of the cell nucleus while the territories of gene poor chromosomes preferentially appear

underneath the nuclear envelope. Each pair of homologous chromosomal territories thus preferentially occurs in a specific concentric shell of the cell nucleus with a defined mean distance from the nuclear center (further referred to as "radial distance"). This mean distance increases with the overall genetic activity of the particular chromosome, is characteristic for each pair of homologous chromosomes, and, to some extent, also depends on the cell type.[1,2] The same rule holds true also for the internal organization of subchromosomal chromatin domains within chromosomal territories and causes their structurally functional polarization.[1,2] The nuclear topology of chromosomal territories in flat cells, such as fibroblasts, follows similar organization principles, but their radial distribution is based on the size of chromosomal territories rather than gene density/activity. In contrast to the radial distribution, the mutual arrangement of chromosomes at the surface of imaginary concentric spheres is random.[1,2] Genetic activity influences and, in turn, is influenced by the level of chromatin condensation.[3] Genetically (transcriptionally) active chromosomal territories and their chromatin subdomains are less condensed and occupy bigger nuclear volumes compared with the genetically inactive counterparts of a comparable molecular size.[3] In some cases, genetically active chromatin even protrude outside of the territory's core area. Therefore, genetically active territories are more irregular than inactive ones and, to a larger extent, intermingle with their neighbors.

The higher-order chromatin structure is disturbed in different ways in tumor cells and actively contributes to disease development.[6–10] For instance, we discovered that an oncogenic protein may initiate cancer just by generating changes in the higher-order chromatin structure.[11] As another example, we revealed that there is incomplete chromatin maturation (composition and condensation) in the terminally differentiated granulocytes of acute and chronic myeloid leukemia (AML, CML) patients. Importantly, this immature status persists in patients' granulocytes even after a successful cancer treatment, leading to complete clinical and molecular remission with the disappearance of the Philadelphia chromosome. Importantly, this defect is of serious functional relevance because it prevents AML/CML granulocytes' immune functioning.[6] Defects in the higher-order chromatin organization appear even in the cells isolated from a morphologically normal tissue adjacent (e.g., 10 cm distant) to the colon tumor. These changes could thus be considered either as premalignant epigenetic defects or feedback chromatin alterations provoked by the tumor in the surrounding cells.[10]

Finally, the specific features of the higher-order chromatin structure could be the explanation for why both spontaneous and radiation-induced DNA breaks, which cause the chromosomal aberrations causative of myelodysplastic syndromes (MDSs), emerge at specific chromosomal loci. Nevertheless, these breakpoint loci are not as sharply defined as, for instance, in the case

of leukemia, and only a few of them colocalize with chromosome fragile sites. This suggests that specific higher-order chromatin structures could be responsible for (or at least contribute to) the susceptibility of MDS breakpoints to DSB formation.[8,9] Indeed, our preliminary data show more frequent colocalization of the γH2AX foci (DSB marker) with some MDS breakpoints in cells exposed to γ-rays (Falk et al., manuscript in preparation). Interestingly, in this context, MDSs frequently appear as the secondary cancer developed as a result of previous radiotherapy history. These results thus form the logical bridge to the next chapter dedicated to the relationship between the higher-order chromatin structure and effects of IR.

Chapter **Chyba! Nenalezen zdroj odkazů.** demonstrated in detail the importance of the higher-order chromatin structure for fundamental cellular processes. Based on this, it is reasonable to hypothesize that the organization of chromatin into structurally and functionally distinct chromatin domains can influence the sensitivity of DNA to radiation-induced damaging,[12,13] the mechanisms of DSB repair,[14–18] and, in turn, the mechanisms for the formation of chromosomal aberrations.[14–18] These studies are the subject of the second chapter of results (Chapter 2.2); here, we focused on DNA double-strand breaks (DSBs) because they are the most deleterious type of DNA lesions generated by IR. It should be noted that IR is the most potent DSB inducer among other DNA-damaging agents. Even a single DSB can result in cancer or cell death if repaired improperly or left unrepaired, respectively.

We showed[13,14] that decondensed, genetically active (eu)chromatin is a more critical target for low-LET radiation than its condensed, genetically inactive counterpart (hetero)chromatin. This difference in the radiosensitivity between the “heterochromatin” and “euchromatin” domains may be because sparse IR types mostly attack DNA through the production of reactive oxygen species (ROS), and as we observed, heterochromatin is better shielded against ROS than euchromatin because of its abundant heterochromatin-binding proteins[12,13] (reviewed in [15,17,18]). Moreover, because ROS arise from water radiolysis and are very short lived (i.e., can only damage biomolecules in their immediate surroundings), heterochromatin is protected against ROS also by its lower hydration compared with euchromatin[12,13] (reviewed in [15,17,18]). On the other hand, heterochromatin, with its higher DNA density, provides more targets per volume for high-LET particles, which mostly attack chromatin directly, and thus without regard to chromatin structure.[16–18]

The higher-order chromatin structure has important consequences for the mechanism of DSB repair and the formation of chromosomal aberrations[14] (reviewed in [15,17,18]). The repair of heterochromatic DNA breaks is more complicated than the repair of euchromatic breaks and requires decondensation of the affected chromatin domains before the process can continue. This decondensation allows for better access of repair factors to the damaged chromatin,

mediating the relocation of DSBs into nuclear areas with a low density of chromatin that probably represent more suitable subcompartments for repair than condensed chromatin. Indeed, our confocal microscopy experiments on live cells with condensed chromatin domains that were labeled with HP1β-GFP and damaged by UV laser micro-irradiation revealed that although small sensors of DSBs (NBS1-RFP) can freely penetrate into these dense chromatin structures, large proteins (53BP1-RFP) acting in the later phases of DSB repair can do it only after preceding decondensation of the domain.[16] Despite the spatial relocation of some of DSB repair foci during the postirradiation time, it should be noted that their movement has no features of the targeted migration of multiple DSBs into putative repair factories,[14,14,17,18] the existence of which has been proposed by several authors. In fact, most damaged chromatin sites remain rather stable and DSB clustering—observed only occasionally after low-LET exposure—represents an unavoidable side effect of repair.[12,13] Our results indicate that DSB clustering provoked by repair processes increases the risk of broken DNA ends misrejoining, perhaps explaining how complex chromosomal translocations occasionally form even in cells that are irradiated with low-LET IR.[13] Thus, the scenario described above adopts some aspects of both the "breakage-first" and "position-first" hypotheses, originally postulated as the opposite views regarding the involvement of chromatin dynamics in the mechanism of the formation of chromosomal translocations (or aberrations in general). We can conclude that chromosomal translocations usually appear between broken chromosomal loci that have been located in mutual proximity in the cell nucleus before damage induction; however, in some cases, illegitimate rejoining can proceed also between originally distant DSBs if they are mobilized by repair processes.[12,13,15]

The higher-order chromatin structure (texture) influences the probability of chromosomal translocations between particular DSBs in an even more complex way—it determines the vectors (extent and direction) of damaged chromatin movements and thus the possibility of their mutual meeting in the cell nucleus.[13,15] For instance, a heterochromatic "barrier" separating two DSBs can prevent their association and chromatin exchange between the affected loci. This challenges the current hypothesis presupposing that the probability of a translocation event occurring between specific genomic loci is simply proportional to their spatial separation in the cell nucleus, an assumption taken because of the nuclear architecture.

The relationship between the higher-order chromatin structure and repair processes described above is relevant also for high-LET irradiation;[16–18,18,19] however, high-LET particles generate a large number of DNA fragments along their track, that is, in a very limited volume of the cell nucleus.[19] With this condition, the higher-order chromatin structure can be locally lost so that (complex) chromosomal translocations can easily form between numerous free

DNA fragments randomly. Complex aberrations in cells exposed to high-LET IR thus mostly appear as the result of the microdosimetric character of radiation energy deposition.[19] Based on these described findings, we propose a new model for the complex relationship between the properties of IR, microscale higher-order chromatin structure, sensitivity of distinct chromatin domains to radiation damage, DSB repair processes, and mechanism of formation of chromosomal aberrations.[13,14,16–19]

Even deeper insights into the mechanisms of the functional architecture of the cell nucleus and processes of DNA damage induction and repair could be obtained with super-resolution microscopy, technology that emerged only recently because of tremendous progress and that represents a breakthrough in cell research. In the frame of the presented research, in cooperation with Prof. Michael Hausmann from the Kirchhoff Institute in Heidelberg, Germany, we have adapted single molecule localization microscopy (SMLM) with a resolution of 10–20 nm for detailed analyses of chromatin and DSB repair focus (IRIF) nanostructures.[18,20–22] The obtained nanoscale results are relevant for more chapters of the current thesis; nevertheless, to prevent redundancy, they are only discussed in Chapters 2.3.1 and 2.3.2 in the context of particular research topics.

The last chapter presents our research on the diversity and mechanisms of tumor cell radioresistance[23–25] and the development of new approaches to therapeutically overcome tumor cell radioresistance.[19,20,22,25–32] Introduced are also the results of the opposite way to improve tumor radiotherapy, that is, normal cell radioprotection.[33–35] Thus, the results are also partially relevant for civil/military radiation protection.

One crucial factor with potentially strong influence on cell radioresistance is DSB repair, which from different points of view has been explored in the frame of the previous chapter. Therefore, we studied here how DSB repair efficiency varies between different normal and tumor cell types[22,25] and between the cells of the same type but that were obtained from different cancer patients[25] or that carried alterations in important repair proteins.[23,24]

Tumor cells are known to have various mutations in the genes that are involved in DNA repair, cell cycle control, and cell death pathways, which can modify their response to radiotherapy. However, cancer cells also carry genetic alterations of other types; the effect these alterations have on DSB repair and cell radioresistance is still unexplored. Here,[23,24] we focused on the relevance of the alternative splicing variants of the BRCA1 protein, which functions in the decision making for a particular repair mechanism (NHEJ, nonhomologous end-joining; HR, homologous recombination; or alternative backup pathways) at individual DSB lesions. We revealed that cancer-specific misregulation of the splicing process may lead to the formation of irregular alternative splicing variants (ASVs) of BRCA1, for instance, with BRCA1Δ14–15 and

BRCA1Δ17–19 ASVs, which according to our observations, undermines NHEJ activity and delays the repair of ionizing radiation-induced DSB damage; BRCA1Δ17–19 also impairs HR. Our results suggest that the alternative splicing variants of BRCA1 (and thus ASVs in general) may negatively influence genome stability, thereby contributing to enhanced probability of cancer development in the affected individuals. This finding could have important implications for the prevention and treatment of breast cancer.

Concerning the cell-type-specific and individual (tumor) radioresistance, we are running a study with primocultures of different cell types isolated from tumors of head and neck cancer patients. Head and neck tumors (HNT) were selected because half of them responded to radiotherapy, while the remaining half was highly radioresistant. The reasons behind this different radiosensitivity are unknown, as are the clinically usable markers of radioresistance, which strongly impairs the current tendency in HNT oncology to shift from surgery to noninvasive (chemo)irradiation to improve patients' post-treatment quality of life.

To shed more light on these issues, we prepared primocultures of $CD90^-$ (tumor) cells, $CD90^+$ (tumor-associated) fibroblasts (TAF), and their mixed ($CD90^-$ (+) $CD90^+$) cultures[25] (Vicar et al., CSBJ, submitted; Falk et al., manuscript in preparation). Consequently, we compared the DSB repair efficiency and postirradiation cell survival between the primocultures of these different cell types that were isolated from a single tumor and between the primocultures of the same cell type isolated from different tumors. The preliminary results revealed that many tumor primocultures could repair DSBs, with the kinetics and efficiency comparable to normal cells (cultured fibroblasts and fibroblasts taken from morphologically normal tonsil tissue); nevertheless, the deviations in both directions—faster or slower repair—were detected and frequently correlated with a higher or lower tumor cell radioresistance. In many cases, however, the DSB repair kinetics were found to remain unchanged, even if the cells exhibited increased or decreased radioresistance. This indicates that although DSB repair definitely represents a critical contributor to tumor cell radioresistance, the response of HNT cells to irradiation is in fact a very complex phenomenon. The search for other factors substantially influencing this response in addition to DSB repair is just beginning, taking advantage of RNA chips designed by the author (in collaboration with J. Gumulec, M. Raudenska, and M. Masařík) for more than 350 of the genes involved in different relevant aspects of cell life (DSB repair, cell cycle regulation, apoptosis initiation, etc.).

Importantly, tumor-associated fibroblasts (TAFs) often repaired DSBs with similar kinetics as tumor cells isolated from the same tumor, even when the tumor cells extensively diverged from normal repair velocity. This held true also for cells obtained from morphologically normal tissues spatially separated from the tumor by about 10 cm. Although these observations need to still be interpreted, at least three interesting possible explanations are possible and not

unprecedented in cancer biology. First, premalignant changes may exist in the tumor-surrounding tissue although it still preserves normal morphological features. This idea has already been proposed in our earlier work on colon cancer.[10] Second, the functions of normal cells in tumor proximity could be altered by tumor cells. Finally, faster or slower DSB repair of TAF- and tumor cell primocultures (compared with the average for normal cells) could reflect the genetic background of individual patients, that is, appear independently of cancer.

In many patients, large numbers of DSBs appear in nonirradiated tumor cells. This points to their permanent genomic instability (of a still unknown origin but most likely related to replication stress or telomere damage), which seems to be a quite frequent factor leading to an initially positive response of HNT cells to irradiation but that leads to the development of potentially resistant clones in a long-term perspective.

A substantial body of the presented research concerns new approaches capable of improving current radiotherapy by decreasing tumor cell radioresistance and/or by selectively protecting normal cells against the deleterious effects of irradiation. First, we investigated DSB induction, DSB repair, and cell survival upon irradiation with protons of different energies and various accelerated ions.[19,22,26] An enhanced capability of ion beams to kill tumor cells (compared with γ-rays or X-rays) follows from the well-understood physics behind this phenomenon. However, the real biological effects remain to be determined in terms of both their mechanism and extent. Consequently, the curing protocols are built up on empirical knowledge rather than on a solid body of experimental data, which prevents maximal therapeutic benefit from the physical advantages of ion beams.

As expected, we observed that the complexity of DSB clusters correlates with radiation LET and significantly influences both the reparability of DSB lesions and the survival of cells upon irradiation. Surprisingly, the complexity and reparability of DSBs also varied for different accelerated particles that have a similar LET and energy. This could be explained by the slight but significant differences in the microdosimetric character of DNA damage induced by the studied particles. The diameter of the track core seems to be an interesting parameter in this respect.[19]

The relationship between the DSB structure and reparability was further studied at the nanoscale, here again taking advantage of SMLM. This attempt represents an important feature of the novelty of the present thesis. In cooperation with Prof. Michael Hausmann (KIP Heidelberg, Germany), we have adapted SMLM for analyses of the structuro-functional and spatio-temporal aspects of DSB damage induction and repair, producing a resolution of up to about 10–20 nm.[21,22]

Our motivation to study the nanostructure of DSB repair foci in the context of (tumor) cell radiosensitivity followed from earlier reports suggesting that different types of cells and DNA damage can activate nonhomologous end-joining (NHEJ) and homologous recombination (HR)—the two main DSB repair mechanisms in human cells—but with different preferences. Because NHEJ, HR, and possibly the backup repair pathways operate with incomparable kinetics and fidelity, it is of the utmost importance to find out how various cell types pick a particular repair mechanism at each single DSB site. Our observations and those of other groups suggest that the decision-making mechanism could be based, at least partially, on the structural characteristics of a damaged chromatin domain and the DSB itself. These characteristics may regulate the attraction and accessibility of individual repair proteins to DSB sites[16] and thus the assembly and structure of DSB repair complexes (DSB repair foci, IRIFs). The structure of DSB repair foci could be an important factor further driving the repair mechanism to NHEJ, HR, or the backup pathways. Nevertheless, other cell-type-specific factors, such as intracellular levels and/or mutations of repair proteins, can influence the composition of DSB repair foci and thus their (nano)structure; these factors could differ significantly among cells, especially between normal and different tumor cells. DSB repair-focused (nano)structures can be more or less directly related to the mechanism of repair and, consequently, to cell radioresistance.

In the study of Depeš et al. (2018),[21] we demonstrated the applicability of SMLM as one highly resolving method for analyses of dynamic repair protein distribution and repair-focused internal nanoarchitecture in intact cell nuclei. This study is the first report on SMLM visualization of γH2AX and 53BP1 repair foci induced by low-LET and high-LET radiation, respectively. Thanks to a "trick"—we irradiated the cell monolayer at a sharp angle (10°)—we were able to analyze the numbers and distributions of individual γH2AX and 53BP1 molecules inside microscopically defined foci and along the particle tracks. DSB repair foci generated by high-LET ions were considerably more complex than those appearing after γ-irradiation and showed an internal nanostructure. Although the research is just in its infancy, the preliminary results revealed that this focus nanostructure and its spatio-temporal dynamics could depend on the cell type, as we have demonstrated for normal human skin fibroblasts and highly radioresistant U87 tumor cells. Hence, DSB repair-focused nanostructures may be functionally relevant and correlate with cell-specific radiosensitivity.[22] Methodologically, the study proved SMLM as being a highly appropriate method for investigating spatio-temporal (DNA repair) protein distributions in cell nuclei and their subcompartments, such as DSB repair foci. We suppose that SMLM can provide deeper insights into how chromatin and DSB repair-focused structures influence the decision making for a particular repair pathway at a given DSB site.

As another approach that could improve both the efficiency and (tumor cell) specificity of radiation-based therapies and that can be combined both with standard radiotherapy and the ion-beam cancer therapy introduced above, we studied the extent and mechanism of the radiosensitizing effect of metal nanoparticles (NPs).[20,27,28,36,37] The radiosensitization from NPs has been predicted based on their physical properties, specifically the ability to emit showers of secondary electrons upon irradiation and thus increase the absorbed dose at the microscale. Moreover, NPs are preferentially internalized by tumor cells, even passively because of the so-called *enhanced permeabilization and retention* (EPR) effects. The original hypothesis thus counts the DNA molecule as the primary target for NP-mediated radiosensitization and increased induction of DSBs as the mechanism of this effect.

Our results confirmed that various metal NPs can be used to radiosensitize even very radioresistant (e.g., U87) tumor cells, at least *in vitro*. The biological mechanisms of this radiosensitization and their dependence on DNA damage remain obscure. At the nanoscale, we recorded higher numbers of γH2AX molecule signals in the nuclei of cells irradiated in the presence of 10 nm gold NPs than in cells irradiated in their absence. On the other hand, neither the numbers of microscopically defined DSB repair foci (γH2AX + 53BP1) increased, nor did the DSB repair kinetics decrease, in cells incubated prior to irradiation with other NP types, even though the radiosensitizing effect was obvious. Therefore, we propose that more phenomena participate in the nanoparticle-mediated (tumor) cell radiosensitization, with the individual contributions depending on the nanoparticle, cell, and radiation properties. Because the nanoparticles in our experiments were mostly encapsulated in lysosomes and did not colocalize with mitochondria—the only cytoplasmic organelles containing DNA in human cells—we hypothesize that lysosomal damage could represent a new mechanism of NP-mediated radiosensitization. This is compatible with new findings highlighting the important role of lysosomes in intracellular signaling, which also includes the initiation of apoptosis. Hence, depending on the extent of lysosome disruption, the compounds released from these organelles into the cytoplasm may either alter cellular signaling and initiate apoptotic cell death or directly digest the cytoplasm and therein the dispersed organelles. Current opinions on metal nanoparticle-mediated radiosensitization are discussed in Pagáčová et al. (2019) and Falk et al. (2019).[28,37]

Freezing is known to kill unprotected cells, has been proven to be effective (cryoablation) in the treatment of several cancers, and is crucial in reproductive medicine (cryopreservation). However, uncertainty remains about its effects on chromatin. The majority of studies point to chromatin fragmentation in frozen/thawed cells because of extensive DSB formation, while other studies recognize DSBs only in cells with somehow defective chromatin already prior to freezing/thawing, with the few remaining studies reporting a failure to observe DSBs at all.

Hence, we have analyzed how freezing/thawing influences chromatin condition[29–32] in the context of cell viability and have considered the potential of this approach for tumor cell radiosensitization.

To clarify the mechanism of chromatin cryo-damaging, we analyzed changes in the chromatin integrity and higher-order chromatin structure in normal and tumor cells frozen/thawed in the absence or presence of cryoprotectants of different types; we then correlated them to cell viability after defrosting. The results we obtained support the hypothesis that freezing/thawing causes DSBs only under specific conditions—as we discovered,[32] in cells just undergoing DNA replication. In these (S-phase) cells, dozens to hundreds of colocalized γH2AX and 53BP1 DSB repair foci can be seen because of a collapse of replication forks, which is probably followed by their conversion into DSBs. Non-S-phase cells, on the other hand, lack DSBs, but together with S-phase cells, suffer from extensive alterations to the higher-order chromatin structure. In some cells, ruptures of the nuclear envelope even lead to chromatin leakage into the cytoplasm. Interestingly, although the extent of nuclear envelope and chromatin structure damage depends on the method of cryoprotection, the collapse of replication forks could not be reduced by the cryoprotectants studied. Taken together, our results on freezing/thawing show that it seriously damages chromatin; however, the induction of DSBs is restricted to S-phase cells that are mostly affected by freezing/thawing. Because tumors contain more S-phase cells in principle than normal tissues, this discovery could provide a mechanistic explanation for why cryoablation could efficiently eradicate tumor cell populations. In addition, we showed that chromatin condensation provoked by some cryoprotectants before freezing can efficiently reduce cell cryo-damage and improve post-thaw cell survival. Whether and how freezing/thawing influences the ability of irradiated cells to repair DSBs and it possibly sensitizes tumor cells to irradiation is under exploration.

The last approach studied in the present thesis—to enhance radiotherapy—follows the opposite strategy than those described above: it is based on selective radioprotection of normal cells. In our research, we focused on the biological effects of amifostine (WR-2721),[33] currently the only drug approved for clinical use that is capable of improving the survival of normal, not tumor, cells after irradiation. In normal cells, amifostine is converted to its active ROS scavenging metabolite WR-1065 by alkaline phosphatase (ALP), the levels of which are decreased in many cancers. Nevertheless, more mechanisms of amifostine action have been proposed that remain to be explored. Hence, we were interested in how amifostine influences DSB induction and repair in normal and tumor cells, respectively. Interestingly, although amifostine reduced the radiation damage to DNA only in normal cells, as expected, it also supported DSB repair in γ-irradiated normal cells and altered it at least in some (MCF7) tumor cell types. Thus, amifostine not only protected normal cells from the deleterious effects

of radiation in multiple ways, but also disturbed DSB repair in tumor cells. Hence, we have confirmed that the selective functioning of amifostine in normal and tumor cells can be ascribed to the common differences between these cells in their ability to convert amifostine. Nevertheless, we propose new scenarios, named here the "good and bad," "Jekyll and Hyde," and "third player" hypothesis (Hofer et al. 2016),[33] theoretically interconnecting the networks of already known and newly discovered amifostine effects, ensuring its double-edged activities. Other possibilities of (combined) radioprotection are reviewed in papers (Hofer et al. 2017a, 2017b).[34,35]

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