Induction of cell death by radiotherapy

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Abstract

Ionising radiation remains one of the most effective tools in the therapy of cancer. It combines the properties of an extremely efficient DNA-damaging agent with a high degree of spatial specificity. Nonetheless, there remain considerable differences in the outcome for treatment of tumours of differing histological type treated by radiotherapy. Tumours arising from lymphoid or germ cells are significantly more radiocurable than most solid tumours of epithelial origin. The molecular mechanisms underlying such differences in cellular radiosensitivity are the subject of current research. When normal mammalian cells are subjected to stress signals - e.g. radiation, chemotherapeutic drugs, oxygen deficiency - a range of gene products involved in the sensing and signalling of such stresses are activated. The response of eukaryotic cells to ionising radiation includes activation of DNA repair pathways and cell cycle checkpoints, with subsequent full 'biological' recovery or cell death. Radiation induces two different modes of cell death termed mitotic or clonogenic cell death, and apoptosis. Until recent years, there was surprisingly little mechanistic understanding of the events following induction of physical damage by radiation and biological outcome for the cell. There have been recent major advances in our understanding of the signal transduction pathways involved in determining the fate of cells after irradiation.

Induction and fate of DNA damage by ionising radiation

Photons generated by clinical linear accelerators for therapy induce a range of biochemical lesions in genomic DNA, thought to be the most important subcellular target molecule. The class of lesion whose fate most closely correlates with cell death is the DNA double strand break (dsb), which can be induced by direct ionisation of DNA or indirectly via the generation of free radicals. The majority of lesions are repaired rapidly by highly conserved enzymatic pathways. Misrepaired lesions, due to 'structural' repair of breaks without fidelity of the genetic message, lead to mutation, with possible change of cell phenotype. Frank unrepaired breaks may generate chromosomal aberrations. Subsequent cell death may occur after a variable number of cell cycles (Fig. 1). This mode of cell death is exhibited by most non-haematopoietic cell lineages in response to ionising radiation, and is referred to as mitotic or clonogenic cell death. It is considered to be the major mechanism by which the majority of solid tumours respond to clinical radiotherapy.

The correlation between the amount of mitotic cell death, DNA lesion induction and chromosomal aberrations suggests that this pattern of cell death probably results from failure of cells to completely or accurately repair DNA damage. Recent work in mammalian and yeast model systems suggests that at least two distinct pathways involving some four discrete complexes facilitate the repair of dsbs. In one such pathway, so-called non-homologous recombinational repair, little or no homology of DNA sequence is required for rejoining of breaks. The DNA-dependent protein kinase (DNA-PK) and the RAD50 complex act predominantly during G1/early S phase. By contrast, in homologous recombination extensive homology is required between the region of DNA with a dsb and the repair template. This pathway requires the RAD52 complex, acting mainly at late S/G2, or the breast cancer predisposition genes BRCA1/2 complex, acting in S phase (for review see Hendrickson (1997)).

Apoptosis is an alternative mode of cell death after irradiation, but appears to be preferentially expressed in embryonal and haematopoietic cells, with significantly lower levels of induction in epithelial cell types. Radiation-induced apoptosis was initially considered not to require cell division, and therefore regarded as a form of death expressed in interphase cells. Further investigation revealed that it could be induced readily in the cells of the small intestine, lymphocytes and salivary glands.
and in experimental tumour systems (Nomura et al. 1992, Hendry et al. 1995). Given the apparently low levels of radiation-induced apoptosis in the cell types which give rise to most solid human cancers, its relevance to the determination of the response in clinical radiotherapy has been questioned. Studies quantitatively correlating apoptosis events with loss of clonogenic cell survival in non-haematopoietic cells have suggested that the major pathway leading to cell death is mitotic rather than apoptosis. Necessarily many of these data relate to outcomes observed in cell lines. Some mouse tumours treated in vivo demonstrate correlations between radiosensitivity and the amount of spontaneous or induced apoptosis.

Clinical regression of solid tumours after completion of therapy is observed over many months, whereas treatment of lymphoid tumours can result in dramatic regression during a course of treatment, perhaps offering circumstantial evidence that cell lineage might be a major determinant of the mode of cell death in response to radiotherapy. This does not preclude a contribution of spontaneous and induced apoptosis in solid tumours to treatment outcome; however, there is a paucity of clinical data to indicate the true contribution of apoptosis to radiosensitivity (Dewey et al. 1995).

**Signal transduction of the radiation response and the DNA damage surveillance network**

Recently it has been recognised that the cell membrane may be important as a target for at least one pathway of radiation-induced apoptosis (Santana et al. 1996). Ionising radiation activates sphingomyelinase, which catalyses the hydrolysis of sphingomyelin to the lipid second messenger, ceramide, thereby inducing interphase death by apoptosis. This pathway has been demonstrated to be deficient in the lymphoblasts of patients with Niemann-Pick disease, a condition in which there is an inherited lack of acid sphingomyelinase. In *vitro*, radiosensitivity can be restored by retroviral transfer of human acid sphingomyelinase cDNA.
Although the cell membrane is a target for some forms of radiation-induced apoptosis, the nucleus is also a critical target. A major pathway of radiation-induced apoptosis involves DNA damage and subsequent induction of a range of genes including ataxia telangiectasia (AT) and p53 (Fig. 2). In the presence of DNA damage, p53-dependent gene transcription is increased and ubiquitin-dependent degradation of the protein is blocked leading to induction of apoptosis and/or cell cycle arrest. Activation of p53 is mediated via stress-activated protein kinases. In its latent state p53 cannot bind DNA, and it requires phosphorylation to function as a transcription factor. Recent data suggest that DNA-PK is required for the p53 response (Woo et al. 1998). DNA-PK modifies the amino-terminal region of p53, which controls its interaction with the transcriptional apparatus and with MDM2. In lymphocytes isolated from p53 knockout mice, clonogenic cell survival is dramatically modulated by inactivation of the p53 response. Expression of a retrovirally transferred mutant p53 transgene in the human ovarian tumour line A2780 produced a significant but lesser increase in radiation resistance, in comparison with the control A2780 cells with an intact p53 response (McIlwrath et al. 1994). However, clinical studies examining the relationship between clinical radiosensitivity and tumour p53 status have largely failed to demonstrate a significant effect.

The AT gene is emerging as another key participant in the cellular response to ionising radiation (Meyn 1995). The phenotype of the rare autosomal recessive disorder ataxia telangiectasia is complex, but key clinical features include progressive cerebellar degeneration, oculocutaneous telangiectasias, immunodeficiency, premature ageing and lymphoreticular malignancies. One per cent of the population is estimated to be heterozygous, with a possible increased risk of solid tumours. Several studies have shown an increased incidence of breast cancer in AT heterozygotes, with a relative risk estimated to be 3.9 (Easton 1994). The AT gene was cloned in 1995, and is 150 kb in length spread over 66 exons. A 13 kb mRNA transcript contains an open reading frame of 9168 bp encoding a 350 kDa nuclear protein (Savitsky et al. 1995). Mutations have been detected in over 100 AT patients; over 80% result in truncation of the protein. The similarity of the phenotypes suggests that most of the mutations are functionally equivalent. Sequence comparisons between human mutated in ataxia telangectasia (ATM) and mouse ATM suggest that the gene is a member of a family of genes involved in cell cycle regulation (TOR1, TOR2, MEC1 of Saccharomyces cerevisiae and rad3 of S. pombe), telomere length monitoring (TEL1 of S. cerevisiae), meiotic recombination (MEC1 of S. cerevisiae and mei41 of Drosophila melanogaster) and DNA repair (DNA-PK), supporting a key role for ATM in the DNA damage surveillance network.

Figure 2 The DNA damage surveillance network.

ssb, single strand DNA breaks
DNA dsb repair and cell cycle regulation. In vitro, cells derived from AT heterozygotes demonstrate increased radiosensitivity in comparison with normal individuals, as measured by chromosomal damage (Chen et al. 1994). However, as yet there is no clinical evidence that such individuals have abnormal clinical responses to radiotherapy.

A role for BRCA1 and BRCA2 in the radiation response?

Although analysis of the nucleotide sequence of BRCA1 and BRCA2 failed to yield insight into the likely functions of these genes, recent data have provided compelling evidence for a role in DNA damage response pathways, including the response to ionising radiation (Zhang et al. 1994). Both proteins are co-regulated in the cell cycle and associate with human RAD51, the eukaryotic equivalent of the bacterial recombination protein, recA, which is involved in repair of dsbs and chromosome maintenance. The first indirect evidence for a role of BRCA2 in DNA repair was the observation that disruption of BRCA2 in embryos produced ionising radiation hypersensitivity. Direct biochemical evidence for an involvement of the BRCA2 gene product in DNA repair comes from the observation that embryonic fibroblasts isolated from mice bearing a truncating mutation in BRCA2 appear to have altered kinetics in the rejoining of DNA dsb as measured in the single cell gel electrophoresis assay (Connor et al. 1997). These mice show a remarkable similarity to Atm-deficient mice, namely, growth retardation (in vitro and in vivo), absence of mature gametes and shortened lifespan due to the development of thymic lymphoma (Barlow et al. 1996). This similarity could indicate a role for both genes in the same DNA damage response pathway, either directly involved in repair, or via low level overexpression of p53 induced by failure of repair. In support of this hypothesis, the growth failure of Atm null fibroblasts is rescued in a p53 null background. We await evidence that the Atm-/– background can rescue the BRCA2 phenotype. Significant questions remain as to the potential relevance of haploid insufficiency in ATM and BRCA2 and implications for cancer predisposition and response to DNA-damaging therapy.

Summary

Knowledge is rapidly increasing of the pathways involved in determining cell fate after exposure to ionising radiation. Many of the genes involved play key roles in genomic stability, and may even participate in determining cancer proneness. Hopefully some of these gene products will provide new targets for therapeutic modulation of the radiation response and facilitate rational molecular radio-sensitisation.

References


