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Mini-review

Molecular determinants of radiosensitivity in normal and tumor tissue: A bioinformatic approach

Athanasia Pavlopoulou [a, **], Pantelis G. Bagos [b], Vasiliki Koutsandrea [c], Alexandros G. Georgakilas [c, *]

[a] Izmir International Biomedicine and Genome Institute (iBG-Izmir), Dokuz Eylül University, 35340, Turkey
[b] Department of Computer Science and Biomedical Informatics, University of Thessaly, Papasiopoulou 2-4, Lamia, 35100, Greece
[c] Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens (NTUA), Iroon Polytechniou 9, Zografou, 15780, Athens, Greece

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ABSTRACT

Although radiation therapy is a treatment of choice for cancer, a high percentage of patients develop adverse effects in normal tissue following radiotherapy, mainly, due to genetic factors. Notably, although it is established that a lower dose of ionizing radiation can minimize the tumor cell population in radiosensitive cancer patients, the sensitivity of tumor cells to radiation has not gained enough attention. In this mini-review, the molecular pathways/mechanisms and the related molecules involved in clinically relevant radiotoxicity, as well as normal and tumor cell radiosensitivity, were investigated for various types of cancers employing bioinformatics approaches. A total of 255 genes/gene products were retrieved and investigated in this study, which are implicated in pathways related mainly to DNA damage repair, oxidative stress, apoptosis and fibrosis. Furthermore, a novel molecular gene signature of normal tissue radiotoxicity was identified. The findings of our study could be utilized by healthcare professionals in personalized clinical decision-making, in order to efficiently sensitize tumor cells to radiation and yet minimize adverse effects in the adjacent normal tissues as well as to improve the quality of life in cancer patients undergoing radiotherapy.

Introduction

Radiotherapy (RT) represents a major modality in the treatment of cancer, either alone or combined with other treatment regimens, such as chemotherapy. It is estimated that approximately half of all cancer patients worldwide have undergone radiotherapy during their illness [1,2]. The optimal goal of radiotherapy is to enhance tumor specificity and radiation response, decreasing at the same time the toxic effects of radiation exposure manifested usually in the surrounding normal cells and tissue [3]. Nevertheless, as with all cancer treatment modalities, patients receiving RT may experience several adverse treatment effects (radiation injury) in the adjacent or distant normal tissues. Acute or early normal tissue toxicity usually occurs within weeks after the treatment. Late normal tissue toxicity develops within three months or several years after radiotherapy [4]. Consequently, the risk for radiation-induced normal tissue toxicity is the dose-limiting factor in radiotherapy protocols [5]. Lately, the role of systemic or 'out-of-field' effects has gained a lot of attention. For example, it was recently shown that both localized thoracic radiotherapy and chemo-radiotherapy can induce significant systemic DNA damage in normal tissues [6]. Therefore, it is suggested that individual assessment of an organism's response to IR during radiotherapy may enable optimized personalization of therapy [6].

There is a large variation (more than 80%) in normal tissue radiosensitivity, both in incidence and severity, among patients

Abbreviations: BER, base excision repair; DSB, DNA double-strand breaks; DSBR, DNA double-strand break repair; ECM, extracellular matrix; HR, homologous recombination; IR, ionizing radiation; MMR, DNA mismatch repair; NHEJ, non-homologous end-joining; QOL, quality of life; RT, radiation therapy; ROS, reactive oxygen species; SSBs, DNA single-strand breaks.

* Corresponding author. DNA Damage Laboratory, Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens (NTUA), Iroon Polytechniou 9, Zografou, 15780, Athens, Greece.
** Corresponding author. Izmir International Biomedicine and Genome Institute (iBG-Izmir), Dokuz Eylül University, 35340, Turkey.
E-mail addresses: athanasia@compgen.org (A. Pavlopoulou), alexg@mail.ntua.gr (A.G. Georgakilas).

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administered identical radiation treatment. This interpatient variability is largely due to patients' genetic background rather than stochastic factors [7,8]. The first evidence for a genetic basis for radiation-induced side effects was based on the observation that patients with specific rare genetic syndromes, such as ataxia telangiectasia, Nijmegen breakage syndrome and DNA ligase IV deficiency, exhibited extreme hypersensitivity to IR [9]. Nevertheless, these high-penetrance rare mutations are not overrepresented among patients with extreme normal tissue radiosensitivity, leading to the suggestion that low-penetrance common genetic variants, as well as multiple genetic pathways, may account for individual normal tissue radiotoxicity [5,10]. In general, DNA repair efficiency is currently regarded as one of the most critical determining factors of radiosensitivity [11,12], since IRs (from X-rays to high-LET protons and carbons) used in RT induce highly complex DNA damage [13], thereby inducing different DNA repair pathways and especially DNA double-strand break repair (DSBR) [14]. The role(s) of inflammatory and immune response interconnected with DNA damage response cannot be disregarded, especially when it comes to RT adverse effects [15].

Of note, radiotherapy of radiosensitive cancer patients may also lead to the eradication of tumor cells using a lower dose of radiation. This is important in enabling personalized therapy, that is, the decision-making process for choosing the optimum radiation dose for treating individual cancer patients, thereby improving the therapeutic potential of radiation [3,5].

In the present review, we have investigated the genetic risk factors for radiation-induced toxicity in normal tissues and cells, as well as the genetic pathways/mechanisms underlying cancer radiosensitivity. Therefore, assessing the genetic factors and the molecular pathways associated with radiosensitivity of both normal and cancer cells and tissues would be essential in order to minimize normal tissue toxicity and yet prevent tumor recurrence. Moreover, these results could be utilized for a pre-treatment genetic testing towards the improvement of the overall quality of life (QOL) in cancer patients [16] following radiotherapy, as well as the prevention of early discontinuation or interruption of radiotherapy due to adverse effects [17].

Methodological issues

A bibliographic search was performed in order to retrieve gene/gene products related to clinical and cellular radiosensitivity. To this end, extensive manual searches were conducted on the biomedical bibliographic database PubMed [18] by using a combination of keywords: ‘radiosensitivity’, ‘radiation therapy sensitivity’, ‘normal tissue’, ‘cancer cells’, ‘tumor cells’, ‘radiation toxicity’, ‘radiation late effects’, ‘radiation adverse reactions’, ‘radiation adverse effects’, ‘radiation toxic effects’ and ‘normal tissue injury’. A total of 255 genes/gene products were retrieved and investigated in this study. The official HGNC [19] gene symbols were used (Table S1). In addition, the different types of radiosensitivity in normal and tumor tissue, that is, normal cellular radiosensitivity, tumor cell radiosensitivity and clinically relevant radiosensitivity, are described. A signature of genes/gene products known to be associated with the adverse effects of radiation therapy was generated. Moreover, the associations, either direct or indirect, among these molecules were examined. The prominent molecular pathways in which the genes retrieved in this study participate were also investigated. Furthermore, the genes or proteins considered to be implicated both in normal and tumor tissue radiosensitivity were found and their potential application in a clinical setting is discussed (Fig. 1).

Normal and tumor tissue radiosensitivity

Normal cellular radiosensitivity

Several approaches have been employed to develop a predictive assay for normal tissue radiation-induced toxicity based on the *in vitro* radiosensitivity of various cell types. Normal skin fibroblasts are the most common cells of the connective tissue that can be grown and manipulated efficiently *in vitro*. To this end, several studies investigated whether fibroblasts from patients' skin samples can be tested for their ability to predict an individual's risk of developing radiation-induced fibrosis by measuring the fraction of surviving fibroblasts in colony survival assays [8,10,20]. In overall, a significant relationship was not found between fibroblast radiosensitivity and the risk of developing fibrosis upon exposure to radiation [8,10]. This is partially due to the fact that the response of cultured cells to radiation cannot be compared to that of whole tissues which is largely affected by the microenvironment [8].

A number of experimental studies have used apoptosis of lymphocytes to predict adverse reactions to radiotherapy. There is an inverse correlation between lymphocyte apoptosis and normal tissue toxicity which can be explained by the fact that lymphocytes undergoing slow apoptosis may promote inflammation or immune response at the irradiated site [10,21]. A significant inverse correlation was found between radiation-induced apoptosis of $CD8^+$ T-lymphocytes and the risk of late reaction in a large cohort of irradiated patients [21].

Tumor cell radiosensitivity

It is suggested that the inherent radiosensitivity of tumor cells is the principle determinant of tumor response to radiation therapy [22]. Targeted radiotherapeutic regimens aim to sensitize tumor cells to radiation and eradicate them, thereby preventing post-therapy tumor recurrence and relapse. Radiation affects tumor cells either directly, through the induction of complex DNA lesions, or indirectly, through the generation of DNA damaging reactive oxygen species (ROS) [23,24], eventually leading to cell death or senescence [25].

Nevertheless, tumor cells can often become resistant to RT. This is either due to their enhanced response to and repair of DNA damage [23,24] or evasion of radiation-induced apoptosis due to mutations in key apoptotic genes like *TP53* [26], thereby increasing significantly cancer patients' probability of post-irradiation recurrence or relapse. Of note, the presence of a subpopulation of cancer cells in solid tumors, namely cancer stem cells, which are characterized by increased resistance to radiation, renders tumors more resistant to radiation [27].

Clinically relevant radiosensitivity

The radiation-induced acute normal tissue effects tend to occur in rapidly proliferating cells such as in epithelial loci (the epithelial surfaces of the skin and the gut, hair and hematopoietic system) [28,29]. The underlying pathogenesis of acute radiotoxicity includes inflammation and oedema. Acute effects are often reversible, while late effects may be permanent [28,29]. The late adverse effects usually occur in slowly proliferating cells such as in the subcutaneous tissue, brain, liver, fatty tissue and heart. Their clinical manifestations include fibrosis, necrosis, neural and vascular damage, atrophy and development of secondary malignancies. Fibrosis is the prominent hallmark of radiotherapy's late adverse effect on normal tissue. A series of events begin immediately after irradiation leading to fibrotic changes in the connective tissues. Irradiation induces the sequential release of proinflammatory