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Mini-review

Emerging molecular networks common in ionizing radiation, immune and inflammatory responses by employing bioinformatics approaches

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ABSTRACT

Efficient radiation therapy is characterized by enhanced tumor cell killing involving the activation of the immune system (tumor immunogenicity) but at the same time minimizing chronic inflammation and radiation adverse effects in healthy tissue. The aim of this study was to identify gene products involved in immune and inflammatory responses upon exposure to ionizing radiation by using various bioinformatic tools. Ionizing radiation is known to elicit different effects at the level of cells and organism i.e. DNA Damage Response (DDR), DNA repair, apoptosis and, most importantly, systemic effects through the instigation of inflammatory 'danger' signals and innate immune response activation. Genes implicated both in radiation and immune/inflammatory responses were collected manually from the scientific literature with a combination of relevant keywords. The experimentally validated and literature-based results were inspected, and genes involved in radiation, immune and inflammatory response were pooled. This kind of analysis was performed for the first time, for both healthy and tumor tissues. In this way, a set of 24 genes common in all three different phenomena was identified. These genes were found to form a highly connected network. Useful conclusions are drawn regarding the potential application of these genes as markers of response to radiation for both healthy and tumor tissues through the modulation of immune and/or inflammatory mechanisms.

Introduction

Interaction of ionizing radiation with any living organism induces a variety of responses and topical or generalized stress in the organism. Some of these responses are but not limited to: DNA damage response (DDR), DNA repair, pro-inflammatory pathway initiation and free radical production like reactive oxygen and nitrogen species (ROS/RNS) in the irradiated area or more generalized in the whole body through systemic (non-targeted) effects [1,2]. Radiation exposure, like other types of stresses, affects the development of the immune system through radiation-induced apoptosis, differentiation and induction of an inflammatory environment [3]. Evidence from atomic bomb (A-bomb) survivors suggests long-lasting alterations of the immune system by radiation exposure like perturbation of one or more of the primary processes regulating T-cell homeostasis resulting therefore in persistent inflammation [4]. This so called 'non-resolving' inflammation is a major driver of disease and late effects including genomic instability [5]. Although multiple mechanisms attempt resolution of this status like for example macrophages switching phenotypes and other secreted molecules switch impact from pro- to anti-inflammatory, persistence of inflammatory status may occur. At the same other important components securing organism homeostasis like DNA repair seems to also be severely affected like in radiation therapy (RT) [6]. In other cases, persistent DNA damage in nucleotide excision repair (NER) deficient tissues has been shown to induce innate immune response [7] and persistent DNA damage-driven inflammation in animals resulting in tissue generation and an aging phenotype [8]. Overall, concluding evidence supports the notion that locally delivered radiation damage at clinical setup (RT doses) in most cases elicits some type of activation of the innate and adaptive immune system [9]. At the same time dose fractionation seems also to play an additional but weaker immuno-modulatory role and in many times with contradicting results especially for human patients [9]. In the case of a tumor tissue environment, responses are

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more complicated and there are several different ways in which radiation can influence 'tumor immunogenicity' as reviewed in ref. 10. Currently, the prevalent idea is that radiation exposure can only augment a pro-immunogenic phenotype and can very rarely inverse an immune suppressing environment into an immune stimulating one [10]. Usually it is accepted that relatively low doses of ionizing radiation like doses of X-rays below 1 Gy can have some anti-inflammatory activities and for higher doses (clinically relevant) a radiation-induced immune modulation and response resulting primarily from the cellular (DNA and protein) damage and various non-targeted or out-of-field abscopal effects [11,12].

Therefore inflammation is closely associated with radiation, since, during radiation treatment, depending on the area of interest, i.e. the tumor or healthy tissue is inevitably exposed to radiation and injured directly or indirectly through non-targeted effects. Inflammatory response is initiated by ionizing radiation usually very shortly after exposure with the primary function of controlling damage and repairing lesions. In various cases of radiation therapy, an early increase in different cytokines like MCP-1, IL-6 has been associated with radiation toxicity in non-small cell lung cancer (NSCLC) patient undergoing radiation therapy (RT) [13]. Adverse effects arise when inflammation sustains for a long time after the completion of radiation treatment and subsequently turns from acute response to chronic late effect(s). In this review, we discuss the current status of knowledge on the genes and proteins involved in immune and inflammatory responses after irradiation based on analytical bibliographical search using bioinformatics tools. We conclude with the identification of specific genes expressed upon radiation treatment in healthy and/or tumor tissue. We anticipate that this work, in the case of cancer tissues, will lead to the discovery of useful and reliable radiation biomarkers relating to the optimization of immune response and enhanced tumor immunogenicity. At the same time we envision, based on this work, a possible optimization of RT protocols to spare normal tissue and develop better pharmacological anti-inflammatory strategies in radiation therapeutics [14].

Employing bioinformatics to identify genes implicated in immune and inflammatory response upon ionizing radiation treatment

Among hundreds of known and unknown genes up- and down-regulated following irradiation, a key position is held by those involved in the regulation of expression of cytokines, chemokines, growth factors and cell surface receptors that alter the interaction between the tumor cells and the immune system [9,15] and often stimulate local or systemic anti-tumor effects [16]. Due to the high importance of covering as completely as possible these phenomena we have instigated ways to search all current relating literature. To this direction and in order to more accurately collect the most possible genes implicated both in radiation response and immune/inflammatory response, we performed thorough and extensive manual searches in the scientific literature. To this end, PubMed [17], Gene Ontology [18], OMIM [19], GeneCards [20] and UniProtKB [21] databases were searched with a combination of keywords first targeting different types of radiation response i.e. the word "radiation response" itself but also "radiosensitivity", "radioresistance" and the more general "ionizing radiation". In addition, we added "immune response" and "inflammatory response" in turns as a combination of key search terms with radiation response terms described above. The search results were manually curated, including rigorous literature review, and by going through each reference, we only selected the genes that were experimentally validated to participate in radiation and immune/inflammatory response and show in each case a differential expression upon irradiation of cells or tissues. The biological function of these genes was further confirmed using PANTHER [22], a comprehensive database for gene function inference based on ontology terms. In this way, we were able to identify the genes which are associated with radiation and immune or inflammatory response in healthy or cancer tissue, as shown in the complete and analytical form under the supplementary material. Specifically, in Table S1, Supplementary Data, we list all retrieved genes with increased or decreased expression in radiation and immune response in healthy tissue, while in Table S2, Supplementary Data, we list all genes implicated in radiation and immune response for cancer tissue. Next, we have included all genes relating to radiation and inflammatory response in healthy (Table S3, Supplementary Data) and cancer tissues (Table S4, Supplementary Data). For the construction of these gene sets, we used the official HGNC [23] gene symbols and gene names, and for the visualization of the overlaps between the gene sets, we constructed a Venn diagram (Fig. 1). In this case we can see that there is a gradual decrease in the number of genes associated with two or more responses. According to this diagram, 24 genes were found to participate in immune and inflammatory mechanisms as part of the radiation response, both in healthy and tumor tissues. This gene set will be henceforth referred to as "common genes".

Protein interaction network

In order to elucidate the role and mechanistic aspects of the 24 common genes, and in particular to discover known or predicted, direct (physical) or indirect (functional) associations among their gene products, this gene set was used as input in STRING v9.1 [24]. STRING is a database of experimental and predicted protein interactions, the entries of which are derived from different sources such as genomic context, high-throughput experiments, co-expression, biomedical text mining, etc. As shown in Fig. 2, a highly interconnected protein interaction network is formed, suggesting a possible mechanism of functional association among the 24 common genes or gene products in immune/inflammatory and radiation response. The expression patterns for all of these genes based on available experimental data are shown in Table 1.

Functional term enrichment analysis

Over-represented WikiPathways [55] in the 243 (150 + 27 + 66) and 131 (68 + 4 + 59) genes that correspond to exclusively healthy and cancer tissues, respectively (Fig. 1), were identified using WebGestalt [56] (the threshold for FDR adjusted p-value was set at 10^{-5}) and a Venn diagram was generated based on these findings (Fig. S1, Supplementary Data). By a first look, one can see that for cancer tissue, there are much less 'unique' pathways (only five), while for healthy tissue a much higher number was found. Among these pathways for healthy tissue, we shall underline for example: oxidative stress, miRNA regulation of DNA damage response, hypertrophy of the heart, apoptosis modulation by HSP70, and different sets of interleukins (IL-2, IL-3, IL-5, IL-7, and IL-9). For the 37 common pathways, the TGF-β signaling pathway holds according to our opinion an important role. For example, TGFB1 has been found to be up-regulated in response to radiation quite differentially between cancer and normal cells and plays a role in the control of tumor growth in the case of radiation-induced carcinogenesis [57].

Enriched human cytogenetic bands in the set of 458 genes that corresponds to the union of the gene sets listed in Tables S1–4, Supplementary Data, were found using WebGestalt [56] (Table 2). This analysis was carried out to identify potential cytogenetic regions which would be particularly active in immune or inflammatory response upon radiation. The three statistically significant cytobands may have been emerged due to the fact that they contain one or two gene clusters i.e. neighboring genes that are produced by successive gene duplication events. Such genes are highly homologous

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