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Overview

Radiation-induced Cell Death and Dendritic Cells: Potential for Cancer Immunotherapy?

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ABSTRACT:
Dendritic cells are key orchestrators of the immune system. There is considerable interest in their use for treating cancer. Whether they initiate an effective cytotoxic response against antigen-bearing cells, or produce tolerance, depends on the context in which those antigens are presented. Ionising radiation, and the cell death it causes, has several properties that may facilitate such an effective response. A range of *in-vitro* and *in-vivo* data supports this, although potential problems exist that may require concurrent strategies. Hatfield, P. *et al*. (2005). *Clinical Oncology* **17,** 1–11

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Introduction

There has been an explosion of interest in the mechanisms of cell death in recent years. This has been driven by the realisation that organised cell death is crucially important for normal tissue turnover, and that derangements of the pathways that control it can lead to disease.

In oncology, cell death is a critical process governing the rate of tumour growth and the response to treatment. Certainly many tumours have developed mechanisms to evade physiological or treatment-related death signals, which contributes to their ability for uncontrolled growth.

Cell death has a complex relationship to the immune system. Under appropriate circumstances, it can prompt a vigorous immune response to eradicate infectious agents in the process of damaging tissues. At other times, however, it can be immunologically silent or even suppress the immune system. Unravelling these complex interactions is important to oncologists as they strive to enhance the immune response to cancer.

Radiation-induced cell death is clearly an important method for treating cancer. It is also, perhaps, an opportunity for the immunotherapist. In this paper, we discuss how cells die, why that matters in immunotherapy strategies, and how radiotherapy could be used to stimulate a clinically useful immune reaction.

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Mechanisms of Cell Death

Various mechanisms of cell death have been described. A classical distinction is between the apoptotic mechanism of 'programmed' cell death and necrosis, but it is becoming apparent that things are not quite so clear-cut.

Apoptosis was originally described in 1972 by Kerr *et al.* [1]. They described isolated cells within tissues undergoing typical morphological changes, such as cell shrinkage, membrane 'blebbing' and compaction of chromatin with segregation. Ultimately, the nucleus developed granular masses of chromatin arranged marginally around the nuclear envelope before fragmenting completely. Apoptotic cells are typically removed rapidly from tissues by phagocytes, without any accompanying inflammation [2]. Indeed, the phagocytes can actively inhibit an inflammatory response in surrounding tissues by the release of cytokines. This process is distinct from necrosis, which often affects many adjacent cells in response to an external insult. Necrotic cells typically swell and rupture their membranes, releasing intracellular contents into the surrounding tissue. Necrosis is usually associated with surrounding inflammation.

The rapid advances in molecular biology have generated a wealth of information about the cellular pathways leading to apoptosis [3–6]. Key processes are the activation of

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caspases (a family of intracellular proteases) and an organised breakdown of DNA into oligonucleotide fragments. Caspases are a highly conserved group of enzymes that act as the co-ordinators of apoptosis. The main triggers for their activation are the binding of specific ligands to death receptors of the tumour necrosis factor (TNF) receptor family [7] on the plasma membrane (e.g. FAS ligand, TNF, TRAIL-TNF-related apoptosis-inducing ligand) and mitochondrial release of cytochrome-*c*, binding with other molecules to form what is known as an apoptosome. Figure 1 shows how many stimuli can trigger this process, with key mediators being the Bcl-2 family of proteins (e.g. Bax, Bak, Bcl-X_L, Bcl-X_S, Bcl-2). This large family of proteins can be divided into suppressors and activators of apoptosis. They all share sequence homology in four α-helical segments (BH1–BH4), although vary in the total number of subunits they contain [8]. One group, for instance, only contain the BH3 subunit. The subtle and complex interplay between these molecules is a major determinant of whether apoptosis occurs. Other important influences are the 'inhibitors of apoptosis' protein family, which inhibit various caspases, and apoptosis-inducing factor, which is also released from mitochondria.

Although the distinction between apoptosis and necrosis seems simple, and is relatively useful, the reality is likely to be more complicated. *In vivo*, both processes may occur in a tumour, with the ratio depending on the type or intensity of the toxic stimulus, the maintenance of a viable circulation, the inherent tendency of the tumour cells to undergo apoptosis and the ability of phagocytes to clear apoptotic bodies (without which 'secondary necrosis' may occur, as apoptotic bodies ultimately rupture). Furthermore, other variants of cell death are known. For instance, various forms of caspase-independent programmed cell death have been described [9], such as autophagy, in which large lysosome-derived vacuoles develop and consume cytoplasmic contents with or without nuclear changes.

In clinical practice it is also clear that many of the common solid tumours take a long time to respond to cytotoxic treatments such as radiotherapy or chemotherapy. The rate usually correlates with the turnover of cells within the tumour, and this fits with a model of 'reproductive' or 'mitotic' cell death rather than an immediate 'interphase' death. In other words, treated cells remain viable until such time as they try to divide, either initially or at some later point, when the accumulated genetic damage makes the cell non-viable. Such a death is frequently described as 'necrotic' because of the increased membrane permeability and cellular swelling that occurs [10], but the importance of programmed mechanisms, such as apoptosis, as this process progresses remains unclear [11]. Such a model fits with the classical radiobiological technique of clonogenic assay, in which the ability of plated cells to form viable colonies is assessed, rather than their immediate death, and where it is clear that some cells divide a few times before the daughter cells ultimately become incapable of further division. It also fits with the conflicting data on the importance of apoptotic markers such as p53 or bcl-2 on the sensitivity of cells to typical cytotoxic strategies such as radiotherapy and chemotherapy [12–14].

Radiation-induced Cell Death

Ionising radiation is not selective in the damage it causes in a cell. All cellular structures can be affected, but it is generally considered that DNA damage (and in particular double strand breaks) is the key process leading to cell

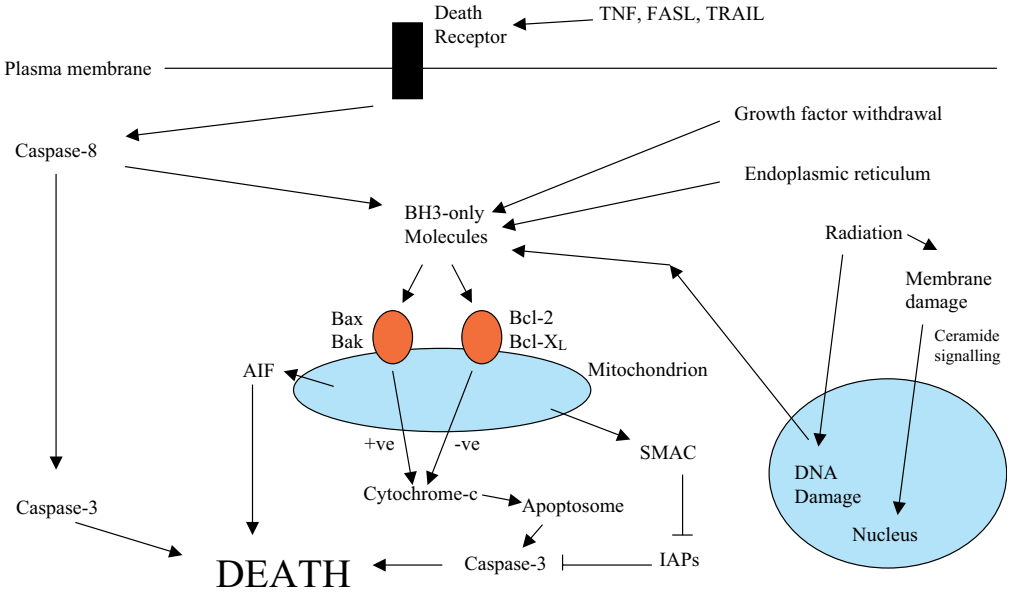

Fig. 1 – Pathways leading to apoptosis. AIF, apoptosis-inducing factor; IAP, inhibitors of apoptosis; SMAC, a mammalian IAP inhibitor; TNF, tumour necrosis factor, TRAIL, TNF-related apoptosis-inducing ligand.