Tumor control and normal tissue toxicity: The two faces of radiotherapy

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Citation for published version (APA):
CHAPTER 7

Summary and General Discussion

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SUMMARY AND GENERAL DISCUSSION

This thesis discussed the two contrasting sides of radiotherapy: tumor control and normal tissue toxicity. On one hand, radiation treatment aims to kill the tumor with the highest possible radiation dose, by inducing as much lethal DNA damage as possible, eventually eradicating the whole tumor. On the other hand, escalation of the radiation dose will also result in an increase of normal tissue damage and hence of the normal tissue complication rate. Unfortunately, ionizing radiation cannot distinguish cancer cells from normal, healthy cells and will cause damage to all cells in the irradiated area. However, the biological differences between the tumor and normal cells in particularly proliferation rate and DNA-repair capacity provide a therapeutic window for radiation treatment. This therapeutic window is widest at a relatively low dose, typically at 2Gy per day. Therefore, dividing the required dose in daily small fractions, so-called fractionated radiotherapy, allows optimal time for normal cells to repair the induced DNA damage while cancer cells are destroyed efficiently. Next to these biological differences, new image-guided technologies are improving the precision of radiotherapy significantly, and minimize the irradiated normal tissue volume. Improvements in radiotherapy remain however necessary as several tumors are still poorly controlled by radiation treatment alone and on the other hand, in cases where tumor control is achieved the resulting improved survival rates make the issue of quality of life in cancer survivors, which may experience complications from radiation damage, more and more important. Exploring the molecular events after radiation treatment, not only in cancer cells but also in normal cells, might unravel mechanistic insight of the radiation responses and possibly lead to the identification of new therapeutic targets or biomarkers.

In the studies described in this thesis, the molecular events involved in the repair of radiation induced DNA damage were investigated. By interfering with the DNA double strand break repair processes, we aimed to sensitize tumors and cancer cells to ionizing radiation and we measured the activity of these processes to predict tumor sensitivity and normal tissue toxicity. Chapter 1 provided a general overview of the biological effects of ionizing radiation and the DNA damage response in mammalian cells. Furthermore, this chapter described important factors involved in the radiation response in cancer cells and in normal cells separately, introducing the two contradictive sides of radiotherapy. Both sides will be discussed in the following sections.

PART I: RADIATION RESPONSE IN CANCER CELLS

The combination of ionizing radiation with other therapies is the focus of many studies that aim to improve currently applied radiation treatment. The combination of radiotherapy with chemotherapy is widely used for the treatment of cancer, and in the Academic Medical Center
there is a long tradition of combining radiation with hyperthermia, i.e. heating of the tumor to 41-43⁰C. Heat sensitizes cancer cells to radiation by altering proteins involved in the DNA damage response.

Compared to radiotherapy alone, the combination of radiotherapy with chemotherapy has been shown to improve local tumor control and to prevent local recurrences or metastasis [1, 2]. In rectal cancer patients, neoadjuvant chemo-radiation treatment before surgery even reduced the local recurrence rate to less than 10% [3]. However, adding chemotherapy to radiation treatment also results in a significant increase of acute and late normal tissue damage [1, 4]. As the quality of life after cancer becomes increasingly important, we instigate the search for a combined radiation treatment strategy that can improve local disease control without increasing the normal tissue toxicity rate. The effect of hyperthermia on normal tissues might be minimal due to the fact that the tumor is locally targeted, but long term effects need to be studied in clinical trials. In recent years, there has been an exponential growth in the knowledge of molecular mechanisms and gene mutations involved in the treatment response of tumors; this knowledge could contribute to the development of molecular-targeted drugs that might augment the effect of ionizing radiation in cancer cells [5-7]. Due to the wide heterogeneity of tumors between patients and cancer types, personalized medicine and targeted therapies becomes more important. The first part of this thesis discussed the role of key factors involved in the DNA damage response, possible therapeutic targets and biomarkers for clinical use.

Targeting the DNA damage response to enhance tumor control.

In chapter 2 study results on the role of tumor suppressor p53 in the repair of potential lethal damage (DNA double strand breaks (DSBs)) after radiation treatment are described and it is concluded that functional p53 is necessary for the repair of DSBs [8, 9]. The important role of p53 as tumor suppressor, by inducing cell cycle arrest, cell cycle checkpoints, apoptosis or senescence, is widely appreciated and chapter 2 further confirmed this role by showing its involvement in the repair of potentially lethal damage. It is thought that without functional p53, normal cells lose the ability to control their growth and death and are able to proliferate regardless of the amount of DNA damage. Uncontrolled dividing cells with unrepaired DNA damage could give rise to a mutagenic cell type and may eventually lead to cancer [10, 11]. In addition, more than 50% of all cancers harbor mutated or functionally deficient p53 [12], explaining several malignant features of cancer cells: aberrant cell cycle progression and loss of apoptotic capability [13]. Furthermore, it is thought that mutated p53 not only loses its tumor suppressive function but also gains oncogenic activities [14], contributing to chemo- and radiotherapy resistance and metastasis. Targeting p53 has therefore been proposed as possible anti-cancer treatment. Targeting strategies either aim to reactivate the loss of functional p53 to induce death in cancer cells, to deplete mutated p53 to reduce oncogenic processes [15], or to inactivate p53 to protect normal cells from death and possible normal tissue toxicity [16].
Unfortunately, the complexity of the p53 pathway and the large variety in mutation types make the development and implementation of p53 target drugs challenging. Whether the mutant p53-depleting drugs or p53-reactivators could have an effect on all p53 mutants or only specific ones, and the possible effects of these drugs on other proteins and pathways needs to be further investigated. In addition, the underlying mechanisms on how mutant p53 promotes tumorigenesis are also not completely understood. Moreover, the effects of p53-reactivation seem to be tumor specific: restoration of p53 in lymphomas led to increased apoptosis levels, whereas other solid tumors only showed an induction in cell cycle arrest [17]. The induction of cell cycle arrest could indicate that tumors with a low apoptotic response will progress again once the reactivating drug is withdrawn. Despite the above mentioned challenges, some promising p53 therapeutics have been developed and are currently emerging in clinical trials [16]. Optimization of p53-targeted drugs could lead to an increase in cell death and reduction of oncogenic activities, thereby possibly preventing therapy resistance and improve tumor control.

Repair of potentially lethal DNA damage may also undermine the efficacy of radiation treatment. Because of the multiple functions of p53, genes and proteins involved in the actual repair of DNA DSBs may be more suitable as targets. Many studies investigated the inhibition of either of the two main DNA DSB break repair pathways: homologous recombination (HR) or non-homologous end joining (NHEJ) [6, 18-21] with varying outcomes. One of the most promising targeted treatments so far are the PARP1 inhibitors. PARP1 is one of the protein enzymes involved in the repair of single strand breaks, and has been suggested to play a role in the alternative form of DNA DSB repair. Inhibition of PARP1 can lead to unrepaired single strand breaks, resulting in the formation of lethal DSB after DNA replication [22]. PARP1 inhibitors are shown to be extremely toxic in homologous recombination deficient cancer cells [23], and may therefore be useful or the treatment of for example the BRCA1/BRCA2 mutated breast and ovarian cancers. Several clinical trials are being conducted, and the PARP1-inhibitor olaparib has already progressed to phase III-studies. The first results show a longer progression-free survival of high-grade ovarian patients [24], but effects on overall survival need to be yet determined as well as the effects in low or intermediate grade cancer patients. Furthermore, the development of dose-limiting hematologic toxicities after olaparib treatment in combination with standard chemo- and radiotherapy make successful implementation for curative treatment more complex [20, 25-27]. In this thesis however, we did not only want to interfere with HR but also with the DSB pathway that repairs the majority of the DNA DSB breaks: NHEJ [28]. Blocking one repair pathway is thought to lead to the compensation by the other repair pathway [29, 30]. Therefore, inhibiting both at the same time could lead to a more complete, and more pronounced radio-sensitization. In chapter 3, the combination of hyperthermia and the DNA-PKcs inhibitor NU7441 – to block HR [31] and NHEJ [32] respectively – before radiation treatment is exploited to destroy cancer cells more efficiently. Our results show that either one of the treatments already enhances the effect
of radiation, but the combination of both increased radio-sensitivity tremendously. Not only the ‘normal’ cervical- and breast cancer cells are sensitized, but also the assumed radio-resistant cancer stem cells are affected by the triple combination treatment. Hyperthermia is already routinely applied in combination with radiotherapy in some medical institutes, and the addition of DNA-PKcs inhibition might support further use of both in the clinic.

As cancer cells and cancer stem cells have been shown to have an increased DNA damage response activity [33, 34] and that this increased activity contributes to the promotion of cancer and therapy resistance, the rationale to block the DNA damage response is comprehensible. Blocking DNA repair will enhance the effect of treatments that induce DNA damage, resulting in an increase of cell death and hence tumor control. Unfortunately, most targeted therapies are not tumor specific and normal healthy cells will be affected during treatment as well [35]. Inhibition of DNA repair processes in normal cells could result in the induction of translocations, mutations leading to unacceptable normal tissue toxicity or even contribute to the development of new malignancies [11]. At this moment, no significant increase in normal tissue complications after hyperthermia treatment are recorded [36], but further studies are necessary as large randomized clinical trials are still lacking. In addition, the effect of DNA-PKcs inhibition on normal tissue toxicity needs to be elucidated.

The precise implementation of DNA repair-targeting drugs must be carefully investigated and optimized for each specific tumor type. Depending on the molecular features of the tumor, the best therapeutic strategy should be determined exploiting synthetic sensitivity or lethality [37]. Targeting the repair pathway that is crucial for the tumor, but less active in normal cells, might lead to a more selective and less toxic treatment strategy. Furthermore, specific combinations of these repair-targeting drugs might be able to induce a synthetic lethal feature in a tumor that would otherwise not be sensitive. For example hyperthermia and PARP1 inhibitors: hyperthermia degrades BRCA2, introducing (temporarily) homologous recombination deficient cancer cells that therefore become sensitive for PARP1 inhibitors. This could augment further use of PARP1 inhibitors in tumors with the specific BRCA1/BRCA2 mutation.

Use of biomarkers for individualized radiation treatment.

The molecular events of the DNA damage response can be studied by several DNA damage markers. Phosphorylated γ-H2AX is the most widely used marker of DNA-damage and the number of γ-H2AX foci per nucleus closely correlates with the number of radiation induced DSBs [9, 38-41]. Although the γ-H2AX foci assay is very sensitive, its potential to assess the efficacy of DNA DSB repair is still a matter of debate [40, 42]. Since dephosphorylation of γ-H2AX is thought to occur more gradually rather than sudden, the detection of γ-H2AX foci a few hours post radiation treatment might not necessarily present an actual, unrepaired DSB [43]. Furthermore, several studies suggest that residual foci could also mark changes in chromatin structure after
repaired or misrepaired DSBs or chromosomal translocations [44, 45]. In Chapter 4, we explored the use of γ-H2AX as determinant for DNA DSB repair and concluded that γ-H2AX foci numbers could determine the DNA-repair capacity of different cell types [46]. Monitoring the induction and disappearance of γ-H2AX foci after ionizing radiation elucidated large differences in foci decay ratios between repair proficient and repair deficient cells. Therefore, the decay ratios (i.e. the initial number of foci divided by the residual number of foci) can be a useful biomarker to predict the radio-sensitivity of tumors. Furthermore, we found that, apart from tumor sensitivity, γ-H2AX foci decay ratios were also able to measure the sensitivity of normal cells to radiation treatment (chapter 4, 5 and 6). Significant differences in decay ratios were observed between patients with and without late normal tissue toxicity after radiotherapy. Knowledge of the sensitivity of tumors and normal tissues to ionizing radiation before start of treatment could contribute to optimization of individual dose- and treatment planning. The possible identification of patients who are susceptible to develop severe late side effects is further discussed in chapters 5 and 6, part II of this thesis.

PART II: RADIATION RESPONSE IN NORMAL TISSUES

Part II of this thesis describes the radiation response in normal tissues and the development of severe late side effects, illustrated by the correlation between cellular responses to ionizing radiation of normal lymphocytes and patient’ toxicity status. Late radiation toxicity is the limiting factor for dose escalation in the radiation treatment and can significantly affect the quality of life of patients. Moreover, technical improvements in cancer treatment over the last decades [7, 47] have led to increased local control rates and a better overall survival of cancer patients [48]. The resulting increase in life-span, endorses the need of identifying clinical and genetic risk factors to reliable predict for late radiation toxicity. Approximately 5 to 10% of irradiated patients will develop severe late complications (grade≥3) and another 10% will develop moderately severe complications (grade 2). Taken together, up to 20% of these patients develop moderate to severe late side effects. Clinical factors like age, diabetes, comorbidities or radiation dose and – volume can only partly explain the varying incidence and severity of normal tissue toxicity [49]. Therefore, it was hypothesized that there might be a genetic predisposition for late radiation toxicity. Genome wide, the role of several genetic variations and gene expression levels in the development of late radiation toxicity have been investigated [50-59]. However, overall results are conflicting [60] and no reproducible or reliable prognostic markers [61-63] associated with late radiation toxicity have been identified. In this thesis, the efficacy of the DNA damage response is correlated to the development of normal tissue toxicity.
Altered DNA damage response in severe late radiation toxicity.

First of all, we retrospectively investigated gene expression profiles and DNA DSB repair capacity in *ex vivo* irradiated lymphocytes of prostate cancer patients (chapter 5) [64]. The radiation response of lymphocytes is shown to reflect patient’s normal tissue reaction after radiotherapy [65-67] and cellular sensitivity to ionizing radiation was assessed with the γ-H2AX assay and gene expression profiling. Significant reduced activity of DSB repair genes and lower foci decay ratios were found in patients with (over-responding, OR) severe late radiation toxicity compared to those without late toxicity (non-responding, NR). Results of both assays indicate a less efficient repair of DNA DSBs in OR patients. In chapter 6, these initial observations were extended in a prospective study of 200 prostate cancer patients. Interestingly, we were able to confirm the strong association between late radiation toxicity and the DNA double strand break repair efficiency. After similar *ex vivo* irradiation of normal lymphocytes, the value of foci decay ratios declined with increasing toxicity grade. This is consistent with our earlier finding that an impaired DNA DSB repair contributes to the development of late radiation toxicity. Furthermore, gene expression analysis designated genes of the homologous recombination pathway to be responsible for the less efficient DSB repair in patients with late radiation toxicity. More specifically, HR repair genes were overall less induced in patient with severe late radiation toxicities compared to patient without complications. No correlations were found between toxicity grade and induction levels of the NHEJ repair genes. A threshold γ-H2AX decay ratio determined from the retrospective study, could correctly classify 82% of the patients with severe radiation toxicity in the prospective study. Unfortunately, there is a large overlap with patients experiencing milder toxicities. Therefore, the γ-H2AX foci decay assay needs to be further optimized and validated in other studies before possible clinical use can be warranted.

In this thesis, the development of normal tissue damage was only assessed for prostate cancer patients. As we use patient lymphocytes to examine their normal tissue response, results obtained in the prospective study might also be indicative for the development of normal tissue damage in other types of cancer. Although a recent study showed that genetic variants associated with overall toxicity in breast cancer patients, were not associated with overall toxicity in prostate cancer patients [56], several studies have correlated levels of residual DNA DSB with increased risk for late normal tissue toxicity in other types of cancer [68-70]. The underlying causes to explain this altered repair capacity remain however unclear. Differences in repair efficiency might be caused by a combination of polymorphisms in DNA damage responsive genes or in their regulation, influencing expression of multiple connected genes. Furthermore, it has been shown that the variation in the baseline expression level of many genes has a heritable component [71], indicating that gene expression differences and hence pathway activity can to some extent be regarded as a hereditary trait.
Clinical implications of late toxicity prediction.

Identification of patients at high risk to develop severe normal tissue damage prior to irradiation exposure is important for patient-tailored therapy. The lack of reproducibility between studies that aim to find predictive markers for late radiation toxicity could be due to the differently used toxicity grading systems. Outcomes between scoring systems (e.g. EORTC, RTOG, CTCAE, LENT-SOMA) can vary considerably, making meaningful comparison between different studies and results more difficult. No standardized implementation of scoring systems exists and interpretation of symptoms might vary among clinicians or institutes. Furthermore, some studies use patient self-administered questionnaires to assess toxicity status [72]. It has been reported that patients can efficiently report toxicity using patient-reported outcomes [73, 74], but the results seem to be more subjective compared to clinicians and reflect daily health status [75]. The majority of currently reported clinical trials assess toxicity signs and symptoms according to the CTCAE system. Nevertheless, the studies discussed in chapter 5 and 6 are important for the possible identification of patients who are likely to have a genetic predisposition to develop late radiation toxicity. Especially the possible use of γ-H2AX foci decay ratios could contribute to improved patient-tailored treatment planning.

Markers with high predictive power can lead to improved therapy and better patient outcome, as well as an increased quality of life after radiation treatment. Patients at high risk to develop severe side-effects may receive a different treatment such as surgery (prostatectomy) or brachytherapy. Brachytherapy is a form of internal radiation treatment that places radioactive material in close vicinity of the tumor, making it a very local technique with minimal radiation damage in surrounding tissues. Unfortunately, brachytherapy is limited to small, slow growing tumors as the radiation source can only reach a small area. In combination with hyperthermia however, patients with low or high grade tumors could be alternatively treated with lower radiation dose while maintaining clinical effectiveness [36, 76]. The results obtained in our prospective study suggest a less active homologous recombination repair to be responsible for the development of late radiation toxicity. Therefore, targeting this pathway with hyperthermia might not have an extra effect in normal cells but it does sensitize the response to radiation in cancer cells (chapter 3). On the other hand, patients who are less susceptible to radiation toxicity might be treated with higher doses, leading to higher cure rates. However, care must be taken with correct classification of the patients and possible change of treatment plan. Although for patients without toxicity a different treatment regime will not be a disadvantage, if it results in similar outcome, patients who are more prone to develop late toxicities should definitely not be irradiated to higher doses.
CONCLUSIONS

All studies presented in this thesis were designed to investigate mechanisms involved in the sensitivity to radiation treatment, either in tumors or in normal tissues. Evidently with the ultimate aim to improve radiation treatment. The indispensable role of effective DNA DSB repair in diminishing the adverse effects of radiation treatment is highlighted in this thesis. To date, we used DNA repair markers to assess tumor response, targeted DNA repair pathways to sensitize cells to radiation treatment and we assessed DNA repair activity to predict the development of normal tissue toxicity.

Blocking DSB repair pathways with for instance hyperthermia or DNA-PKcs inhibition has been shown to contribute to improved tumor control by killing cancer cells more effectively. Because cancer cells and cancer stem cells are thought to possess a highly active, though aberrant, DNA damage response, blocking the involved pathways with molecular-targeted therapies could significantly contribute to reduced treatment resistance and hopefully spare normal cells to some extent. Furthermore, there is supportive evidence of a genetic predisposition for the risk of developing late radiation toxicity. Results obtained in this thesis suggest that late complications are caused by altered, less efficient DNA damage repair. Therefore, expression levels of DNA repair genes and functional DNA damage markers may be useful to better predict late complications. Although conflicting results among studies remain present, differences in individual expression profiles, mutational status and DNA repair capacity are worthwhile topics for further investigation. Exploring the DNA damage response to elucidate possible targets or mechanisms which underlie to differences in radiation response are warranted. Overall, for successful improvement of currently applied radiation treatment, we stress that both tumor control and normal tissue toxicity should be taken into account.

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