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Mini-review

Targeting the heat shock response in combination with radiotherapy: Sensitizing cancer cells to irradiation-induced cell death and heating up their immunogenicity

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ABSTRACT

Radiotherapy represents an essential treatment option for the majority of cancer patients in different stages of their disease. Physical achievements of the recent years led to the implementation of high precision treatment planning procedures, and image-guided dose delivery is current state of the art. Yet, radiotherapy still faces several limitations with cancer intrinsic radioresistance being a key driver of therapeutic failure. Accordingly, the mechanisms orchestrating radioresistance and their therapeutic targeting by combined modality approaches are in the center of attention of numerous radiation oncologists. In the present review, we summarize and discuss therapeutic approaches that exploit the heat shock response, either by hyperthermia or by pharmacological heat shock protein inhibition, in combination with radiotherapy. These strategies appear particularly promising, since they sensitize cancer cells to irradiation-induced cell death and at the same time have proven the potential to promote systemic anti-tumor immune mechanisms, which may target not only locally surviving tumor cells, but also distant out-of-field metastases.

Radiotherapy: current use and limitations

Radiotherapy (RT), alongside surgery and chemotherapy, is one of the cornerstones of cancer treatment. It has been used in cancer therapy for almost a century, and today more than half of all cancer patients receive RT at one point during their treatment. RT is employed in different settings, including adjuvant, neoadjuvant, definitive, and palliative ones, highlighting its central role in all stages of cancer treatment.

For most treatment regimes, the total irradiation dose is administered in week-daily fractions of 1.8–2 Gy up to a cumulative dose of 45–70 Gy. The rationale underlying this fractionation strategy is the observation that tumor cells are commonly less capable to repair irradiation-induced damage as compared to normal tissue cells. Furthermore, tumor cells can redistribute to more radiosensitive phases of the cell cycle between two fractions, and re-oxygenation of formerly hypoxic regions of the tumor can occur, thus rendering them

Abbreviations: 17-AAG, 17-(Allylamino)-17-demethoxygeldanamycin; *17-DMAG*, 17-Dimethylaminoethylamino-17-demethoxygeldanamycin; *AKT*, v-akt murine thymoma viral oncogene homolog; *APC*, antigen presenting cell; *ATM*, Ataxia telangiectasia mutated; *ATR*, Ataxia telangiectasia and RAD3 related; *BAX*, BCL-2 associated X protein; *BCL-2*, B cell lymphoma 2; *BRCA1/2*, breast cancer 1/2; *CDK*, cyclin-dependent kinase; *CDKI*, cyclin-dependent kinase inhibitor; cGAS, cyclic GMP-AMP synthase; *CTLA-4*, cytotoxic T lymphocyte-associated protein 4; *DAMP*, damage-associated molecular pattern; *DC*, dendritic cell; *DDR*, DNA damage response; *DNA-PKcs*, DNA-dependent protein kinase catalytic subunit; *DSB*, double strand break; *EGF-R*, epidermal growth factor receptor; *EPHA2*, ephrin receptor tyrosine kinase A2; *ERBB1/2/3*, v-erb-b2 erythroblastic leukemia viral oncogene homolog 1/2/3; *FLIP*, FLICE-inhibitory protein; *GM-CSF*, granulocyte macrophage colony stimulating factor; *GRP94*, Glucose-related protein 94; *HER2/NEU*, human epidermal growth factor receptor 2; *HIF-1*, hypoxia inducible factor 1; *HLA-DR*, human leukocyte antigen DR; *HMGB-1*, high mobility group box 1 protein; *HRR*, homologous recombination repair; *HSF*, heat shock factor; *HSP*, heat shock protein; *HT*, hyperthermia; *hTERT*, human telomerase reverse transcriptase; *IAP*, inhibitor of apoptosis protein; *ICAM-1*, intercellular adhesion molecule 1; *IFN*, interferon; *IGRT*, image-guided radiation therapy; *IL*, interleukin; *IMRT*, intensity-modulated radiation therapy; *IORT*, intraoperative radiation therapy; *IR*, ionizing radiation; *LCK*, lymphocyte specific protein tyrosine kinase; *LHT*, local hyperthermia; *MAPK*, mitogen-activated protein kinase; *MDSC*, myeloid-derived suppressor cell; *MHC*, major histocompatibility complex; *MIC-A/B*, MHC class I-related chain A/B; *MNHT*, magnetic nanoparticle-mediated hyperthermia; *MRN*, MRE11-RAD50-NBS1; *MSU*, monosodium urate; *NHEJ*, non-homologous end joining; *NK cell*, natural killer cell; *NKG2D*, natural killer group 2 member D; *nu/nu mouse*, athymic nude mouse; *PARP*, poly-ADP ribose polymerase; *PD-1*, programmed cell death protein 1; *PD-L1*, programmed cell death ligand 1; *PGE_2*, prostaglandin E_2; *PS*, phosphatidylserine; *PI3-kinase*, phosphatidylinositol-3-kinase; *PUMA*, p53-upregulated modulator of apoptosis; *RAF kinase*, rapidly accelerated fibrosarcoma kinase; *RHT*, regional hyperthermia; *RPA*, replication protein A; *RT*, radiotherapy; *SCID mouse*, severe combined immunodeficiency mouse; *ssDNA*, single-strand DNA; *STING*, stimulator of interferon genes; *STK38*, serine/threonine kinase 38; *TGF-β*, transforming growth factor β; *TLR*, toll-like receptor; *TNF*, tumor necrosis factor; *TRAP1*, TNF receptor-a35ssociated protein 1; *Treg*, regulatory T cell; *UA*, uric acid; *VCAM-1*, vascular cell adhesion protein 1; *VEGF*, vascular endothelial growth factor; *WBHT*, whole body hyperthermia; *γH2AX*, phosphorylated histone H2AX.

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more susceptible to the next fraction. On the other hand, repopulation phenomena – particularly in case of fast proliferating, highly aggressive tumors, such as squamous cell carcinomas of the head and neck region, or sarcomas – can counteract the fractionation effect [1]. Considering that not all cancer entities exhibit truly compromised repair capacity often accompanied by rapid repopulation, strategies to reduce the overall treatment period are emerging. In this regard, hypofractionation regimes, in which the total dose is split into fewer fractions with higher doses, are becoming part of clinical practice. In the UK and Canada for instance, breast cancer patients are irradiated in 16 fractions with doses of 2.66 Gy per fraction [2]. Additionally, for specific tumor entities, ablative irradiation is employed, i.e. one to five high doses of 8–25 Gy. Intraoperative radiotherapy (IORT) of breast cancer or stereotactic irradiation of lung and liver metastases are examples of these ablative treatment regimes [3,4]. Furthermore, brachytherapy complements the spectrum of radiotherapeutic techniques. Here, radioactive γ-emitters are inserted into natural body cavities or interstitial catheters that have been implanted during tumor debulking surgery. This type of radiotherapy is often used for the treatment of cervical cancer [5].

Physical achievements of the recent years have led to significant improvements in terms of treatment planning procedures and accuracy of dose delivery. Modern radiotherapeutic techniques, such as intensity-modulated radiation therapy (IMRT) and image-guided radiation therapy (IGRT) employing integrated computed tomography, ultrasound, or surface scanners, enable the design and administration of target volumes that reflect the clinical tumor volume with a high degree of preciseness while sparing the organs at risk [6]. The implementation of other radiation qualities, including protons and heavy ions, whose physical properties allow for very steep dose gradients, appears to take treatment planning and dose delivery to an even higher level of accuracy. Nevertheless, further in-depth radiobiological validation and long-term clinical follow-up studies are required to identify those cancer entities, whose treatment would benefit most strongly from proton and heavy ion radiotherapy. Besides, the high costs of the required instrumentation compared to photon radiotherapy hamper the ubiquitous availability in the clinical routine [6].

Despite its central role in cancer treatment and the tremendous advances of the recent years, radiotherapy still faces several limitations. Intrinsic radioresistance of different cancer cells is one of them. In this regard, dose escalation studies have revealed that the radiotherapeutic efficacy cannot be increased by higher overall doses beyond a certain point due to accumulating side effects [7]. These can be acute as well as chronic. The occurrence of side effects is closely related to the irradiated body region and the position of organs at risk in proximity to the irradiated volume. For example, irradiation of cancers of the head and neck region can cause mucositis and sialadenitis. Besides, general side effects are also described, including fatigue or anorexia. Overall, the occurrence and severity of side effects are critically dependent on the total dose administered as well as biological, physiological, and individual factors.

Understanding and overcoming cancer cell radioresistance is a vividly evolving field in molecular radiation oncology and radiation biology. A plethora of data have already been collected and numerous studies are still underway, including efforts to characterize the molecular mechanisms and pathways that orchestrate radioresistance as well as their specific targeting by combined modality approaches with classical chemotherapy or molecularly designed agents. In the present mini review, we want to focus on approaches addressing the heat shock response, either by hyperthermia (HT) or by pharmacological heat shock protein inhibition. These strategies reportedly do not only sensitize cancer cells to irradiation-induced cell death, but also have proven the potential to promote systemic anti-tumor immune mechanisms. The latter may target locally surviving tumor cells as well as out-of field metastases.

Molecular radiation oncology: DNA damage response, irradiation-induced cell death, and its immunological consequences

The cytotoxic consequences of ionizing radiation (IR) are predominantly caused by the induction of DNA damage as mediated by direct and indirect effects. Whereas direct effects of IR are characterized by immediate ionization of the DNA by IR itself, indirect effects involve radiolysis of water molecules and the generation of highly reactive free radicals, including hydroxyl and hydrogen radicals that damage the DNA secondarily. In the presence of oxygen, hydrogen radicals can further form peroxide radicals and hydrogen peroxide, which contribute to indirect DNA damage. Additionally, oxygen can interfere with repair processes by adding to and thereby stabilizing DNA radicals. That is why highly perfused and oxygenated tumor areas are more prone to IR-induced DNA damage than hypoxic areas [8]. The relative effectiveness of IR under normoxic conditions is two to three times higher than under hypoxic conditions – a phenomenon known as the oxygen effect [9].

DNA damage response

Irradiation-induced DNA damage stimulates the activation of a network of DNA repair pathways, the so-called DNA damage response (DDR). The DDR is essential for the maintenance of genomic integrity, resulting in cell survival and successful transmission of genetic information to daughter cells. It comprises a complex interplay of signaling pathways, and since inheritance of DNA lesions to progeny must be prevented, activation of DDR also transiently arrests the cell cycle at its transition from G1- to S-phase, during S-phase, or at the G2/M-boundary, respectively [10–12]. This is realized by cyclin-dependent kinase inhibitors (CDKIs), which are upregulated upon irradiation and inhibit the activity of cyclin-dependent kinases that control cell cycle progression and transit into the next cell cycle phase [13]. The cell cycle phase at the time point of irradiation has an essential impact on the efficacy of IR: Cells in mitosis are most sensitive to IR, because here the cell cycle can only be arrested for a short time and different DDR mechanisms are inactivated in order to prevent telomere fusion [14]. In contrast, cells within the S-Phase are rather radioresistant, since multiple DDR pathways are pre-activated due to their requirement during DNA replication, and sister chromatids as well as the enzymatic repertoire for homologous recombination repair (HRR) are available [15].

The most severe DNA damage events induced by IR are DNA double strand breaks (DSBs), which may result in chromosomal aberrations if left unrepaired. A dose of 1 Gy causes approximately 40–60 DSBs per cell [16]. DSBs can be processed by two different repair pathways: Homologous recombination repair (HRR) or non-homologous end joining (NHEJ) [12]. As HRR relies on intact homologous DNA sequences from the sister chromatid in order to remove the DNA damage, it is utilized only during G2- and S-phase of the cell cycle. HRR reveals a high degree of fidelity and is considered as an essentially error-free DNA damage repair mechanism. In contrast, NHEJ is highly error-prone. The ends of broken DNA sequences are brought into close proximity, trimmed, and finally ligated [10,12,15]. NHEJ does not depend on homologous sequences as template for DNA damage repair, and thus can occur throughout the cell cycle.

On a molecular level, the activation of the DDR is orchestrated by diverse DNA damage sensing factors and a cascade of protein kinases. The DSB recognizing MRE11–RAD50–NBS1 (MRN) complex recruits and activates the protein kinase Ataxia telangiectasia mutated (ATM), which subsequently phosphorylates histone H2AX

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