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How tissues respond to damage at the cellular level: orchestration by transforming growth factor-β (TGF-β)

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Abstract. When the human body is exposed to high doses of radiation, a complex, rapidly evolving, deleterious biological response is initiated that may culminate in multi-organ failure (MOF). Although this process begins with energy deposits in cellular targets, it is propagated and amplified by the tissue response to cell damage. I will argue that if the biology of wound healing is at the root of MOF following surgical trauma, and inflammation is the basis for MOF in sepsis, then the biology of the irradiated tissue uniquely initiates radiogenic MOF. The present review summarises data suggesting that tissue response to radiation damage is initiated and co-ordinated by extracellular signalling. In particular, research from the author's laboratory demonstrates that transforming growth factor-β1 orchestrates the biology of irradiated tissue via a novel function as a tissue level sensor of oxidative stress, and is integral to the cellular DNA damage response. Thus, the means to therapeutically control radiogenic MOF lies in the mechanisms by which tissues respond to global cellular damage.

How do tissues respond to damage at the cellular level?

One of the most exciting aspects of modern biology is the technology that permits intricate manipulations and observation of cells. Expansion of the knowledge space has recently grown from genetic sequence to molecular composition to integrated signalling pathways. The irradiated cell has yielded particularly critical information about the cell cycle, DNA damage, DNA repair and cell fate determination that broadly impact every aspect of cell biology. However, after a century of study, the pathology that ensues from high dose radiation exposure is not well understood. The current challenge is to integrate fundamental knowledge of cellular response into the prediction and ultimately, the manipulation of the pathological response to significant radiation damage. This challenge is further complicated by multiple cell types within each organ, and cellular heterogeneity within a given cell type. Thus, it is as yet unknown how the organism balances cell and tissue response to restore homeostasis following ionising radiation (IR) exposures.

IR is a unique stress, particularly in the case under consideration in the proceedings of this Workshop, namely of humans exposed to uniform, or nearly uniform, doses of more than 1 Gy (Sv). As detailed elsewhere in these Proceedings, high dose radiation exposure of the human body elicits a complex, rapidly evolving and deleterious biological response that can, even with heroic medical support, culminate in multi-organ failure (MOF). Given that the biology of wound healing is at the root of MOF following surgical trauma, and inflammation is the basis for MOF in sepsis, does the biology of the irradiated tissue uniquely initiate radiation MOF?

Unlike wounding, which even in severe trauma is localised, or inflammation, which often begins gradually and expands, acute, external source, sparsely ionising IR exposure deposits energy in all cells, causing essentially immediate and ubiquitous damage to macromolecules. DNA damage, lipid peroxidation and protein oxidation result directly from energy and indirectly from secondary reactive oxygen species (ROS) generated from electron interactions with water [1]. The cellular damage response program is initiated within minutes via a network of protein sensors that rapidly induce an altered state of the cell, preparing it to repair and live, or to die.

However, at the level of the organism, the fate of an individual cell is inconsequential, whilst the maintenance of tissue functions is essential. Thus, multicellular organisms have evolved additional supracellular responses to damage. Indeed, those tissues that are classically considered most sensitive to radiation, *i.e.* bone marrow, the gastrointestinal tract and central nervous system, are critical for acute survival. To address the dichotomy between mechanisms leading to cell versus organism survival, we have postulated the existence of an orchestrated program of damage response in tissues that is directed towards limiting damage and restoring homeostasis [2]. Understanding how and why such programs operate following low doses of IR is likely to provide the best avenue for predicting how the program is corrupted by high doses or in susceptible individuals.

The cell biology of irradiated tissue

The potential contributions from basic research using model organisms under controlled conditions to the medical management of accidental radiation exposures is still far from clinical reality in most cases. Yet some research, such as understanding which radiation-induced cytokines support re-establishment of homeostasis in the bone marrow, is in use (see review in these Proceedings). What features of the irradiated tissue have long-term consequences? And at what doses? How do stem cells respond, and is their response intrinsic or a function of the irradiated microenvironment? Is there a characteristic phenotype inherited by progeny of irradiated cells, and does it contribute to tissue dysfunction?

Answers to such questions will eventually lead to questions asking how the effects of whole body radiation

exposures can be mitigated. Experimental studies of mice with different genetic backgrounds or expressing modified genes suggest that the response to a significant radiation dose total body is not an inexorable process. Gudkov and Komarova [3] proposed that the p53 DNA damage sensor system which dictates cell fate is also a mediator of organismal response, albeit by different mechanisms. In the first case, the cell fate decisions are a direct consequence of the activation of p53 molecular functions, whilst in the second case tissue responses to p53-mediated cell fate decisions indirectly influence the capacity of the haematopoietic system to recover.

Will blocking one step in the DNA damage response be sufficient to stop the evolution of MOF? Acute radiation-induced lethality due to haematopoietic damage is decreased in mice by knocking out Tp53 [4] or by using a small molecule inhibitor to block p53-dependent apoptosis [5]. Clearly, once initiated MOF is multi-factorial, with contributions from inflammation, immune responses, bone marrow failure and vascular defects, among others. But what initiates the collapse? Not DNA damage *per se*, since inhibition of p53, for example, does not alter the initial radiation damage, yet mice survive nearly twice the LD_{50} dose [5]. Indeed, the same could be said of all types of immediate damage that occur from energy deposition in cells. Rather than the initial cellular damage, or even its lack of repair, the root cause of catastrophic systemic failure is the response of tissues to damaged cells.

Tissue responses to ionising radiation

Several studies support the existence of a rapid, programmed response to radiation that may be initiated at the cellular level but is carried out at the tissue level. Although the organisation of multicellular organisms into purpose-specific tissue is achieved through differential expression of the genome, cells receive information about how to behave from signals that are conducted through the tissue microenvironment, which consists of other cells, insoluble extracellular matrix proteins, soluble hormones and cytokines [2]. In response to damage, the flow of information both locally between cells and tissues, and distantly between organs, is mediated in large part by cytokines [6]. Tissue pathology and organ failure can arise from the lack of orchestrated communication between cells and among different cell types. IR damages individual cells, thus one can argue that radiation response is the sum of individual cell responses, such as cell death. However, recent data support the view that tissues respond to radiation in a co-ordinated and multifactorial fashion, and that radiation exposure ultimately compromises tissue integrity by altering the flow of information among cells [7]. As in cells, tissue responses to IR depend on dose, dose rate, radiation quality and context (*e.g.* genotype, age, pre-existing conditions). We have identified several general features in recent studies in mouse mammary gland and skin (Table 1). Thus, tissue response to radiation is a composite of the results of genetic damage, cell loss and induced gene products. An integrated view of the varied and complex cellular processes governing tissue response to radiation exposure would provide new insight into the basis of radiation susceptibility and new targets for effective intervention.

Transforming growth factor-β

One of the consequences of this integrated multicellular response is rapid activation of the cytokine transforming growth factor-β1 (TGF-β). TGF-β was isolated on the basis of its ability to stimulate anchorage-independent growth in rodent fibroblasts [8], but it has since been shown to be a potent modulator of cellular phenotype depending on cell type, concentration and context [6, 9, 10]. TGF-β is important in a variety of primary processes such as wound repair, inflammation, tissue morphogenesis and immune response. It elicits physiological responses at nanomolar to picomolar concentrations yet can be detrimental at higher concentrations.

A primary mechanism controlling TGF-β activity, whilst making it available for rapid responses such as wounding, is its secretion as a latent complex that is sequestered in the extracellular space (reviewed in [11]). Latency is conferred during protein processing by the association of TGF-β with its precursor peptide. TGF-β1, the best-studied protein of the three differentially expressed and regulated TGF-β mammalian isoforms, is derived from a 390 amino acid precursor. During processing, the peptide is cleaved to produce a 112 amino acid carboxy terminal peptide [12]. The homodimer of this peptide is non-covalently associated with a dimer of the processed N-terminal pro-segment, called the latency-associated peptide. This secreted latent TGF-β complex is unable to bind to TGF-β receptors until TGF-β is dissociated from the latent complex [13]. Physical alterations or protease degradation of latency-associated peptide releases TGF-β, which then binds to widely distributed cell surface receptors. Thus, the biological activity of TGF-β is controlled by its release from the latent complex. This activation is considered to be the critical control mechanism for TGF-β function *in vivo*. As a result, elevated expression of latent complex is not likely to have biological consequences, whereas increased activation, even without changes in synthesis rate, will profoundly affect physiological events [9].

Activation occurs during tissue damage, at which point TGF-β orchestrates complex tissue responses such as inflammation and repair [14, 15]. TGF-β activation *in situ* was first demonstrated in irradiated tissue [16]. By using an immunodetection protocol that discriminates between active and latent TGF-β [17], the pattern of staining indicates that latent TGF-β is abundant throughout the tissue but that active TGF-β is restricted to the epithelium. This pattern changes rapidly when tissues respond to damage. Within an hour of exposure to IR, TGF-β increases in the epithelium and is induced in the adipose stroma, whilst latent TGF-β is concomitantly decreased. This rapid shift also occurs in skin following wounding or phorbol ester application (unpublished data). TGF-β signalling begins with ligand binding to its serine–threonine kinase receptors (reviewed in [18]). The type II receptor forms a heterodimeric complex with, and phosphorylates, the type I receptor, which in turn phosphorylates two cytosolic proteins, Smad2 and Smad3. Once activated, these proteins form a complex with Smad4 and translocate to the nucleus, where they bind to DNA and regulate gene transcription. The translocation of Smad 2/3 to the nucleus in irradiated tissue is further evidence of activation [19]. These data

Table 1. The program of tissue response to ionising radiation (IR)

- Microenvironment (*i.e.* cytokines, growth factors and extracellular matrix) is a target of radiation action.
- Tissue response is global yet innately organ- and tissue-type specific.
- Tissue response is evident very rapidly after radiation exposure.
- Some components are clearly secondary to others, indicative of a dynamic network.
- Tissue response can be detected after exposure to low whole body doses (0.1 Gy).
- Radiation-induced changes can be persistent.
- Microenvironment remodelling is dependent on radiation quality.

indicate that although activation is usually a regulated and restricted event, tissue damage widely elicits latent TGF-β activation [11].

IR results in the production of various oxidants. We demonstrated a novel and efficient mechanism of TGF-β activation via ROS [20]. Latent TGF-β1 exposed in solution to ROS generated by classic Fenton chemistry released active TGF-β as measured by bioassay. Addition of radical scavengers suggests that the hydroxyl radical is the critical ROS. The data using this model system indicate that oxidation of the latent complex produces a conformational change resulting in activation [20].

Redox-mediated activation offers a novel route for TGF-β1 involvement in chronic tissue processes in which oxidative stress is implicated, and would endow latent TGF-β1 with the ability both to sense extracellular oxidative stress and to transduce the signal by eliciting changes in diverse cell types (Table 2). We propose that TGF-β functions as an extracellular sensor and signal of oxidative stress. In the context of radiation exposure, inflammation or ischaemia/reperfusion, TGF-β is rapidly activated by ROS, and in turn, because all cells have TGF-β receptors, initiates a complex multicellular response to the damage. Furthermore, TGF-β can induce further ROS production and thereby create positive feedback to perpetuate its activity. Under the circumstances of limited volume exposure or relatively low dose, like the DNA damage programs within cells, tissue response to IR is a multicellular program that limits genetic damage and cell loss [2]. However, this homeostatic mechanism can be derailed, particularly following high dose exposures.

TGF-β is integral to multiple levels of programmatic response to IR. The pleiotropic actions of TGF-β are well suited to orchestrate cellular radiation responses that would facilitate re-establishment of homeostasis. Other DNA damaging agents, including PALA [21], cisplatin [22] and alkylating agents [23], also induce TGF-β1 activity in cell culture. Since TGF-β is a potent regulator of epithelial proliferation and apoptosis, we asked whether the IR-induced activation of TGF-β contributes to the cell fate decisions in response to DNA damage [19]. To do so, we used *Tgfβ1*+/− mice, in which protein levels of TGF-β1 are chronically depressed by more than 90% in adult tissues [24, 25], or administration of TGF-β neutralising antibodies to cause transient depletion.

Table 2. Functions of transforming growth factor-β (TGF-β) in DNA damage response

Sensor functions	Signal functions
Rapid	Inhibit epithelial proliferation
Efficient and sensitive	Stimulate mesenchymal cells
Generation of diffuse response	Initiate ECM remodelling
Independent of cell type or processes	Recruit inflammatory cells
Positive feedback via ROS	
Generate ROS	

ROS, reactive oxygen species; ECM, extracellular matrix.

Radiation induced a three-fold increase in apoptosis in the mammary epithelium of C57BL/6/129Sv *Tgfβ1*+/+ mice 6 h following whole body exposure to a dose of 5 Gy γ-radiation. In contrast, the frequency of *Tgfβ1*+/− mammary epithelial apoptosis was one-eighth the level of irradiated wild-type mice. Apoptosis is not generally depressed in *Tgfβ1*+/− mammary epithelium since levels are similar to wild-type at puberty and are even increased during pregnancy [24]. Interestingly, radiation-induced apoptosis in lymph node and spleen was similar in *Tgfβ1*+/− and wild-type mice, which suggests that TGF-β1 affects cell fate decisions in response to DNA damage in a cell-type-dependent manner.

Although the radiation response of *Tgfβ1* null adult mice cannot be determined because *Tgfβ1*−/− genotype mice commonly die *in utero* [25], several embryonic tissues exhibit both a robust apoptotic response and cell cycle inhibition shortly after irradiation *in utero* [5]. Therefore, mid-pregnant *Tgfβ1*+/− dams were irradiated with a whole body dose of 5 Gy and the embryos were collected 6 h later. Apoptosis increased 2–3-fold in the epidermis and liver in irradiated wild-type embryos, but radiation-induced apoptosis was significantly decreased in *Tgfβ1*+/− embryos, and *Tgfβ1*−/− embryos lacked an apoptotic response. Similarly, proliferation was unaffected by radiation in null embryos, although wild-type embryos responded by a 2–3-fold decrease in the liver and epidermis following irradiation.

Consistent with the lack of appropriate cell fate decisions, we also found that p53, which is critical to the DNA damage signalling within a cell, was hypophosphorylated following radiation in *Tgfβ1*+/− mice or in mice transiently depleted of TGF-β by administration of neutralising antibodies. Together with our previous observations [16, 17], and those showing that expression of constitutively active TGF-β increases radiosensitivity [26] whilst suppressing its action can protect from radiation [27], these data indicate that TGF-β1 signalling initiated via the extracellular activation of latent TGF-β1 is essential for the primary cellular fate decisions following radiation damage [19].

Co-ordination of multicellular responses to DNA damage

Whilst our recent studies have focused on the immediate cellular response to radiation, chronic stimulation of TGF-β may be the root of late radiation effects such as fibrotic responses in irradiated tissues [28]. Mice lacking Smad 3 have been shown to be remarkably resistant to radiation dermal fibrosis [29]. This may be due to the requirement for Smad 3 in the transcriptional control of TGF-β1 [30],

which in its absence fails to maintain TGF-β at high levels post radiation. Studies in irradiated rat lung by Vujaskovic and Anscher support the role for chronic TGF-β feedback via ROS in contributing to organ pathology. In these studies, blocking TGF-β resulted in less ROS and less tissue damage [31], whilst blocking ROS led to less TGF-β and less tissue damage [32].

One may think that there is a paradox in that a program directed to restoring homeostasis can be subverted to generate chronic disease, but the double-edge is a common aspect TGF-β biology. Wound healing elicits abundant TGF-β during platelet degranulation, inflammation and epidermal regrowth, yet surgical wounds heal without scarring if TGF-β is reduced [30, 33]. In cancer, TGF-β is a classic tumour suppressor in its inhibition of epithelial proliferation and stimulation of apoptosis (reviewed in [34]). Yet TGF-β can convert to a tumour-promoting role in late carcinogenesis in the skin, mammary gland and colon [35–38] and recent studies have shown that TGF-β is functionally critical in metastatic behaviour [38–41]. Chronic exposure to TGF-β can elicit phenotypic transformations under certain conditions, or in certain cells, leading to the acquisition of mesenchymal-like transformation of mammary epithelial cells [42], or myofibroblast characteristics in stromal cells [43] that contribute to pathology.

Likewise in radiation damage, TGF-β has both positive and negative consequences depending on the exposure context (*e.g.* radiation dose, dose rate, quality, volume) and the individual's condition (*e.g.* age, other pathology, genotype). The experimental data suggest that at low radiation doses, TGF-β in its latent form is a sensor of oxidative stress and signals to initiate tissue remodelling, to recruit cells to repair tissue and to direct appropriate cell fate decisions. TGF-β appears to be a primary initiator of these events and remains active until the balance between cell and tissue is restored. When large volumes are irradiated acutely at high dose, I suggest that two key processes contribute to the eventual MOF. One is that the tissue program, once initiated, appears to be amplified, either as a function of the total dose or the volume exposed, and becomes a chronic positive feedback loop. The cytokine cascade becomes a torrent, destroying its usual boundaries and sweeping up many mechanisms that would limit tissue toxicity. The second may be the acute loss of cells or their function, which breach a physiological dam. The resulting cell debris appears to elicit accessory responses in macrophages, namely inflammation, leading to further amplification of the process.

Thus, defining appropriate management of MOF could lie in blocking the earliest manifestations, in order to control by limiting cell loss acutely. Novel agents may include biological and small molecule inhibitors of apoptosis, DNA damage signalling and growth factor signalling. The surprising conclusion from this perspective of tissue-based response to radiation may be that the late effects are less about the damage than about the cellular, organ and tissue response to that damage.

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