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Mini-review

The nuclear factor κB pathway: A link to the immune system in the radiation response

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ABSTRACT

Exposure to ionizing radiation modulates immune responses in a complex dose-dependent pattern, with possible anti-inflammatory effects in the low dose range, expression of pro-inflammatory cytokines at moderate doses and immunosuppression after exposure to higher doses due to precursor cell death together with concomitant exacerbated innate immune responses. A central regulator in the immune system is the transcription factor Nuclear Factor κB (NF-κB). NF-κB is involved in the regulation of cellular survival, immune responses and inflammation, resulting in eminent importance in cancerogenesis. After exposure to ionizing radiation, NF-κB activation is initially triggered by ATM which is activated by DNA double strand breaks. Together with the NF-κB essential modulator (NEMO), it serves as a nucleoplasmic shuttle. The pathway converges with the classical NF-κB pathway at IκB kinase (IKK) complex activation. Resulting cytokine expression can activate NF-κB in a positive feed forward loop. Danger signals released from dying cells can activate NF-κB via Toll-like receptors (TLRs). The resulting immune activation can be beneficial or detrimental. In the low dose range, pro- and anticancerogenic effects are possible. In the radiotherapy-relevant dose range, tolerogenic immune responses should be avoided, and an anti-tumor immune response might be supported by TLR agonists activating NF-κB.

Introduction

Since its discovery as a nuclear protein bound to the enhancer of immunoglobulin κ light chain genes in pre-B cells stimulated with bacterial lipopolysaccharide (LPS) [1], a growing list of functions has been attributed to the transcription factor Nuclear Factor κB (NF-κB) and it was shown to be involved in the pathogenesis of a large number of diseases. These include cancer, chronic inflammatory disorders such as rheumatoid arthritis, diabetes, transplant intolerance, cachexia, Alzheimer's disease, and organ ischemia/reperfusion injury [2–4].

Abbreviations: A20 (TNFAIP3), tumor necrosis factor inducible protein 3; RD, ankyrin repeat domain; ATM, ataxia telangiecta-sia mutated protein; β-TrCP, β-transducin repeat containing protein; BIRC, baculoviral IAP repeat-containing; BTG2, B-cell translocation gene 2; CCL3, chemokine (C-C motif) ligand 3; cIAP-1 (BIRC2), cIAP-2 (BIRC3), cellular inhibitor of apoptosis protein 1 and 2; CHO, Chinese hamster ovary; COX-2/PTGS2, cyclooxygenase 2/prostaglandin-endoperoxide synthase 2; CXCL, chemokine (C-X-C motif) ligand; DAMPs, damage-associated molecular patterns; DDR, DNA damage response; DNA-PK, DNA-dependent protein kinase; DSB, double strand break; DUSP, dual specificity phosphatase; EBV, Epstein–Barr virus; ELKS, protein abundant in glutamic acid (E), leucine (L), lysine (K), and serine (S); EMSA, electrophoretic mobility shift assay; EMT, epithelial mesenchymal transition; GADD45B, growth arrest & DNA-damage-inducible, β; h, hours; HEK/293, human embryonic kidney; HMBG1, high-mobility-group box 1; HUVEC, human umbilical vein endothelial cells; IAP, inhibitor of apoptosis; ICAM1, intercellular adhesion molecule; IκB, inhibitor of NF-κB; IKK, IκB kinase; IKK-K, IKK kinases; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; LET, linear energy transfer; LPS, lipopolysaccharide; LRR, leucine-rich repeat; MAP3K (or MAPKKK), MAPK kinase kinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein-1; MD-2, myeloid differentiation factor-2; MEF, murine embryonic fibroblasts; MEKK3 (or MAP3K3), MAPK kinase kinase 3; MCP-1, monocyte chemotactic protein-1; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NEMO, NF-κB essential modulator; NF-κB, nuclear factor κB; NIK, NF-κB-inducing kinase; NK, natural killer; NRE, NF-κB response element; PAMPs, pathogen-associated molecular patterns; PARP-1, poly(ADP-ribose)-polymerase-1; PGE2, prostaglandin E2; PI3K, phosphatidyl-inositol 3-kinase; PIASy, protein inhibitor of activated STAT 4; PIDD, p53-induced protein with a death domain; PRR, pattern recognition receptors; RHD, Rel homology domain; RIP1, receptor interacting protein 1; RNS, reactive nitrogen species; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; SUMO-1, small ubiquitin-like modifier 1; SV40, simian virus 40; TAB, TGF-β activated kinase; TAD, transcriptional activation domain; TAK1, TGF-β-activated protein kinase 1; Th, T helper; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; TNFR, TNF receptor; TRAF, TNF-R associated factor; Tregs, regulatory T cells; Ubc13, ubiquitin-conjugating enzyme 13; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; XIAP, X-linked IAP.

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NF-κB is a master switch in inflammation, the central element of an acute innate immune response. Furthermore, it plays a fundamental role in the adaptive immune response and is involved in the regulation of embryonic development, lymphopoiesis and osteogenesis [5–12]. It contributes to oncogenesis as it can affect most of the hallmarks of cancer through the transcriptional activation of genes associated with cell proliferation, angiogenesis, metastasis, tumor promotion, inflammation and suppression of apoptosis [7]. NF-κB is considered as a crucial promoter of inflammation-linked cancers, with chronic inflammation being a route to carcinogenesis [13]. Aberrant NF-κB regulation, including constitutive or induced activation, has been observed in many cancers. Constitutive NF-κB activation was found e.g. in breast, thyroid, bladder and colon cancer [14–18], and NF-κB is often activated in malignant cells in response to inflammatory stimuli from the microenvironment. NF-κB can be critical for cancer progression [19] and for radio- and chemotherapy resistance in cancer [8]. This resistance can rise from constitutive NF-κB activity in the tumor cells, or by activation of NF-κB in response to DNA damaging agents such as chemotherapeutics and ionizing radiation [15]. Activation of the NF-κB pathway can protect cells from apoptosis after treatment with various genotoxic agents via expression of anti-apoptotic proteins [20]. NF-κB also enhances the expression of degradative enzymes and adhesion molecules supporting the idea that it makes a major contribution to tumor progression and metastasis in various cancers [21]. Therefore, most interest for the role of NF-κB in DNA damage pathways comes from the field of cancer therapy, where resistance of tumors to chemo- and radiotherapy is a major problem [15], and NF-κB was identified quite early as a potential target of innovative cancer therapies [22].

Otherwise, radiotherapy induced NF-κB activation in the tumor microenvironment might be beneficial as it might contribute to an antitumor immune response by modulating cytokine production of tumor cells and of tumor infiltrating lymphocytes [19]. The role of cytokines in radiobiological responses was recently reviewed [23]. High radiation doses result in significant cell death with release of danger signals which could support the rise of an antitumor immune response with strong involvement of NF-κB activation [24].

Low dose radiotherapy, e.g. for different inflammatory diseases, tries to regulate and terminate inflammation by applying a dose at which the anti-inflammatory arm of the response outweighs the pro-inflammatory arm. It acts on already inflamed tissue with upregulated inflammatory responses. A suppression of NF-κB by low-dose radiation therapy was found in allergic asthma with chronic airway remodeling: Whole body irradiation of mice (0.5 Gy per day for three days) with ovalbumin induced asthma reduced NF-κB activity in mast cells [25]. In vitro, exposure to 0.5–0.7 Gy X-rays suppressed NF-κB activation in monocytes [26].

NF-κB's role in responses to chronic low dose ionizing radiation exposure is much less investigated, but modulations in cytokine expression in response to such exposures point to the involvement of NF-κB as crucial regulator. For example, low dose (<0.2 Gy) and high dose exposure generated both pro-inflammatory responses in the thymus of irradiated mice [27]. It is suggested that chronic low dose irradiation creates an inflammatory milieu due to cytokine secretion and production of reactive oxygen and nitrogen species (ROS, RNS) with secondary genotoxic [24] and pro-tumorigenic effects [24,28,29]. On the other hand, some authors claim beneficial effects of low doses (1.2 mGy/h), such as immune activation by chronic exposure [30,31]. Shin et al. argue that low-dose rate irradiation inhibited tumor growth in mice prone to develop thymic lymphoma [32]. The natural killer (NK) cell and T cell stimulating cytokine interleukin-15 (IL-15), which expression is partially regulated by NF-κB [27], was up-regulated in the thymus after low dose rate irradiation of mice [32]. Currently, it is very difficult to judge which effects of chronic low dose ionizing radiation exposure are pro- or anti-inflammatory, pro- or anticancerogenic, or might even cross the border to autoimmune effects.

Seemingly, in both cases, exposure to radiotherapy relevant doses or chronic low dose exposure, the outcome of the inflammatory response involving NF-κB can be desirable or undesirable [24], and requires further investigation. In this review, first, the multitude of pathways leading to NF-κB activation after exposure to ionizing radiation will be highlighted. In the second part, the involvement of NF-κB target genes in survival, inflammatory, immune and bystander responses will be discussed.

NF-κB and IκB families: the basics

NF-κB consists of homo- or hetereodimeric complexes made up of members of the NF-κB/Rel family. These carry the characteristic 300-amino-acid long N-terminal stretch, called the Rel homology domain (RHD), which is responsible for binding of DNA and inhibitory factors and for homo- and heterodimerization: RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2) [33–36], whereby p105 and p100 are precursor proteins. RelA, RelB and c-Rel possess a transcriptional activation domain (TAD), rendering NF-κB a potent transcription factor, which lacks in p50 and p52 [35].

Under resting conditions, retention of NF-κB in the cytoplasm is achieved by the inhibitor of NF-κB (IκB) proteins, which bind through their ankyrin repeat domain (ARD) to NF-κB. Thereby the nuclear localization sequences are masked [35,37]. In their free state, IκB proteins are unstable and rapidly degraded, while binding to NF-κB strongly increases their stability [35]. The three canonical IκB proteins, IκBα, IκBβ, and IκBε, are encoded by the NFKBIA, NFKBIB, and NFKBIE genes [35]. The prototypical p50:p65 heterodimer is mainly bound by IκBα. p105 and p100 proteins, which are involved in the alternative NF-κB pathway, contain the inhibitory part in their C-terminal region in addition to the NF-κB part in the N-terminal half. Two novel IκBs (IκBζ and BCL-3) were described. BCL-3 is a non-inhibiting IκB family member that acts as transcriptional co-activator for p50:p50 and p52:p52 homodimers [2].

Canonical, alternative and atypical NF-κB pathways

The list of NF-κB activators is long and comprises diverse endogenous and exogenous ligands and physical and chemical stresses [4,38,39]. Frequent ligands are inflammatory cytokines or bacteria, viruses and pathogen-derived agents (e.g. LPS). Among the activating cellular stress factors are phorbol esters, ROS, necrotic cell products, growth factor depletion, hypoxia, heat shock, and ultraviolet as well as ionizing radiation [4,40–42].

These activators trigger pathway run-through via activation by membrane receptors (cytokine receptors, toll-like receptors, TLRs), as seen in the canonical (or classical) and alternative (or non-canonical) pathway, or from intracellular sites such as the nucleus in the case of atypical pathways. Ligands bind to their receptors resulting in recruitment of distinct proximal signaling molecules. These use common intermediates (receptor interacting protein 1, RIP1; TNF-R associated factor, TRAF) to activate IκB kinase (IKK) complex, which is as the proteasome a central element of all sub-pathways [43].

Depending on the sub-pathway, the IKK complex is composed by different members of the IKK family. In the canonical and the atypical pathway, the IKK complex is composed of the two catalytic subunits, IKK-α (IKK1) and IKK-β (IKK2), and the regulatory subunit, IKK-γ/NF-κB essential modulator (NEMO) [6,35]. ELKS associates as another regulatory subunit within the IKK complex [44]. It is abundant in glutamic acid (E), leucine (L), lysine (K), and serine (S) [45] and is suggested to recruit IκBα to the IKK complex [44]. The activated IKK phosphorylates IκB in the signal responsive domain at the serine residues 32 and 36 and thereby targets IκB for

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