Chapter 1
Targeting NF-κB to Prevent Radiation-Induced Carcinogenesis

M.A. Christine Pratt

Abstract Ionizing radiation inflicts damage to cells in large part through the generation of chemical intermediates that damage DNA and generate DNA strand breaks. Radiation-induced late normal tissue toxicity is the outcome of changes in tissue following exposure to radiation that precede overt toxicity. These events include mitotic cell death especially in bone marrow and mucosal tissue, and the activation of inflammatory responses that can lead to blood vessel damage, tissue necrosis resulting from lack of oxygen and excessive extracellular matrix deposition (fibrosis). While antioxidants can prevent damage by moderating the chemistry of DNA strand breaks, other agents known as radiation mitigators can be used soon after exposure to protect essential compartments such as the bone marrow from collapse. These include agents that reinforce the rapid development of mature bone marrow and mucosa. Medical management of acute radiation syndrome following accidental exposures to ionizing radiation (IR) involves attempts to reduce the risks of infection and hemorrhage resulting from bone marrow aplasia. This involves stimulating the proliferation and differentiation of residual non-impacted or radio-resistant hematopoietic stem and progenitor cells (HSPC) with hematopoietic growth factors. Soon after irradiation radiosensitive HSPC have been shown to undergo apoptosis. It has therefore been proposed that antiapoptotic cytokines including stem cell factor, Flt-3 ligand, thrombopoietin, and interleukin-3 could be employed acutely to prevent this cell death. Moreover, acute exposure to high doses of IR induces sequential, deleterious effects responsible for a delayed multiple organ dysfunction syndrome. Of course, the caveat of preventing the death of cells with damaged DNA is carcinogenesis resulting from DNA mutations in critical genes. NF-κB constitutes a family of transcription factors best associated with

M.A. Christine Pratt (✉)
Department of Cellular and Molecular Medicine, University of Ottawa,
451 Smyth Rd, Ottawa, ON, Canada K1H 8M5
e-mail: cpratt@uottawa.ca

mediating the inflammatory response. In cancer cells the activation of the inhibitor of-κB kinase (IKK) and consequently the NF-κB pathway increases resistance to ionizing radiation (IR) by facilitating cell survival, despite the presence of DNA damage and mutations. A number of small molecule inhibitors of NF-κB have been described. I discuss the potential benefit of targeting NF-κB for the prevention of radiation-induced cancers.

1.1   Therapeutics of Exposure to Ionizing Radiation

Ionizing radiation inflicts damage to cells in large part through the generation of chemical intermediates that damage DNA and generate DNA strand breaks. Radiation-induced late normal tissue toxicity is the outcome of changes in tissue following exposure to radiation that precede overt toxicity. These events include mitotic cell death especially in bone marrow and mucosal tissue, and the activation of inflammatory responses that can lead to blood vessel damage, tissue necrosis resulting from lack of oxygen and excessive extracellular matrix deposition (fibrosis). While antioxidants can prevent damage by moderating the chemistry of DNA strand breaks, other agents known as radiation mitigators can be used soon after exposure to protect essential compartments such as the bone marrow from collapse. These include agents that reinforce the rapid development of mature bone marrow and mucosa. Medical management of acute radiation syndrome following accidental exposures to ionizing radiation (IR) involves attempts to reduce the risks of infection and hemorrhage resulting from bone marrow aplasia. This involves stimulating the proliferation and differentiation of residual non-impacted or radioresistant hematopoietic stem and progenitor cells (HSPC) with hematopoietic growth factors including granulocyte-macrophage colony stimulating factor (GM-CSF). GM-CSF treatment promotes rapid development of mature bone marrow and mucosa from the stem cell compartment. Colony-stimulating factor remains the therapeutic standard after exposure to less than the lethal dose 50 % (Haematopoietic [H] score 3-H3) while higher scores are treated with stem cell transplantation. Soon after irradiation radiosensitive HSPC have also been shown to undergo apoptosis [1, 2]. It has therefore been proposed that antiapoptotic cytokines including stem cell factor, Flt-3 ligand, thrombopoietin, and interleukin-3 could be rapidly employed to prevent acute cell death [1].

1.2   The NF-κB Pathway and Its Regulation by Receptors and Ionizing Radiation

Of course, the caveat of preventing the death of cells with damaged DNA or promoting their proliferation is carcinogenesis resulting from DNA mutations in critical genes. Tissue damage arising from IR results in activation of the inflammatory process.

Activated macrophages secrete cytokines including TNF-α and IL-1, both of which interact with receptors on T lymphocytes. There are two TNF receptors, I and II, which both result in activation of NF-κB and have been implicated in cancer (reviewed in [3]). NF-κB is a transcription factor that is composed of members of the Rel family including p65RelA and p105/p50 (NF-κB1) and p100/p52 (NF-κB2), RelB and c-Rel. These proteins function as heterodimers or in the case of p52 and p50, can also form homodimers to regulate target genes. The inhibitor of κB (IκBα) kinase (IKK) complex consists of IKK-α,-β and -γ (NEMO) which negatively regulates IκBα activity. The *canonical NF-κB pathway* is activated by numerous cellular stresses and inflammatory cytokines including TNF-α. In this pathway IκBα binds and retains p65 in the cytoplasm until stimulated IKK-β-mediated phosphorylation results in ubiquitin-mediated degradation of IκBα allowing p65/p50 heterodimerization and nuclear translocation (reviewed in [4, 5]). A second pathway, called the *alternative NF-κB pathway* (NF-κB AP), is usually induced by activation of specific members of the TNF receptor family including the lymphotoxin (LT)α/β, B-cell activating factor (BAFF) receptors, CD40, and receptor activator of NF-κB (RANK) and is involved in lymphoid organ development and adaptive immunity [6]. Here, activation of the NF-κB inducing kinase, NIK, activates IKK-α homodimers which phosphorylates p100 to signal partial proteolytic processing to mature p52. p52 then translocates to the nucleus to participate in transcriptional activation [4–6]. RelB associates with unprocessed p100 in the cytoplasm and its nuclear translocation occurs coordinately with p52 [7]. Homodimers of p52 are transcriptionally inactive but can also combine with Bcl-3 to mediate transactivation [8, 9].

Importantly, NF-κB can also be activated in an atypical manner by *DNA damage* (Fig. 1.1). NEMO becomes SUMOylated by PIASy following DNA damage, and then undergoes nuclear translocation where it is phosphorylated at serine 85 by the ATM DNA damage-induced kinase which stimulates cIAP1 E3 ligase-mediated mono-ubiquitination of NEMO resulting in nuclear export and subsequent activation of IKK-α and IKK-β [10].

The NF-κB alternative pathway is also activated by DNA damage as evidenced by induced p100 processing and nuclear localization of the p52 subunit in an IKK-α, ATM and NEMO-dependent manner following DNA damage [11]. While the canonical pathway has been associated with cell survival following DNA damage, the alternative pathway is reported to promote cell death.

1.3   NF-κB Induction by TNFα and Cancer Progression

Interestingly, ATM has been shown to primarily mediate cell survival since downregulation sensitizes cells to IR [12]. In cancer cells the activation of the inhibitor of κB kinase (IKK) and consequently the NF-κB pathway increases resistance to ionizing radiation (IR) by facilitating cell survival, despite the presence of DNA damage/mutations. Importantly, TNFα and IL-1 can stimulate the production of NF-κB-responsive cytokines which further induce growth
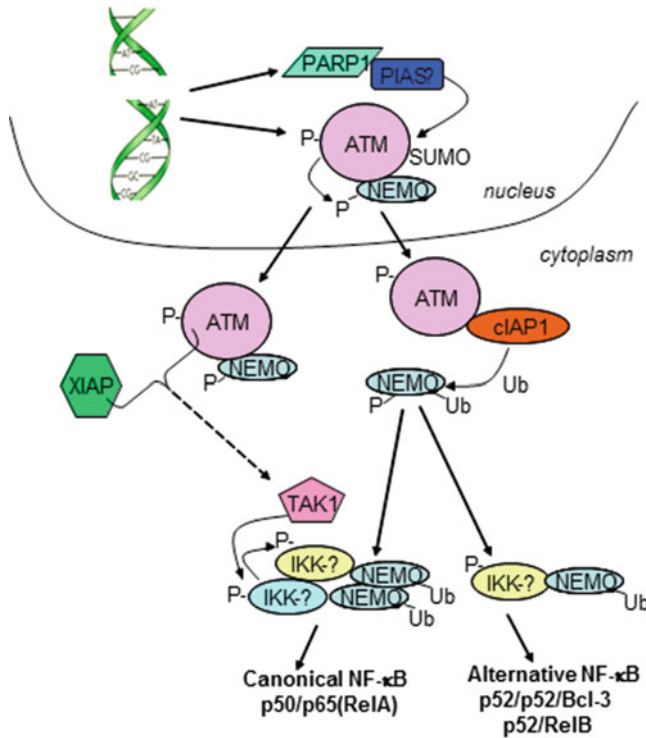
Fig. 1.1 Both the canonical and alternative NF-κB pathways can be activated following IR/DNA damage through a mechanism involving phosphorylation and signalling through the ataxia telangectasia mutated (ATM) protein

factors to promote survival and stem cell expansion. The therapeutic value of promoting bone marrow maturation from stem cells is clear promoting expansion and maturation of cells that have either escaped IR damage or those that have successfully repaired their DNA as shown in Fig. 1.2.

However, survival signalling combined with the establishment of a growth factor: cytokine feedforward pathway may allow the progression of hematopoetic stem cells harbouring cancer initiating mutations through TNF mediated signalling to NF-κB as shown in Fig. 1.3.

Clearly the risk to benefit ratio associated with replenishing the bone marrow compartment and the risk of enhancing cancer progression needs to be carefully considered. Should, for example, both high dose and very high dose radiation exposure be treated similar to multiple myeloma avoid potential expansion of an initiated population? Treatment of multiple myeloma involves induction therapy with high-dose chemotherapy to ablate the haematopoetic stem cell compartment which is then followed by autologous stem cell transplant.
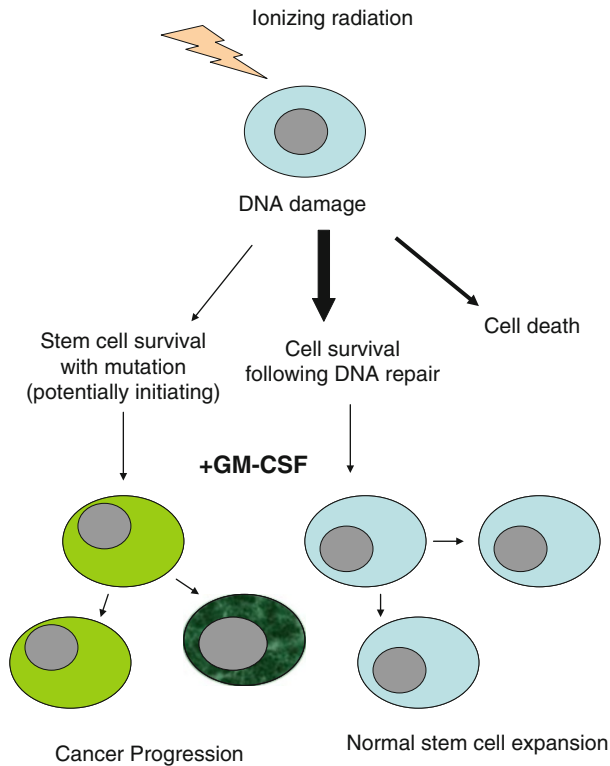
Fig. 1.2 Ionizing radiation induces DNA strand breaks in heamatopoetic stem/progenitor cells. Some cells die outright while DNA repair will render most surviving cells normal. However, repair can introduce mutations in some cells that will be expanded through therapeutic administration of GM-CSF

Maintenance and/or induction can include bortezomib and steroids [13] both of which block NF-κB to prevent further signalling through this pathway. Of course the drawbacks include that applying this approach would be difficult on a large scale and would require further induction treatment to ablate residual cells. Another option could be a more limited exposure to GM-CSF with a set minimum recovery of the haematopoetic index followed by intermittent borte-mozib or steroids to block initiated cell survival and proliferation. Promotion of initiated cells might also be better prevented by measures to block inflammation arising from tissue damage which would increase the production of TNFα and IL-1 from activated macrophages. To this end, therapeutics could include factors such as keratinocyte growth factor and erythropoietin as suggested by Herodin and Drouet [1] to ensure tissue damage repair and mitigate the inflammatory processes.
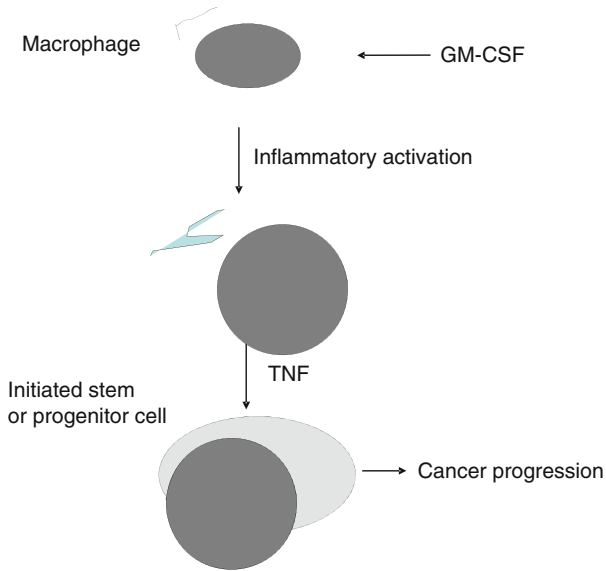
Fig. 1.3 GM-CSF induces the maturation of macrophages which can become activated as a result of tissue damage. Production of TNFα results in activation of TNFRs on lymphocytes and other cell types inducing NF-κB which can promote cancer progression

1.4   Conclusion

It is difficult to establish standard therapies for radiation exposure given the inability to run randomized clinical trials. However, our knowledge of mechanisms of long term cell survival and cancer promotion should guide in the rational treatment of high level exposure to ionizing radiation and efforts to spare damaged cells balanced with the potential for mediating cancer progression.

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