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REVIEW Article

NF-κB as a key player in regulation of cellular radiation responses and identification of radiation countermeasures

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ABSTRACT

Nuclear factor (NF)-κB is a transcription factor that plays significant role in immunity, cellular survival and inhibition of apoptosis, through the induction of genetic networks. Depending on the stimulus and the cell type, the members of NF-κB related family (RelA, c-Rel, RelB, p50, and p52), forms different combinations of homo and hetero-dimers. The activated complexes (Es) translocate into the nucleus and bind to the 10bp κB site of promoter region of target genes in stimulus specific manner. In response to radiation, NF-κB is known to reduce cell death by promoting the expression of anti-apoptotic proteins and activation of cellular antioxidant defense system. Constitutive activation of NF-κB associated genes in tumour cells are known to enhance radiation resistance, whereas deletion in mice results in hypersensitivity to IR-induced GI damage. NF-κB is also known to regulate the production of a wide variety of cytokines and chemokines, which contribute in enhancing cell proliferation and tissue regeneration in various organs, such as the GI crypts stem cells, bone marrow etc., following exposure to IR. Several other cytokines are also known to exert potent pro-inflammatory effects that may contribute to the increase of tissue damage following exposure to ionizing radiation. Till date there are a series of molecules or group of compounds that have been evaluated for their radio-protective potential, and very few have reached clinical trials. The failure or less success of identified agents in humans could be due to their reduced radiation protection efficacy.
In this review we have considered activation of NF-κB as a potential marker in screening of radiation countermeasure agents (RCAs) and cellular radiation responses. Moreover, we have also focused on associated mechanisms of activation of NF-κB signaling and their specified family member activation with respect to stimuli. Furthermore, we have categorized their regulated gene expressions and their function in radiation response or modulation. In addition, we have discussed some recently developed radiation countermeasures in relation to NF-κB activation.

Keywords:
Ionizing radiation, NF-κB, apoptosis, cell proliferation, inflammation, radioprotector

Abbreviations:
Mitogen-activated protein kinase (MAPK); Phosphoinositide 3-kinases (PI3K); Ataxia telangiectasia mutated (ATM); Activator protein 1 (AP1); Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); Growth arrest and DNA damage-inducible gene 153 (GADD153); Rel Homology Domain (RHD); Dimerization (DM); Nuclear Localization Sequence (NLS); DNA-Binding Domain (DBD); Terminal

Transactivation Domain (TAD); B-cell lymphoma 3 (BCL-3); Transcription activation domain 1 (TA1); Transcription activation domain 2 (TA2); Glycine rich hinge region (GGG); Radiation Countermeasures (RC); Ionizing Radiation (IR); Inhibitor κB Kinase (IKK); Reactive Oxygen Species (ROS); Interleukin (IL); Tumour Necrosis Factor α (TNFα); Tumour Growth Factor β (TGFβ); Nuclear Export Signals (NES); NF-κB Inducing Kinase (NIK); Receptor activator of NF-κB (RANK); Clusters of Differentiation (CD); Lipopolysaccharide (LPS); Radiation countermeasure agents (RCA); Single stranded Ribonucleic acid (ssRNA); Double strand break (DSB); DNA-binding domain (DBD); C-terminal transactivation domain (TAD); Protein rich in amino acids E, L, K and S (ELKS); TNF receptor-associated factor (TRAF); Forkhead box transcription factor (FOXO); Interleukin-1 (IL-1); B cell-activating factor (BAFF); B cell lymphoma-2 (Bcl-2); Lymphotoxin beta receptor (LTβR); Antioxidant Response Element (ARE);

SUMMARY

1. Introduction

Deleterious effects of ionizing radiation (IR) may lead to significant morbidity and a possible fatal illness that affects various organs of the organism in a dose and time dependent manner [1]. Exposure of the organism to IR during therapy, or as a result of a radiological/ nuclear incident, or act of terrorism, may symbolize serious health issues. However, this problem remains largely impervious to medical management of IR exposure and therefore, there is a pressing need to develop safe and effective radiation countermeasures agents (RCA) to reduce or mitigate the harmful consequences of IR exposure at cellular, tissue and organism levels. Following exposure of the organism to ionizing radiation, various signaling pathways, such as the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinases (PI3K), and ataxia telangiectasia mutated (ATM) are activated and all these processes are tightly regulated in relation to changes in expression of various transcription factors (AP, NF-κB, p53, ARE, GADD153 etc) along with changes in the functional status of cell organelles [2, 3, 4]. This may trigger alterations in expression of a large number of genes that are mostly related to cell cycle progression, cell survival, DNA repair and apoptosis [4, 5].

NF-κB was first discovered by Baltimore & Sen as a B cell specific nuclear protein that binds to a site in the immunoglobulin kappa (Igκ) light chain gene enhancer [6]. NF-κB is basically a highly conserved and inducible transcription factor, which regulates the expression of over 200 genes involved in a broad range of events, including the immune response[7], inflammation [8], differentiation, proliferation, cell survival, apoptosis [9, 10]. The role of NF-κB in protection of cells from the complement dependent cytotoxicity has been recently reported by Gancz et al [11]. Although there are few exceptions where NF-κB contributes to cell death [12], in most cases, the expression of NF-κB target genes promotes cellular survival. Normally, NF-κB transcription factor is bound to the Inhibitor(s) of kappa B (IκB) and is located in the cytoplasm. The NF-κB is activated by numerous stimuli through a variety of receptors or other intrinsic activation pathways. This recruits unique combinations of scaffolding and signaling proteins, that ultimately converge to the IκB kinase (IKK) complex. There are over 150 different stimuli that can activate NF-κB [13]. Most of the disparate ligands act upon similar cell surface and intracellular receptors including the cytokines (TNF-α, IL-1α/β and TRAIL), [14] bacterial molecules (LPS, flagellin, and non-methylated dsDNA) [15], viral components (dsDNA, dsRNA and ssRNA), DNA damaging agents (ionizing radiation or oxidative stress and chemotherapeutic drugs) [16, 17]. A majority of NF-κB activators are functionally related to either pathogenic cellular invasion or a cellular insult that initiates an immune response. Overall, NF-κB is activated in parallel with other mitogenic pathways, through induction of its genetic network (Figure 1).

Abnormal activation of NF-κB subsidizes in many human diseases, such as in cancer and

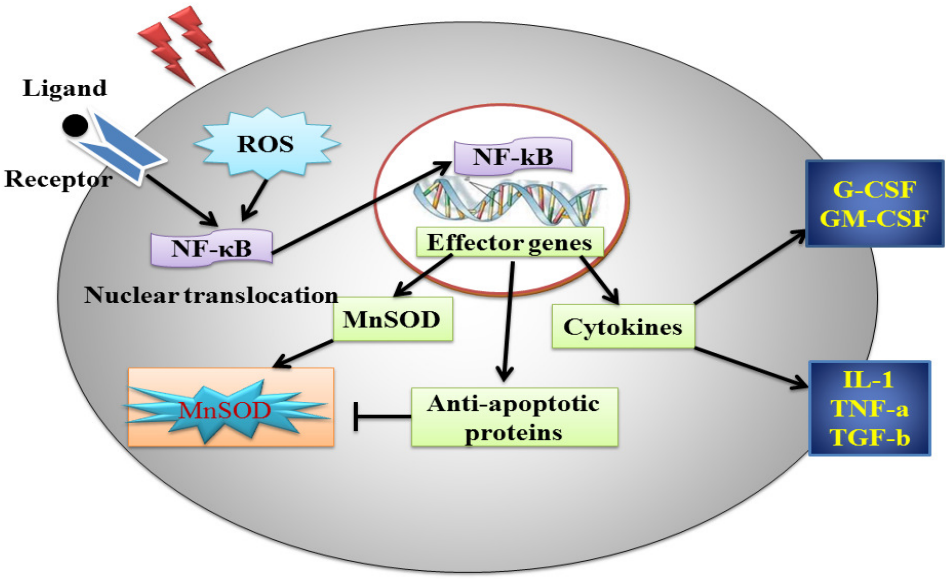

Figure 1. Schematic picture of nuclear factor (NF)-κB signaling events that influence the cellular responses to IR

inflammatory diseases. Hence, elucidating how NF-κB signaling is regulated in different contexts is important for the identification and development of therapeutics for various ailments, such as atherosclerosis, asthma, arthritis and cancer [18, 19].NF-κB is one of the major targets for the screening and identification of promising radiation countermeasure agents (RCAs). In this review, we have mainly focused on NF-kB modulation following IR exposure and associated target genes for NF-kB in relation to identification of RCAs. We have also discussed the current status of RCAs ,specifically their role in NF-κB activation.

2. NF-κB/IκB family members & associated proteins

The mammalian NF-κB/Rel family possesses five different related monomers (RelA (p65), c-Rel, RelB, NF-κB1 (p50; p105), and NF-κB2 (p52; p100)) that form homo- and hetero-dimers, and bind to 10-base pair kappa B site of promoter region of target genes [20]. The N-terminus of these proteins contains the structurally conserved 300 amino acid sequence called the RHD region, which possesses the dimerization domain (DM), nuclear localization sequence (NLS), DNA-binding domain (DBD) and interaction site with IκBs [21, 22]. Three of the family members, RelA, c-Rel, and RelB, have a C-terminal transactivation domain (TAD) that regulates expression of genes. RelA and RelB have two subdomains (TA1/2) of C-terminal transactivation domain [23]. NF-κB1/p105 and NF-κB2/p100 are the inactive precursors of the p50 and p52 proteins, respectively (Figure 2) [14]. All monomers of Rel family are capable to form 14 types of homo- or heterodimers and thereby determine the intrinsic NF-κB specificity and its regulation[24, 25, 26, 27], with the exception of RelB, which can only form heterodimers (Figure 3).

Different NF-κB dimeric complexes are formed as per cell type and stimulus; some of the physiological important dimers are RelA/p50, cRel/p50 and RelB/p52[22]. RelA and p50 exists in a wide variety of cell types [28]; while c-Rel expression is limited to hematopoietic cells and lymphocytes. The RelB expression is highly specific, being found in the thymus, lymph nodes, and Peyer's patches [20]. Each NF-κB dimer has the ability to bind with varying affinities to κB sites bearing the consensus sequence GGGRNNYYCC (R, purine: Y, pyrimidine: N, any base) and exhibit their unique functions [29]. However, NF-κB complexes composed only of the family members lacking TAD, such as the p50 homodimers, are known to impose transcriptional repression [30]. For all diverse functions of NF-κB in general, the activity is controlled by a family of regulatory proteins, called inhibitors of NF-κB (IκBs; IκB-α, IκB-β, IκB-ε, IκB-ζ, Bcl-3 etc) [14, 30] (Figure 2).

Three of "typical" IκBs (IκB-α, IκB-β, and IκB-ε), bind to NF-κB proteins and mask their nuclear translocation and DNA binding activity. IκBs also regulates the export of NF-κB proteins from the nucleus, and are thus known for inhibitory processes in multiple ways [31]. Recent investigations suggest that p100, when located in a

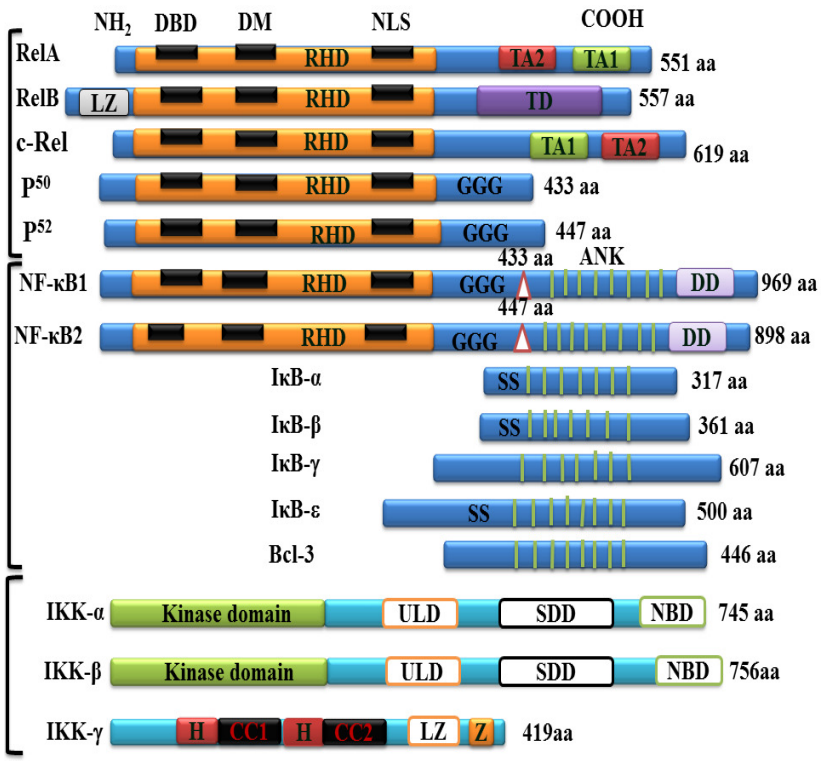

Figure 2. Schematic drawings of NF-κB/Rel proteins. Structures of the mammalian NF-κB, IκB, and IKK proteins.

The number of amino acids in each protein is indicated on the right. Presumed sites of cleavage for p105/NF-κB1 (amino acid 433) and p100/NF-κB2 (amino acid 447) are shown on the top of each protein. The positions of functional domains are indicated, including the Rel homology domain (RHD), DNA binding domain (DBD), dimerization domain (DM), nuclear localization signal (NLS), transactivation domains (TD). TA1 and TA2 subdomain of TD presented in RelA and cRel, glycine-rich hinge region (GGG), ankyrin repeats (ANK), double serine phosphorylation sites (SS), leucine zipper (LZ), helix-loop-helix (HLH), NEMO-binding domain (NBD), α-helix (H), coiled coil (CC), and zinc finger (Z).

multimeric complex, may also mediate NF-κB inhibition *in trans*; this activity is termed as IκBδ [32, 33]. The complex of IκBs proteins and NF-κB dimers weas originally thought to be retain in the cytoplasm by the NF-κB super repressor IKK. IKK complex is formed by three different subunits: two catalytic subunits IKKα (IKK1 or CHUK), IKKβ (IKK2) and the regulatory subunit IKKγ. IKKγ is also known as NF-κB essential modulator (NEMO) protein (Figure 2). Although IKKα and IKKβ cooperate for IκBs phosphorylation, these proteins differ in the signals that they mediate.

3. NF-κB Activation Pathways

There are four models that have been proposed to explain NF-κB activation [34]. NF-κB is activated by numerous pathological and physiological conditions in a very efficient manner. NF-κB also regulates expression of various genes by modulating promoter activity of targets genes [35].

3.1. The IKKβ dependent (classical) pathway The IKKβ dependent NF-κB activation has been a well studied signaling event. It is also known as the classical or NEMO (IKK-γ)-dependent or canonical pathway (Figure 4). It is induced by several of innate and adaptive immunological agents, and can be turned on within minutes. It principally requires IKKβ components [36, 37]. Phosphorylation of IKKβ at Ser177 and Ser181 may occur after stimulation by TNFR, IL-1R, TLR agonists, radiation exposure, TNF-α (tumour necrosis factor-α), PMA (phorbol 12-myristate 13-acetate), interleukins and other factors, which regulate downstream phosphorylation of IκB-α at Ser32 and Ser36, or IκBβ at Ser19 and Ser23, through the function of ubiquitin-dependent protein kinases. Phosphorylated IκB proteins are then ubiquitinated at nearby lysine residues (lysines 21 and 22 of IκBα and lysine 9 of IκB-β), and thus triggers a rapid degradation of IκB proteins by 26S proteasome [38, 39]. The rapid degradation of IκB-α, IκB-β, and IκB-ε occurs during classical NF-κB signaling pathway. Phosphorylated p65/p50 (phosphorylation of p65 at Ser536) complex quickly translocates into nucleus and binds to 10-bp kB site or interacts with other transcription factors and regulates expression of various target genes. IκBα is a well known regulatory protein (providing a feedback control) for this pathway. The newly synthesized IκBα enters into the nucleus and prevents NF-κB DNA binding activity and transports NF-κB back into the cytoplasm.

3.2. The IKKα dependent (alternative) pathway

Alternative or NEMO-independent or non-canonical pathway is mainly activated during secondary lymphoid organ development, homeostasis and adaptive immunity, and it turns on in few hours [40]. Senftleben et al. first described

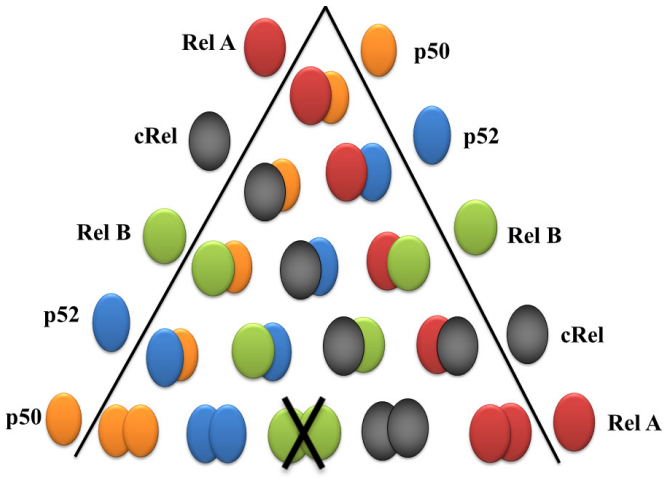

Figure 3. Different stimuli induce specific formation of known homo and hetero NF-κB dimers.

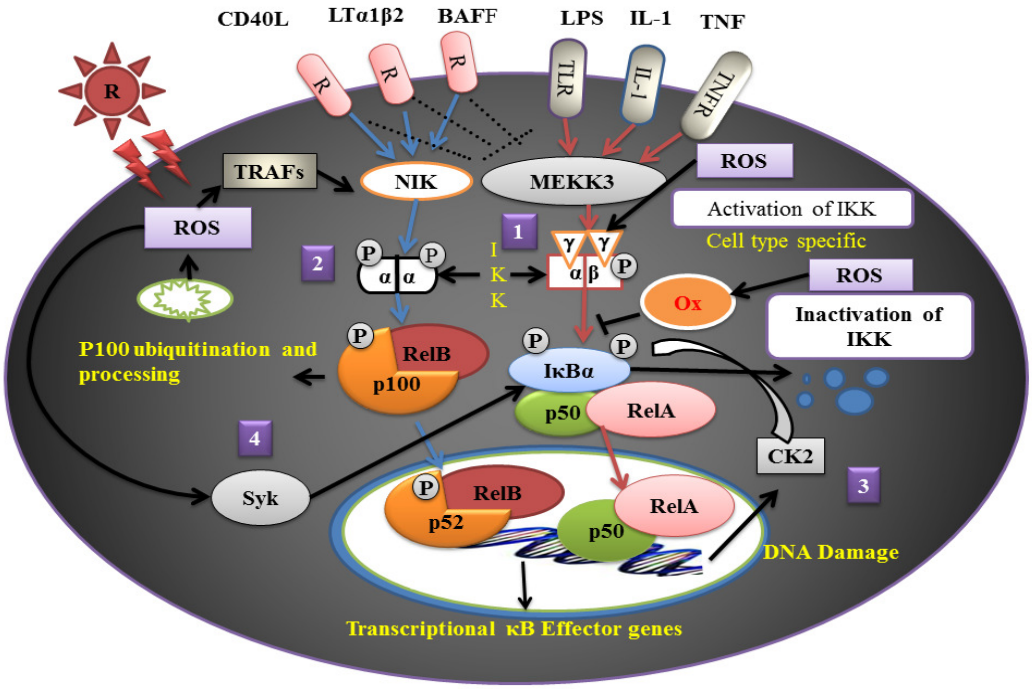

Figure 4. There are four proposed NF-κB signaling pathways in response to various stimuli.

(1) The canonical pathway (2), the non-canonical pathway (3), atypical pathway and (4) oxidative stress-induced pathway. Downstream binding of the NF-κB proteins to DNA regulates downstream transcriptional of many potential antioxidant, pro-oxidant, cell cycle regulation and anti-apoptotic targets that have been shown in Supplementary Table 1.

IKKα dependent pathway in which processing of p100 and activation of p52/RelB is defined as the alternative pathway (Figure 4) [39]. In this pathway phosphorylation of IKKα homodimer at Ser176 and Ser180 occurs through the upstream kinase NIK, (NF-κB inducing kinase). This pathway is stimulated by specific TNF receptor family members, such as LTβR, CD40, CD27, CD30, BAFF-R, RANK and others [41], that signal through the recruitment of TRAF2 and TRAF3. In the resting cells, continuous degradation of NIK prevents non-canonical NF-κB activation [42].

3.3 Atypical pathway

This pathway is essentially independent of IKK and it is mainly triggered in case of UV or chemical-induced DNA damages [43, 44]. Evidence suggests that CK2 (formally known as casein kinase II) is a stress-activated protein kinase involved in the transduction of survival signals (Figure 4) [45, 46]. CK2-mediated IκBα phosphorylation has an important UV-protective function. Jung et al. demonstrated a correlation of ATM with NF-κB in cellular radiosensitivity [47] and suggested that the loss of ATM function promotes radiosensitivity by activation of NF-κB [47]. Recently, Wu et al. [48] demonstrated that the cytosolic activation of signaling and sensor complexes (ATM, NEMO, IKK catalytic subunits, and ELKS - an IKK regulatory subunit) are associated with nuclear DNA damage-induced NF-κB activation. This model was proposed on their findings that ATM interacts with NEMO and phosphorylates NEMO at Ser85 after DSBs.

3.4. Oxidative stress-induced pathway

Oxidative stress-induced activation of NF-κB signaling is achieved *via* IκB-α tyrosine phosphorylation without degradation of IκB-α by Syk protein tyrosine kinase (Figure 4) [49, 50]. H_2O_2 is one of the central free radical, involved in different cellular processes, including NF-κB activation [51].The redox-sensitive pathways triggering this activation may vary with everh cell and cell-type [50]. NF-κB is also sensitive to oxidative modifications of Cys62 in p50, which are essential for DNA binding [52, 53]. Activation and translocation of NF-κB is stimulated by oxidative circumstances, while its DNA binding affinity is inhibited by the redox sensitive cysteine residue [54, 55]. The tyrosine phosphorylation of IκBα by most agents does not lead to IκBα degradation. However, Pervanadate (it is a protein tyrosine phosphatase inhibitor)-induced activation of NF-κB signaling, tyrosine phosphorylation and degradation of IκB-α has been documented [56]. Surprisingly, UV-C induced NF-κB

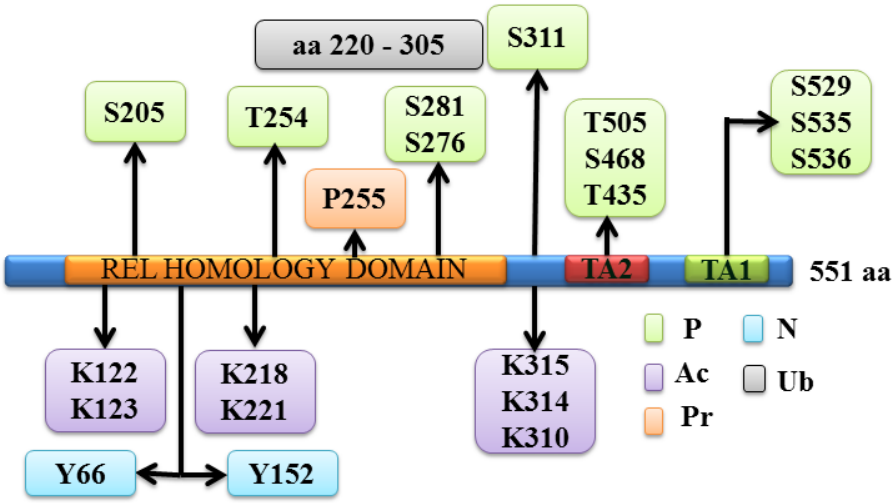

Figure 5. Phosphorylation and acetylation sites within NF-κB p65.

Eight Serine three Threonine residues phosphorylation and seven acetylation sites have been identified in the NF-κB p65 subunit. Abbreviations: Ac, acetylation; K, lysine; N, tyrosine nitration; P, phosphorylation; Pr, proline isomerization; S, serine; T, threonine; Ub, ubiquitination; Y, tyrosine.

activation is mediated through the degradation of IκB-α, that involves neither phosphorylation of serine nor the tyrosine residue of IκB-α [57].

4. Post translational modifications of NF-κB proteins

The mammalian transcription factor NF-κB is activated by over 150 diverse stimuli and thousands of potential NF-κB DNA binding sites have been marked across the genome [13, 58]. After degradation of IκBs, activated NF-κB complex moves into nucleus and binds to 10bp defined sequence GGGRNWYYCC (N represents any base, R represents a purine; W represents an adenine or a thymine and Y represents a pyrimidine), which is present in the promoter and enhancer regions of

Table 1. The phosphorylation sites of p65, and responsible kinases

Site	Location	Kinase	Function	Reference
Ser 205*	RHD	unknown	Transcriptional activity	66
Ser 276	RHD	PKAc MSK1	Transcriptional activity Captivator binding Transcriptional activity	67, 68
Ser 281*	RHD	unknown	Transcriptional activity	66
Ser 311	RHD	PKCζ	Transcriptional activity	69
Ser 468	TA2	GSK3β IKKβ IKKα	Transcriptional activity Transcriptional activity Transcriptional activity	70, 71, 72
Ser 529	TA1	CK II	Transcriptional activity	73
Ser 535	TA1	CaMKIV	Transcriptional activity	74
Ser 536	TA1	IKKα IKKβ IKKε TBK1 RSK1	Transcriptional activity and stabilization Transcriptional activity and nuclear import Transcriptional activity Nuclear localization Affinity to IκBα	75-81
Ser 547*	unknown	ATM-DSB	Transcriptional inhibition of target genes by HDAC recruitment	82
Thr 254*	RHD	unknown	Stabilization and Nuclear localization	83
Thr 435*	TA2	unknown	Transcriptional activity	84
Thr 505	TA2	ATR ChK1	Transcriptional activity	85, 86
Tyr 66 Tyr 152	RHD	NO treatment	p65 dissociation from p50 and association with IκBα	87

Site	Location	Enzyme	Function	Reference
Lys 122	RHD	P300, PCAF	Inhibition DNA binding	88
Lys 123	RHD	P300, PCAF	Inhibition DNA binding	88
Lys 218	RHD	CBP/p300	Unknown	89
Lys 221	RHD	CBP/p300	Promoting DNA binding Inhibition IκBα binding	89
Lys 310	RHD	CBP/p300	Enhancing transactivation	89
Lys 314	RHD	P300	Transcriptional activity	90
Lys 215	RHD	P300	Transcriptional activity	90

Acetylation sites of p65 and the corresponding enzymes

* Recently discovered phosphorylation sites

target genes [59]. Moreover, activity and DNA binding affinity of NF-κB transcription factor are spatially and kinetically controlled, thereby regulating expression of its target genes [60]. Within the nuclear compartments, various posttranslational modifications (PTMs) of NF-κB occurs, such as: ubiquitination, acetylation and phosphorylation [61]. Among all NF-κB subunits, most of the post-translational modifications take place in the p65 subunit, which is known to be modified by phosphorylation, acetylation, prolylisomerization, nitrosylation and ubiquitination (Figure 5 and Table 1) [12]. Phosphorylation of p65 unit takes place either in the cytoplasm or in the nucleus, and is mediated by numerous protein kinases. These sites can be modified in a stimulus- and/or cell type-specific fashion by several kinases (Table 1) [62-65].

PTMs of p65 can regulate the interaction with co-activators [91], co-repressors [92] promoter-bound degradation [93] and interactions with the basal transcriptional machinery [94]. According to the NF-κB barcode hypothesis the differential modifications of the DNA-binding subunits generate distinct arrays that function through transcription in a highly target gene-specific manner [95]. Other than p65 post-translation modifications, NIK and IKKα (IKK1)-mediated phosphorylation of p105 NF-κB occurs at multiple sites (Ser921, 923, 927, and 932) on its carboxyl-terminus. SCF/β-TrCP-mediated processing of p105 NF-κB produces the 50 kDa active form product, p50 [96, 97]. NF-κB p50 serine 337 is phosphorylated in response to PKA, which regulates the binding affinity of NF-κB p50 and impacts the NF-κB transcriptional activity. In addition to post-translational modifications, recent studies showed the ability of NF-κB to bind the DNA (NF-κB: DNA) is also regulated by other proteins. A recent report suggests that RPS3 (ribosomal protein subunit 3) interacts with RelA *via* its KH (K Homology) domain and specifically enhances p50: RelA binding affinity with DNA (p50:RelA:DNA) [98].

5. NF-κB regulated proteins and their functions in oxidative stress

Exposure of mammalian cells with low doses of ionizing radiation is known to have variable effects and may generate valuable effects within cells [99, 100]. The correct cellular response to ROS following low doses of IR is consequently critical, in order to reduce further oxidative damage, and to maintain cell survival through initiation of cellular signaling, including NFkB pro-survival signaling. Therefore ROS-mediated NF-κB response and thereby regulation of NF-κB target genes may attenuate cell survival. One important way in which NF-κB activity influences ROS levels is by increasing expression of antioxidant and anti-apoptotic proteins. Since NF-κB is known to play a central role in inflammation, some enzymes that promotes the production of ROS are also controlled as well as its targets, particularly in cells of the immune system [31]. A few known or possible NF-κB target genes that may contribute to the protection of cells from ROS-induced cellular damage are mentioned in Supplementary Table 1[101-191].

6. Radiation Countermeasures in relation to NF-κB activation

The radioprotective agent can be described as the "molecule(s) or compound(s) that protects against radiation-induced cellular, tissue injury, when applied before, during, or after irradiation in a specified time period" [192, 193]. A number of chemical compounds that are identified and evaluated for radio-protective efficacy may be classified as (Supplementary Table 2[198-258] and Figure 6):

- Prophylactic agents,
- Mitigators and
- Therapeutic agents

To date, there are no safe and effective drugs for the protection against ionizing radiation damage. Therefore, a great need exists to identify and develop non-toxic agents that will be useful as radio-protectors or post-irradiation therapies under a variety of operational scenarios. Suppressing of IR-induced cell death or enhancing survival, proliferation, differentiation of cells are the major ways to obtain protection mechanisms against radiation, addressing the massive cell loss in radiosensitive tissues specifically hematopoietic system (HP) and gastrointestinal tract [194, 195, 196]. Some of radio-protective agents that are currently in clinical trials are listed in Supplementary Table 2. NF-κB plays important roles in immunity and cellular survival in response to radiation exposure and oxidative stress. It is known to reduce programmed cell death or apoptosis by promoting the expression of anti-apoptotic proteins and antioxidant molecules associated with enhanced radio-resistance, whereas its deletion in mice results

in hypersensitivity to ionizing radiation-induced GI damage [21, 197].

NF-κB also regulates the production of a wide variety of cytokines in a cell type specific manner. Some of these cytokines induce proliferation and survival of hematopoietic stem cells, thereby promoting bone marrow recovery and tissues regeneration following irradiation. Therefore, pharmacological activation of NF-κB may be considered as a possible approach for radioprotection / mitigation. In this review, we have discussed some radioprotectors/ mitigators, specifically in relation to their efficacy for activation of NF-κB. Great efforts have been directed towards recognizing the role of TLRs (Toll Like Receptor)-mediated responses to microbes (viruses, bacteria, fungi) for the development of novel therapies in autoimmune allergic diseases, malignancy and other infections [259]. Investigations of TLR agonists are one of the global recent interests, for use in the preparation of immune-modulators. TLR agonists include: small molecules, pathogen derived DNA, RNA, proteins, lipids, which target one or more of the toll-like receptors, including TLR 2-9. Bacterial flagellin, the natural ligand of TLR5, was found to have radioprotective effects in rodents and nonhuman primates [260]. Recently, Cleveland Bio-Lab has developed the new pharmacological CBLB series, including CBLB502, for radiological emergencies. CBLB502 is a rationally designed derivative of Salmonella flagellin. It is substantially less immunogenic than full length flagellin and possesses its TLR5-dependent NF-κB–inducing activity and radioprotective ability [208]. Moreover, CBLB502 protected mice from dermatitis and mucositis associated with local fraction irradiation of head and neck area modelling radiation treatment of patients with head and neck cancer and also was shown to be effective as a tissue protectant in mouse models of renal ischemia-reperfusion injury[261]. A single dose of CBLB502 (0.2mg/kg body weight) 30 min prior to 13 Gy of TBI to NIH-Swiss mouse offered 87% protection. Administration of CBLB502 even up to 1 h post-irradiation results in greater than 90% survival after 9 Gy. CBLB502 also showed radio-protective efficacy in lethally irradiated rhesus monkeys [208]. Burdelya et al, recently showed that liver was the primary responsive organ for CBLB502 and CBLB502-mediated radioprotection of the HP system. The radioprotection occurred by factors secreted by responsive liver hepatocytes. A strong suppression of growth of tumor cells in the liver, regardless of their TLR5 status, was also observed[209].

Recently, a lipopeptide of Mycoplasma arginini has been reported to act as a TLR 2/6 agonist. This novel radiation countermeasure, CBLB 613, has been observed as possible radio-mitigator for humans against radiation induced lethality [262]. CBLB613 significantly protected mice against a lethal dose of γ-radiation with no observable toxicity at 1.79 mg/kg body weight and 1 mg/kg body weight for single and repeated doses, respectively. In irradiated CD2F1 mice it stimulates bone marrow cellularity, enhances production of cytokines, such as interleukin-1β (IL-1β), IL-6, IL-10, IL-12, keratinocyte-derived chemokine, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumour necrosis factor-1α (TNF-α), and reduces radiation-mediated cytopenia. CBLB613 exhibits substantial dose reduction factor of 1.25.

The baicalein is a bioactive flavonoid, which has been shown to have antioxidant, anti-inflammatory and anti-hepatotoxic properties in both *in vitro* and *in vivo* conditions [263, 264]. Treatment with baicalein inhibits the inflammatory signaling pathways involving ERK (extracellular signal-regulated kinase), Akt and nuclear factor-κB (NF-κB) activities in vascular smooth muscle cells [265]. A recent study showed that γ-irradiation with baicalein reduces lipid and protein oxidation in rat liver. Damaging effects of IR are generally mediated through the production of reactive species (RS), and a substantial increase in RS levels induces cellular damage and decrease in antioxidant enzymes, as well as activates intracellular signaling pathways that activate the expression of many inflammatory genes. The IR induced molecular responses may also be characterized as increased cyclooxygenase-2 (COX-2) level, inducible nitric oxide synthase (iNOS) and vascular adhesion molecule-1 (VCAM-1) expressions that also initiates the activation of the transcription factor NF-κB [266]. The key role played by NF-κB activation in the process of inflammation has been reported to be closely associated with a redox-sensitive signal cascade that includes MAPKs (ERK, c-Jun N-terminal kinase (JNK] and p38) and Akt [266, 267].

However, activation of the Akt signaling pathway has been known to reduce forkhead box-O (FOXO) transcription activity [268], and is involved in cytoprotective effects against oxidative stress [269]. Irradiation of mice showed an enhancement of NF-κB-mediated inflammatory factors due to the oxidative damage, and the inactivation of FOXO and its target genes, such as catalase and SOD. However, baicalein (5mg/kg bw/day) has the ability to suppress radiation-induced inflammatory consequences, by down regulating NF-κB and up-regulating FOXO activation [270]. Furthermore, baicalein inhibited radiation induced phosphorylation of MAPKs and Akt, which are upstream kinases of NF-κB and FOXOs. These observations also suggest that baicalein has a radioprotective effect against NF-κB mediated inflammatory response, through MAPKs and the Akt pathway, which is complemented by the protective effects on FOXO and its target genes, such as catalase and SOD.

DNA double-strand breaks (DSBs) are the most deleterious form of DNA damage and numerous *in vitro* studies have analyzed the DSB repair system that is activated after exposure to ionizing radiation. DSBs rapidly trigger the activation of NF-κB pathway *via* NEMO [48, 271]. The death-domain protein PIDD was originally identified as an early p53-inducible gene and is implicated in p53-induced apoptosis [48]. PIDD is a mediator of the

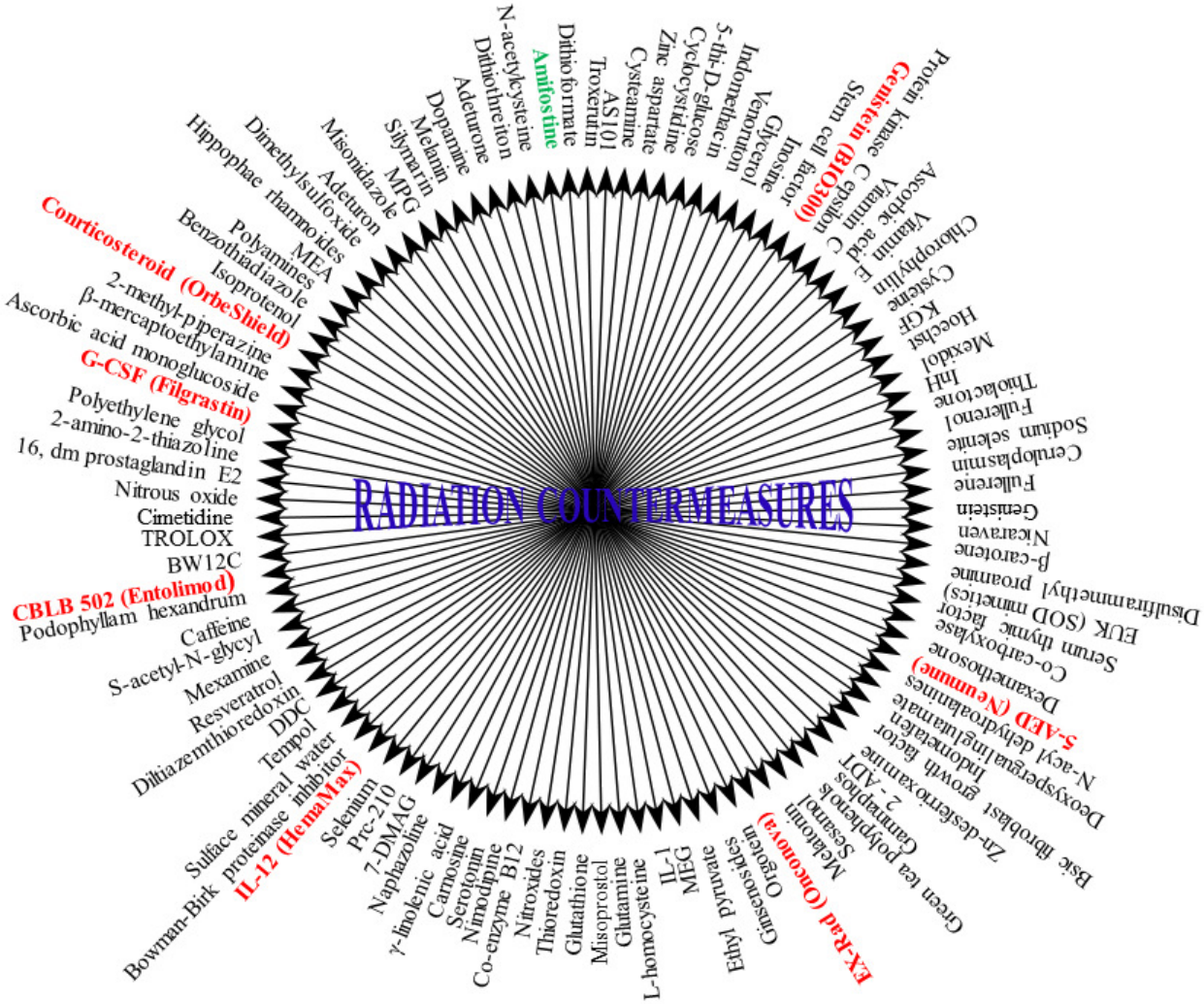

Figure 6. Schematic representation of chemical compounds that have been evaluated for radio-protective potential until today.

DNA-damage-activated stress response and is involved in genotoxic stress-induced NF-κB activation [271, 272]. PIDD expression enhances genotoxic-stress-induced NF-κB activation through augmented sumoylation and ubiquitination of NEMO [272]. Corilagin (ß-1-O-galloyl-3, 6-(R)-hexahydroxydiphenoyl- D-glucose) is a member of the tannin family and has been isolated from medicinal plants, such as the *Phyllanthus* sps [273]. Corilagin has antioxidative, atherogenic, and hypertensive effects in various models [273-276]. A preliminary *in vitro* study suggested that corilagin has anti-inflammatory activity [277]. The activation of microglia and release of pro-inflammatory cytokines post irradiation are regarded as the key effectors of RIBI. Recent, studies demonstrated that corilagin exhibited anti-inflammatory activity in irradiated B7-2 cells by suppressing the release of pro-inflammatory cytokines and mediators. Corilagin suppresses the transcription of pro-inflammatory cytokine genes, through effects on the DSB-triggered NF-κB signaling pathway [278].

Ex-RAD employs a novel mode of action, involving the enhancement of internal DNA repair pathways, which significantly reduces the levels of p53, p21, bax, c-abl and p73 proteins-key players in the DNA damage cascade induced upon exposure to 8.0 Gy gamma irradiation [207]. These mechanisms can cause a halt in cell death pathways and lead to increased recovery and survival of irradiated cells. These novel mechanisms of action attended by minimal side effects suggest that Ex-RAD could be useful both as a prophylactic and mitigative agent. Ex-RAD (4-carboxystyryl-4-chlorobenzylsulfone, sodium salt; or ON 01210.Na] is a synthetic small-molecule radioprotective compound (from Onconova Therapeutics, Inc. (OTI)) that is active in male C3H mice [207] when administered 24 h and 15 min (two injections) before total body irradiation (TBI). Although Ex-RAD had been shown to be an inhibitor of apoptosis *in vitro*, it is not recognized whether a parallel mechanism is occurring *in vivo* [207]. In numerous cell-based and complete animal models, Ex-RAD has revealed to have potential for defense from radiation injury when administered either before or after radiation exposure. The drug is currently in Phase I clinical trials in humans. In decision, Ex-RAD usage mitigates potentially life-threatening neutropenia and bone marrow overthrow and, in turn, stimulates bone marrow retrieval, decreases radiation induced phosphorylation of p53 signaling, and enhances survival of acutely irradiated mice [279]. In addition to mitigating of hematopoietic damage, Ex-RAD also moderates intestinal injury. However, the molecular mechanisms elaborated in Ex-RAD's promotion of recovery of hematopoietic and GI tissues warrant further study.

7. Conclusion

Radiation-induced injuries and lethality are well described at clinical level and understanding of mechanisms of tissue responses in the event of radiation exposure has gained much attention in recent years. The quest to search a potent radiation countermeasure which can ameliorate radiation syndrome and at the time exhibits no toxicity for human consumption is prevalent since past decades. However, even after the existence of a lot of literature available on radiation counter-measures only handful of identified drugs seem promising for human use. Based on prudent dissection of complicated series of signaling changes within multiple pathways, it might be possible to rationally combine inhibitors of these cascades, to repair damaged bio-molecules, activation of intracellular pathways, stress receptor activation, to achieve radiation protection. As a stress sensor, NF-κB is a crucial component of the cell's protective response to radiation and therefore an attractive target in the new therapeutic lines to fight cancer or radiological emergencies. NF-κB is now documented as an important player in several critical steps for development of radiation countermeasures.

Recently, focus of radiation protection has shifted to test the radioprotective potential of plant products and herbs in the hope that one day it will be possible to find a suitable pharmacological agent that could protect humans against the deleterious effects of ionizing radiation in clinical and other conditions as well as during nuclear terror attack. Majority of plants and herbs described in this review have medicinal properties and are being used in traditional Ayurvedic or Chinese systems of medicine to treat various ailments in humans. Our review provides a broad idea on the physicochemical role of ionizing radiation on cellular systems and highlights the importance of developing new natural radioprotectants. Medicinal plants like *Aconitum heterophyllum, Bergenia stracheyi, Bunium persicum, Dactylorhiza hatgirea, Ephedra gerardiana, Pichorrhiza kurroa*, etc., are

some of the plants that need elaborate investigations. Furthermore, some radioprotectants may boost their own efficacy in combination therapies Fractionation guided evaluation may result in the development of ideal radioprotectors in the near future.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Hall EJ, Giaccia AJ. *Radiobiology for the Radiologist*. Lippincott Williams & Wilkins, 2006.
2. Park WY, Hwang CI, Im CN, Kang MJ, Woo JH, Kim JH*, et al.* Identification of radiation-specific responses from gene expression profile. *Oncogene* 2002, 21(55): 8521-8528.
3. Dent P, Yacoub A, Contessa J, Caron R, Amorino G, Valerie K*, et al.* Stress and radiation-induced activation of multiple intracellular signaling pathways. *Radiat Res* 2003, 159(3): 283-300.
4. Chaudhry MA. Analysis of gene expression in normal and cancer cells exposed to gamma-radiation. *J Biomed Biotechnol* 2008, 2008: 541678.
5. Chaudhry MA. Biomarkers for human radiation exposure. *J Biomed Sci* 2008, 15(5): 557-563.
6. Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell* 1986, 47(6): 921-928.
7. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol* 1994, 12: 141-179.
8. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997, 336(15): 1066-1071.
9. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2002, 2(4): 301-310.
10. Bours V, Bonizzi G, Bentires-Alj M, Bureau F, Piette J, Lekeux P*, et al.* NF-kappaB activation in response to toxical and therapeutical agents: role in inflammation and cancer treatment. *Toxicology* 2000, 153(1-3): 27-38.
11. Gancz D, Lusthaus M, Fishelson Z. A role for the NF-kappaB pathway in cell protection from complement-dependent cytotoxicity. *J Immunol* 2012, 189(2): 860-866.
12. Perkins ND, Gilmore TD. Good cop, bad cop: the different faces of NF-kappaB. *Cell Death Differ* 2006, 13(5): 759-772.
13. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 1999, 18(49): 6853-6866.
14. Silverman N, Maniatis T. NF-kappaB signaling pathways in mammalian and insect innate immunity. *Genes Dev* 2001, 15(18): 2321-2342.
15. Byrd-Leifer CA, Block EF, Takeda K, Akira S, Ding A. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur J Immunol* 2001, 31(8): 2448-2457.
16. Bender K, Gottlicher M, Whiteside S, Rahmsdorf HJ, Herrlich P. Sequential DNA damage-independent and -dependent activation of NF-kappaB by UV. *EMBO J* 1998, 17(17): 5170-5181.
17. Huang TT, Wuerzberger-Davis SM, Wu ZH, Miyamoto S. Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. *Cell* 2003, 115(5): 565-576.
18. Baldwin AS, Jr. Series introduction: the transcription factor NF-kappaB and human disease. *J Clin Invest* 2001, 107(1): 3-6.
19. Shishodia S, Aggarwal BB. Nuclear factor-kappaB: a friend or a foe in cancer? *Biochem Pharmacol* 2004, 68(6): 1071-1080.
20. Li Q, Verma IM. NF-kappaB regulation in the immune system. *Nat Rev Immunol* 2002, 2(10): 725-734.
21. Ahmed KM, Li JJ. NF-kappa B-mediated adaptive resistance to ionizing radiation. *Free Radic Biol Med* 2008, 44(1): 1-13.
22. Shih VF, Tsui R, Caldwell A, Hoffmann A. A single NFkappaB system for both canonical and non-canonical signaling. *Cell Res* 2011, 21(1): 86-102.
23. Schmitz ML, Baeuerle PA. The p65 subunit is responsible for the strong transcription activating potential of NF-kappa B. *EMBO J* 1991, 10(12): 3805-3817.
24. Kuriyan J, Thanos D. Structure of the NF-kappa B transcription factor: a holistic interaction with DNA. *Structure* 1995, 3(2): 135-141.
25. Ghosh G, van Duyne G, Ghosh S, Sigler PB. Structure of NF-kappa B p50 homodimer bound to a kappa B site. *Nature* 1995, 373(6512): 303-310.
26. Gossen M, Freundlieb S, Bender G, Muller G, Hillen W, Bujard H. Transcriptional activation by tetracyclines in mammalian cells. *Science* 1995, 268(5218): 1766-1769.
27. Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappa B/I kappa B

family: intimate tales of association and dissociation. *Genes Dev* 1995, 9(22): 2723-2735.
28. Nabel GJ, Verma IM. Proposed NF-kappa B/I kappa B family nomenclature. *Genes Dev* 1993, 7(11): 2063.
29. Hayden MS, Ghosh S. Signaling to NF-kappaB. *Genes Dev* 2004, 18(18): 2195-2224.
30. May MJ, Ghosh S. Rel/NF-kappa B and I kappa B proteins: an overview. *Semin Cancer Biol* 1997, 8(2): 63-73.
31. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res* 2011, 21(1): 103-115.
32. Basak S, Kim H, Kearns JD, Tergaonkar V, O'Dea E, Werner SL*, et al.* A fourth IkappaB protein within the NF-kappaB signaling module. *Cell* 2007, 128(2): 369-381.
33. Savinova OV, Hoffmann A, Ghosh G. The Nfkb1 and Nfkb2 proteins p105 and p100 function as the core of high-molecular-weight heterogeneous complexes. *Mol Cell* 2009, 34(5): 591-602.
34. Dejardin E. The alternative NF-kappaB pathway from biochemistry to biology: pitfalls and promises for future drug development. *Biochem Pharmacol* 2006, 72(9): 1161-1179.
35. Sha WC. Regulation of immune responses by NF-kappa B/Rel transcription factor. *J Exp Med* 1998, 187(2): 143-146.
36. Delhase M, Hayakawa M, Chen Y, Karin M. Positive and negative regulation of IkappaB kinase activity through IKKbeta subunit phosphorylation. *Science* 1999, 284(5412): 309-313.
37. Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M*, et al.* The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. *J Exp Med* 1999, 189(11): 1839-1845.
38. Chen JY, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett JE*, et al.* RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO J* 1995, 14(6): 1187-1197.
39. Baeuerle PA, Rupec RA, Pahl HL. Reactive oxygen intermediates as second messengers of a general pathogen response. *Pathol Biol (Paris)* 1996, 44(1): 29-35.
40. Bellezza I, Mierla AL, Minelli A. Nrf2 and NF-kappaB and Their Concerted Modulation in Cancer Pathogenesis and Progression. *Cancers (Basel)* 2010, 2(2): 483-497.
41. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell* 2001, 7(2): 401-409.
42. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. *J Biol Chem* 2004, 279(25): 26243-26250.
43. Kato T, Jr., Delhase M, Hoffmann A, Karin M. CK2 Is a C-Terminal IkappaB Kinase Responsible for NF-kappaB Activation during the UV Response. *Mol Cell* 2003, 12(4): 829-839.
44. Tergaonkar V, Bottero V, Ikawa M, Li Q, Verma IM. IkappaB kinase-independent IkappaBalpha degradation pathway: functional NF-kappaB activity and implications for cancer therapy. *Mol Cell Biol* 2003, 23(22): 8070-8083.
45. Ahmed K, Gerber DA, Cochet C. Joining the cell survival squad: an emerging role for protein kinase CK2. *Trends Cell Biol* 2002, 12(5): 226-230.
46. Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochem J* 2003, 369(Pt 1): 1-15.
47. Jung M, Zhang Y, Lee S, Dritschilo A. Correction of radiation sensitivity in ataxia telangiectasia cells by a truncated I kappa B-alpha. *Science* 1995, 268(5217): 1619-1621.
48. Wu ZH, Shi Y, Tibbetts RS, Miyamoto S. Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. *Science* 2006, 311(5764): 1141-1146.
49. Takada Y, Mukhopadhyay A, Kundu GC, Mahabeleshwar GH, Singh S, Aggarwal BB. Hydrogen peroxide activates NF-kappa B through tyrosine phosphorylation of I kappa B alpha and serine phosphorylation of p65: evidence for the involvement of I kappa B alpha kinase and Syk protein-tyrosine kinase. *J Biol Chem* 2003, 278(26): 24233-24241.
50. Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol* 2006, 72(11): 1493-1505.
51. Schmidt KN, Amstad P, Cerutti P, Baeuerle PA. The roles of hydrogen peroxide and superoxide as messengers in the activation of transcription factor NF-kappa B. *Chem Biol* 1995, 2(1): 13-22.
52. Matthews JR, Wakasugi N, Virelizier JL, Yodoi J, Hay RT. Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. *Nucleic Acids Res* 1992, 20(15): 3821-3830.
53. Matthews JR, Kaszubska W, Turcatti G, Wells TN, Hay RT. Role of cysteine62 in DNA recognition by the P50 subunit of NF-kappa B. *Nucleic Acids Res* 1993, 21(8): 1727-1734.
54. Droge W, Schulze-Osthoff K, Mihm S, Galter D, Schenk H, Eck HP*, et al.* Functions of glutathione and glutathione disulfide in immunology and immunopathology. *FASEB J* 1994, 8(14): 1131-1138.
55. Bowie A, O'Neill LA. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the

evidence in the light of recent discoveries. *Biochem Pharmacol* 2000, 59(1): 13-23.
56. Mukhopadhyay A, Manna SK, Aggarwal BB. Pervanadate-induced nuclear factor-kappaB activation requires tyrosine phosphorylation and degradation of IkappaBalpha. Comparison with tumor necrosis factor-alpha. *J Biol Chem* 2000, 275(12): 8549-8555.
57. Li N, Karin M. Ionizing radiation and short wavelength UV activate NF-kappaB through two distinct mechanisms. *Proc Natl Acad Sci U S A* 1998, 95(22): 13012-13017.
58. Natoli G, Saccani S, Bosisio D, Marazzi I. Interactions of NF-kappaB with chromatin: the art of being at the right place at the right time. *Nat Immunol* 2005, 6(5): 439-445.
59. Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation via the NF-kappaB signaling module. *Oncogene* 2006, 25(51): 6706-6716.
60. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008, 132(3): 344-362.
61. Wietek C, O'Neill LA. Diversity and regulation in the NF-kappaB system. *Trends Biochem Sci* 2007, 32(7): 311-319.
62. Neumann M, Naumann M. Beyond IkappaBs: alternative regulation of NF-kappaB activity. *FASEB J* 2007, 21(11): 2642-2654.
63. Perkins ND. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. *Oncogene* 2006, 25(51): 6717-6730.
64. Viatour P, Merville MP, Bours V, Chariot A. Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. *Trends Biochem Sci* 2005, 30(1): 43-52.
65. Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. *Nat Rev Mol Cell Biol* 2004, 5(5): 392-401.
66. Anrather J, Racchumi G, Iadecola C. cis-acting, element-specific transcriptional activity of differentially phosphorylated nuclear factor-kappa B. *J Biol Chem* 2005, 280(1): 244-252.
67. Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* 1998, 1(5): 661-671.
68. Vermeulen L, De Wilde G, Van Damme P, Vanden Berghe W, Haegeman G. Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). *EMBO J* 2003, 22(6): 1313-1324.
69. Duran A, Diaz-Meco MT, Moscat J. Essential role of RelA Ser311 phosphorylation by zetaPKC in NF-kappaB transcriptional activation. *EMBO J* 2003, 22(15): 3910-3918.
70. Buss H, Dorrie A, Schmitz ML, Frank R, Livingstone M, Resch K*, et al.* Phosphorylation of serine 468 by GSK-3beta negatively regulates basal p65 NF-kappaB activity. *J Biol Chem* 2004, 279(48): 49571-49574.
71. Schwabe RF, Sakurai H. IKKbeta phosphorylates p65 at S468 in transactivaton domain 2. *FASEB J* 2005, 19(12): 1758-1760.
72. Mattioli I, Geng H, Sebald A, Hodel M, Bucher C, Kracht M*, et al.* Inducible phosphorylation of NF-kappa B p65 at serine 468 by T cell costimulation is mediated by IKK epsilon. *J Biol Chem* 2006, 281(10): 6175-6183.
73. Wang D, Westerheide SD, Hanson JL, Baldwin AS, Jr. Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. *J Biol Chem* 2000, 275(42): 32592-32597.
74. Bae JS, Jang MK, Hong S, An WG, Choi YH, Kim HD*, et al.* Phosphorylation of NF-kappa B by calmodulin-dependent kinase IV activates anti-apoptotic gene expression. *Biochem Biophys Res Commun* 2003, 305(4): 1094-1098.
75. Jiang X, Takahashi N, Matsui N, Tetsuka T, Okamoto T. The NF-kappa B activation in lymphotoxin beta receptor signaling depends on the phosphorylation of p65 at serine 536. *J Biol Chem* 2003, 278(2): 919-926.
76. Lawrence T, Bebien M, Liu GY, Nizet V, Karin M. IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation. *Nature* 2005, 434(7037): 1138-1143.
77. Sakurai H, Chiba H, Miyoshi H, Sugita T, Toriumi W. IkappaB kinases phosphorylate NF-kappaB p65 subunit on serine 536 in the transactivation domain. *J Biol Chem* 1999, 274(43): 30353-30356.
78. Mattioli I, Sebald A, Bucher C, Charles RP, Nakano H, Doi T*, et al.* Transient and selective NF-kappa B p65 serine 536 phosphorylation induced by T cell costimulation is mediated by I kappa B kinase beta and controls the kinetics of p65 nuclear import. *J Immunol* 2004, 172(10): 6336-6344.
79. Buss H, Dorrie A, Schmitz ML, Hoffmann E, Resch K, Kracht M. Constitutive and interleukin-1-inducible phosphorylation of p65 NF-{kappa}B at serine 536 is mediated by multiple protein kinases including I{kappa}B kinase (IKK)-{alpha}, IKK{beta}, IKK{epsilon}, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated interleukin-8 transcription. *J Biol Chem* 2004, 279(53): 55633-55643.
80. Fujita F, Taniguchi Y, Kato T, Narita Y, Furuya A, Ogawa T*, et al.* Identification of NAP1, a regulatory subunit of IkappaB kinase-related kinases that

potentiates NF-kappaB signaling. *Mol Cell Biol* 2003, 23(21): 7780-7793.
81. Bohuslav J, Chen LF, Kwon H, Mu Y, Greene WC. p53 induces NF-kappaB activation by an IkappaB kinase-independent mechanism involving phosphorylation of p65 by ribosomal S6 kinase 1. *J Biol Chem* 2004, 279(25): 26115-26125.
82. Sabatel H, Di Valentin E, Gloire G, Dequiedt F, Piette J, Habraken Y. Phosphorylation of p65(RelA) on Ser(547) by ATM represses NF-kappaB-dependent transcription of specific genes after genotoxic stress. *PLoS One* 2012, 7(6): e38246.
83. Ryo A, Suizu F, Yoshida Y, Perrem K, Liou YC, Wulf G*, et al.* Regulation of NF-kappaB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol Cell* 2003, 12(6): 1413-1426.
84. Yeh PY, Yeh KH, Chuang SE, Song YC, Cheng AL. Suppression of MEK/ERK signaling pathway enhances cisplatin-induced NF-kappaB activation by protein phosphatase 4-mediated NF-kappaB p65 Thr dephosphorylation. *J Biol Chem* 2004, 279(25): 26143-26148.
85. Campbell KJ, Witty JM, Rocha S, Perkins ND. Cisplatin mimics ARF tumor suppressor regulation of RelA (p65) nuclear factor-kappaB transactivation. *Cancer Res* 2006, 66(2): 929-935.
86. Campbell KJ, Perkins ND. Regulation of NF-kappaB function. *Biochem Soc Symp* 2006(73): 165-180.
87. Park SW, Huq MD, Hu X, Wei LN. Tyrosine nitration on p65: a novel mechanism to rapidly inactivate nuclear factor-kappaB. *Mol Cell Proteomics* 2005, 4(3): 300-309.
88. Kiernan R, Bres V, Ng RW, Coudart MP, El Messaoudi S, Sardet C*, et al.* Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. *J Biol Chem* 2003, 278(4): 2758-2766.
89. Chen LF, Mu Y, Greene WC. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. *EMBO J* 2002, 21(23): 6539-6548.
90. Buerki C, Rothgiesser KM, Valovka T, Owen HR, Rehrauer H, Fey M*, et al.* Functional relevance of novel p300-mediated lysine 314 and 315 acetylation of RelA/p65. *Nucleic Acids Res* 2008, 36(5): 1665-1680.
91. Hoberg JE, Popko AE, Ramsey CS, Mayo MW. IkappaB kinase alpha-mediated derepression of SMRT potentiates acetylation of RelA/p65 by p300. *Mol Cell Biol* 2006, 26(2): 457-471.
92. Dong J, Jimi E, Zhong H, Hayden MS, Ghosh S. Repression of gene expression by unphosphorylated NF-kappaB p65 through epigenetic mechanisms. *Genes Dev* 2008, 22(9): 1159-1173.
93. Geng H, Wittwer T, Dittrich-Breiholz O, Kracht M, Schmitz ML. Phosphorylation of NF-kappaB p65 at Ser468 controls its COMMD1-dependent ubiquitination and target gene-specific proteasomal elimination. *EMBO Rep* 2009, 10(4): 381-386.
94. Nowak DE, Tian B, Jamaluddin M, Boldogh I, Vergara LA, Choudhary S*, et al.* RelA Ser276 phosphorylation is required for activation of a subset of NF-kappaB-dependent genes by recruiting cyclin-dependent kinase 9/cyclin T1 complexes. *Mol Cell Biol* 2008, 28(11): 3623-3638.
95. Moreno R, Sobotzik JM, Schultz C, Schmitz ML. Specification of the NF-kappaB transcriptional response by p65 phosphorylation and TNF-induced nuclear translocation of IKK epsilon. *Nucleic Acids Res* 2010, 38(18): 6029-6044.
96. Heissmeyer V, Krappmann D, Hatada EN, Scheidereit C. Shared pathways of IkappaB kinase-induced SCF(betaTrCP)-mediated ubiquitination and degradation for the NF-kappaB precursor p105 and IkappaBalpha. *Mol Cell Biol* 2001, 21(4): 1024-1035.
97. Orian A, Gonen H, Bercovich B, Fajerman I, Eytan E, Israel A*, et al.* SCF(beta)(-TrCP) ubiquitin ligase-mediated processing of NF-kappaB p105 requires phosphorylation of its C-terminus by IkappaB kinase. *EMBO J* 2000, 19(11): 2580-2591.
98. Wan F, Anderson DE, Barnitz RA, Snow A, Bidere N, Zheng L*, et al.* Ribosomal protein S3: a KH domain subunit in NF-kappaB complexes that mediates selective gene regulation. *Cell* 2007, 131(5): 927-939.
99. Kelsey KT, Memisoglu A, Frenkel D, Liber HL. Human lymphocytes exposed to low doses of X-rays are less susceptible to radiation-induced mutagenesis. *Mutat Res* 1991, 263(4): 197-201.
100. Feinendegen LE, Bond VP, Sondhaus CA, Muehlensiepen H. Radiation effects induced by low doses in complex tissue and their relation to cellular adaptive responses. *Mutat Res* 1996, 358(2): 199-205.
101. Jones PL, Ping D, Boss JM. Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. *Mol Cell Biol* 1997, 17(12): 6970-6981.
102. Das KC, Lewis-Molock Y, White CW. Activation of NF-kappa B and elevation of MnSOD gene expression by thiol reducing agents in lung adenocarcinoma (A549) cells. *Am J Physiol* 1995, 269(5 Pt 1): L588-602.
103. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL*, et al.* Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese

superoxide dismutase. *Nat Genet* 1995, 11(4): 376-381.
104. Macmillan-Crow LA, Cruthirds DL. Invited review: manganese superoxide dismutase in disease. *Free Radic Res* 2001, 34(4): 325-336.
105. Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H*, et al.* CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* 2005, 24(3): 367-380.
106. Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K*, et al.* Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. *Cell* 2004, 119(4): 529-542.
107. Torti FM, Torti SV. Regulation of ferritin genes and protein. *Blood* 2002, 99(10): 3505-3516.
108. Schreiber J, Jenner RG, Murray HL, Gerber GK, Gifford DK, Young RA. Coordinated binding of NF-kappaB family members in the response of human cells to lipopolysaccharide. *Proc Natl Acad Sci U S A* 2006, 103(15): 5899-5904.
109. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. *Cell Mol Life Sci* 2004, 61(2): 192-208.
110. Djavaheri-Mergny M, Javelaud D, Wietzerbin J, Besancon F. NF-kappaB activation prevents apoptotic oxidative stress via an increase of both thioredoxin and MnSOD levels in TNFalpha-treated Ewing sarcoma cells. *FEBS Lett* 2004, 578(1-2): 111-115.
111. Matsui M, Oshima M, Oshima H, Takaku K, Maruyama T, Yodoi J*, et al.* Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev Biol* 1996, 178(1): 179-185.
112. Tanaka T, Hosoi F, Yamaguchi-Iwai Y, Nakamura H, Masutani H, Ueda S*, et al.* Thioredoxin-2 (TRX-2) is an essential gene regulating mitochondria-dependent apoptosis. *EMBO J* 2002, 21(7): 1695-1703.
113. Nonn L, Williams RR, Erickson RP, Powis G. The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. *Mol Cell Biol* 2003, 23(3): 916-922.
114. Xia C, Hu J, Ketterer B, Taylor JB. The organization of the human GSTP1-1 gene promoter and its response to retinoic acid and cellular redox status. *Biochem J* 1996, 313 (Pt 1): 155-161.
115. Dang DT, Chen F, Kohli M, Rago C, Cummins JM, Dang LH. Glutathione S-transferase pi1 promotes tumorigenicity in HCT116 human colon cancer cells. *Cancer Res* 2005, 65(20): 9485-9494.
116. Dourado DF, Fernandes PA, Ramos MJ. Mammalian cytosolic glutathione transferases. *Curr Protein Pept Sci* 2008, 9(4): 325-337.
117. Salinas AE, Wong MG. Glutathione S-transferases--a review. *Curr Med Chem* 1999, 6(4): 279-309.
118. Hinata K, Gervin AM, Jennifer Zhang Y, Khavari PA. Divergent gene regulation and growth effects by NF-kappa B in epithelial and mesenchymal cells of human skin. *Oncogene* 2003, 22(13): 1955-1964.
119. Kumari MV, Hiramatsu M, Ebadi M. Free radical scavenging actions of metallothionein isoforms I and II. *Free Radic Res* 1998, 29(2): 93-101.
120. Howells C, West AK, Chung RS. Neuronal growth-inhibitory factor (metallothionein-3): evaluation of the biological function of growth-inhibitory factor in the injured and neurodegenerative brain. *FEBS J* 2010, 277(14): 2931-2939.
121. Yao KS, Hageboutros A, Ford P, O'Dwyer PJ. Involvement of activator protein-1 and nuclear factor-kappaB transcription factors in the control of the DT-diaphorase expression induced by mitomycin C treatment. *Mol Pharmacol* 1997, 51(3): 422-430.
122. Ahn KS, Sethi G, Jain AK, Jaiswal AK, Aggarwal BB. Genetic deletion of NAD(P)H:quinone oxidoreductase 1 abrogates activation of nuclear factor-kappaB, IkappaBalpha kinase, c-Jun N-terminal kinase, Akt, p38, and p44/42 mitogen-activated protein kinases and potentiates apoptosis. *J Biol Chem* 2006, 281(29): 19798-19808.
123. Dinkova-Kostova AT, Talalay P. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch Biochem Biophys* 2010, 501(1): 116-123.
124. Wu G, Marin-Garcia J, Rogers TB, Lakatta EG, Long X. Phosphorylation and hypoxia-induced heme oxygenase-1 gene expression in cardiomyocytes. *J Card Fail* 2004, 10(6): 519-526.
125. Prawan A, Kundu JK, Surh YJ. Molecular basis of heme oxygenase-1 induction: implications for chemoprevention and chemoprotection. *Antioxid Redox Signal* 2005, 7(11-12): 1688-1703.
126. Lei XG, Cheng WH, McClung JP. Metabolic regulation and function of glutathione peroxidase-1. *Annu Rev Nutr* 2007, 27: 41-61.
127. Sies H, Sharov VS, Klotz LO, Briviba K. Glutathione peroxidase protects against peroxynitrite-mediated oxidations. A new function for selenoproteins as peroxynitrite reductase. *J Biol Chem* 1997, 272(44): 27812-27817.
128. Ciaccio PJ, Walsh ES, Tew KD. Promoter analysis of a human dihydrodiol dehydrogenase. *Biochem Biophys Res Commun* 1996, 228(2): 524-529.
129. Chen J, Adikari M, Pallai R, Parekh HK, Simpkins H. Dihydrodiol dehydrogenases regulate the

generation of reactive oxygen species and the development of cisplatin resistance in human ovarian carcinoma cells. *Cancer Chemother Pharmacol* 2008, 61(6): 979-987.
130. Anrather J, Racchumi G, Iadecola C. NF-kappaB regulates phagocytic NADPH oxidase by inducing the expression of gp91phox. *J Biol Chem* 2006, 281(9): 5657-5667.
131. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 2004, 4(3): 181-189.
132. Brown DI, Griendling KK. Nox proteins in signal transduction. *Free Radic Biol Med* 2009, 47(9): 1239-1253.
133. Xu P, Huecksteadt TP, Hoidal JR. Molecular cloning and characterization of the human xanthine dehydrogenase gene (XDH). *Genomics* 1996, 34(2): 173-180.
134. Maia L, Vala A, Mira L. NADH oxidase activity of rat liver xanthine dehydrogenase and xanthine oxidase-contribution for damage mechanisms. *Free Radic Res* 2005, 39(9): 979-986.
135. Kolyada AY, Savikovsky N, Madias NE. Transcriptional regulation of the human iNOS gene in vascular-smooth-muscle cells and macrophages: evidence for tissue specificity. *Biochem Biophys Res Commun* 1996, 220(3): 600-605.
136. Nakata S, Tsutsui M, Shimokawa H, Yamashita T, Tanimoto A, Tasaki H*, et al.* Statin treatment upregulates vascular neuronal nitric oxide synthase through Akt/NF-kappaB pathway. *Arterioscler Thromb Vasc Biol* 2007, 27(1): 92-98.
137. Li Y, Zhao Y, Li G, Wang J, Li T, Li W*, et al.* Regulation of neuronal nitric oxide synthase exon 1f gene expression by nuclear factor-kappaB acetylation in human neuroblastoma cells. *J Neurochem* 2007, 101(5): 1194-1204.
138. Morris KR, Lutz RD, Choi HS, Kamitani T, Chmura K, Chan ED. Role of the NF-kappaB signaling pathway and kappaB cis-regulatory elements on the IRF-1 and iNOS promoter regions in mycobacterial lipoarabinomannan induction of nitric oxide. *Infect Immun* 2003, 71(3): 1442-1452.
139. Ahmad R, Rasheed Z, Ahsan H. Biochemical and cellular toxicology of peroxynitrite: implications in cell death and autoimmune phenomenon. *Immunopharmacol Immunotoxicol* 2009, 31(3): 388-396.
140. Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS, Lanzo CA. Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. *J Biol Chem* 1999, 274(33): 22903-22906.
141. Arakawa T, Nakamura M, Yoshimoto T, Yamamoto S. The transcriptional regulation of human arachidonate 12-lipoxygenase gene by NF kappa B/Rel. *FEBS Lett* 1995, 363(1-2): 105-110.
142. Chopra A, Ferreira-Alves DL, Sirois P, Thirion JP. Cloning of the guinea pig 5-lipoxygenase gene and nucleotide sequence of its promoter. *Biochem Biophys Res Commun* 1992, 185(2): 489-495.
143. Kim C, Kim JY, Kim JH. Cytosolic phospholipase A(2), lipoxygenase metabolites, and reactive oxygen species. *BMB Rep* 2008, 41(8): 555-559.
144. Nordblom GD, Coon MJ. Hydrogen peroxide formation and stoichiometry of hydroxylation reactions catalyzed by highly purified liver microsomal cytochrome P-450. *Arch Biochem Biophys* 1977, 180(2): 343-347.
145. Imaoka S, Osada M, Minamiyama Y, Yukimura T, Toyokuni S, Takemura S*, et al.* Role of phenobarbital-inducible cytochrome P450s as a source of active oxygen species in DNA-oxidation. *Cancer Lett* 2004, 203(2): 117-125.
146. Abdel-Razzak Z, Garlatti M, Aggerbeck M, Barouki R (eds). *Determination of interleukin-4-responsive region in the human cytochrome P450 2E1 gene promoter*, 2004.
147. Morgan ET, Li-Masters T, Cheng PY. Mechanisms of cytochrome P450 regulation by inflammatory mediators. *Toxicology* 2002, 181-182: 207-210.
148. Caro AA, Cederbaum AI. Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol* 2004, 44: 27-42.
149. Cederbaum AI, Wu D, Mari M, Bai J. CYP2E1-dependent toxicity and oxidative stress in HepG2 cells. *Free Radic Biol Med* 2001, 31(12): 1539-1543.
150. Deveraux QL, Reed JC. IAP family proteins--suppressors of apoptosis. *Genes Dev* 1999, 13(3): 239-252.
151. Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM*, et al.* IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J* 1998, 17(8): 2215-2223.
152. Kasof GM, Gomes BC. Livin, a novel inhibitor of apoptosis protein family member. *J Biol Chem* 2001, 276(5): 3238-3246.
153. Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 1997, 388(6639): 300-304.
154. Tang G, Minemoto Y, Dibling B, Purcell NH, Li Z, Karin M*, et al.* Inhibition of JNK activation through NF-kappaB target genes. *Nature* 2001, 414(6861): 313-317.
155. Benayoun B, Baghdiguian S, Lajmanovich A, Bartoli M, Daniele N, Gicquel E*, et al.* NF-kappaB-dependent expression of the antiapoptotic factor c-FLIP is regulated by calpain 3, the protein involved

in limb-girdle muscular dystrophy type 2A. *FASEB J* 2008, 22(5): 1521-1529.
156. Golks A, Brenner D, Krammer PH, Lavrik IN. The c-FLIP-NH2 terminus (p22-FLIP) induces NF-kappaB activation. *J Exp Med* 2006, 203(5): 1295-1305.
157. Iyer AK, Azad N, Talbot S, Stehlik C, Lu B, Wang L*, et al.* Antioxidant c-FLIP inhibits Fas ligand-induced NF-kappaB activation in a phosphatidylinositol 3-kinase/Akt-dependent manner. *J Immunol* 2011, 187(6): 3256-3266.
158. Catz SD, Johnson JL. Transcriptional regulation of bcl-2 by nuclear factor kappa B and its significance in prostate cancer. *Oncogene* 2001, 20(50): 7342-7351.
159. Buchholz TA, Garg AK, Chakravarti N, Aggarwal BB, Esteva FJ, Kuerer HM*, et al.* The nuclear transcription factor kappaB/bcl-2 pathway correlates with pathologic complete response to doxorubicin-based neoadjuvant chemotherapy in human breast cancer. *Clin Cancer Res* 2005, 11(23): 8398-8402.
160. Murphy KM, Ranganathan V, Farnsworth ML, Kavallaris M, Lock RB. Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells. *Cell Death Differ* 2000, 7(1): 102-111.
161. Zhu L, Ling S, Yu XD, Venkatesh LK, Subramanian T, Chinnadurai G*, et al.* Modulation of mitochondrial Ca(2+) homeostasis by Bcl-2. *J Biol Chem* 1999, 274(47): 33267-33273.
162. Deng X, Xiao L, Lang W, Gao F, Ruvolo P, May WS, Jr. Novel role for JNK as a stress-activated Bcl2 kinase. *J Biol Chem* 2001, 276(26): 23681-23688.
163. Chen C, Edelstein LC, Gelinas C. The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). *Mol Cell Biol* 2000, 20(8): 2687-2695.
164. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 1998, 281(5381): 1322-1326.
165. Minn AJ, Kettlun CS, Liang H, Kelekar A, Vander Heiden MG, Chang BS*, et al.* Bcl-xL regulates apoptosis by heterodimerization-dependent and -independent mechanisms. *EMBO J* 1999, 18(3): 632-643.
166. Vander Heiden MG, Li XX, Gottleib E, Hill RB, Thompson CB, Colombini M. Bcl-xL promotes the open configuration of the voltage-dependent anion channel and metabolite passage through the outer mitochondrial membrane. *J Biol Chem* 2001, 276(22): 19414-19419.
167. Fan M, Goodwin M, Vu T, Brantley-Finley C, Gaarde WA, Chambers TC. Vinblastine-induced phosphorylation of Bcl-2 and Bcl-XL is mediated by JNK and occurs in parallel with inactivation of the Raf-1/MEK/ERK cascade. *J Biol Chem* 2000, 275(39): 29980-29985.
168. Poruchynsky MS, Wang EE, Rudin CM, Blagosklonny MV, Fojo T. Bcl-xL is phosphorylated in malignant cells following microtubule disruption. *Cancer Res* 1998, 58(15): 3331-3338.
169. Jung M, Dritschilo A. NF-kappa B signaling pathway as a target for human tumor radiosensitization. *Semin Radiat Oncol* 2001, 11(4): 346-351.
170. Zhou D, Brown SA, Yu T, Chen G, Barve S, Kang BC*, et al.* A high dose of ionizing radiation induces tissue-specific activation of nuclear factor-kappaB in vivo. *Radiat Res* 1999, 151(6): 703-709.
171. Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS, Jr. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol* 1999, 19(8): 5785-5799.
172. Hirai H, Roussel MF, Kato JY, Ashmun RA, Sherr CJ. Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. *Mol Cell Biol* 1995, 15(5): 2672-2681.
173. Sherr CJ. Cancer cell cycles. *Science* 1996, 274(5293): 1672-1677.
174. Lukas J, Bartkova J, Bartek J. Convergence of mitogenic signalling cascades from diverse classes of receptors at the cyclin D-cyclin-dependent kinase-pRb-controlled G1 checkpoint. *Mol Cell Biol* 1996, 16(12): 6917-6925.
175. Diehl JA, Zindy F, Sherr CJ. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev* 1997, 11(8): 957-972.
176. Fan M, Ahmed KM, Coleman MC, Spitz DR, Li JJ. Nuclear factor-kappaB and manganese superoxide dismutase mediate adaptive radioresistance in low-dose irradiated mouse skin epithelial cells. *Cancer Res* 2007, 67(7): 3220-3228.
177. Lorca T, Labbe JC, Devault A, Fesquet D, Capony JP, Cavadore JC*, et al.* Dephosphorylation of cdc2 on threonine 161 is required for cdc2 kinase inactivation and normal anaphase. *EMBO J* 1992, 11(7): 2381-2390.
178. Norbury C, Blow J, Nurse P. Regulatory phosphorylation of the p34cdc2 protein kinase in vertebrates. *EMBO J* 1991, 10(11): 3321-3329.
179. Toyoshima-Morimoto F, Taniguchi E, Shinya N, Iwamatsu A, Nishida E. Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase. *Nature* 2001, 410(6825): 215-220.
180. Papa S, Zazzeroni F, Bubici C, Jayawardena S, Alvarez K, Matsuda S*, et al.* Gadd45 beta mediates the NF-kappa B suppression of JNK signalling by

targeting MKK7/JNKK2. *Nat Cell Biol* 2004, 6(2): 146-153.
181. Wang T, Hu YC, Dong S, Fan M, Tamae D, Ozeki M*, et al.* Co-activation of ERK, NF-kappaB, and GADD45beta in response to ionizing radiation. *J Biol Chem* 2005, 280(13): 12593-12601.
182. Wang XW, Zhan Q, Coursen JD, Khan MA, Kontny HU, Yu L*, et al.* GADD45 induction of a G2/M cell cycle checkpoint. *Proc Natl Acad Sci U S A* 1999, 96(7): 3706-3711.
183. Yang Q, Manicone A, Coursen JD, Linke SP, Nagashima M, Forgues M*, et al.* Identification of a functional domain in a GADD45-mediated G2/M checkpoint. *J Biol Chem* 2000, 275(47): 36892-36898.
184. Zhao H, Jin S, Antinore MJ, Lung FD, Fan F, Blanck P*, et al.* The central region of Gadd45 is required for its interaction with p21/WAF1. *Exp Cell Res* 2000, 258(1): 92-100.
185. Liebermann DA, Hoffman B. Gadd45 in stress signaling. *J Mol Signal* 2008, 3: 15.
186. Lim JW, Kim H, Kim KH. Expression of Ku70 and Ku80 mediated by NF-kappa B and cyclooxygenase-2 is related to proliferation of human gastric cancer cells. *J Biol Chem* 2002, 277(48): 46093-46100.
187. Tuteja R, Tuteja N. Ku autoantigen: a multifunctional DNA-binding protein. *Crit Rev Biochem Mol Biol* 2000, 35(1): 1-33.
188. Cao QP, Pitt S, Leszyk J, Baril EF. DNA-dependent ATPase from HeLa cells is related to human Ku autoantigen. *Biochemistry* 1994, 33(28): 8548-8557.
189. Collis SJ, DeWeese TL, Jeggo PA, Parker AR. The life and death of DNA-PK. *Oncogene* 2005, 24(6): 949-961.
190. Cao N, Li S, Wang Z, Ahmed KM, Degnan ME, Fan M*, et al.* NF-kappaB-mediated HER2 overexpression in radiation-adaptive resistance. *Radiat Res* 2009, 171(1): 9-21.
191. Muthuswamy SK, Gilman M, Brugge JS. Controlled dimerization of ErbB receptors provides evidence for differential signaling by homo- and heterodimers. *Mol Cell Biol* 1999, 19(10): 6845-6857.
192. Rosen EM, Day R, Singh VK. New approaches to radiation protection. *Front Oncol* 2014, 4: 381.
193. Citrin D, Cotrim AP, Hyodo F, Baum BJ, Krishna MC, Mitchell JB. Radioprotectors and mitigators of radiation-induced normal tissue injury. *Oncologist* 2010, 15(4): 360-371.
194. Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. *Nat Rev Cancer* 2003, 3(2): 117-129.
195. Potten CS. The significance of spontaneous and induced apoptosis in the gastrointestinal tract of mice. *Cancer Metastasis Rev* 1992, 11(2): 179-195.
196. Kolesnick R, Fuks Z. Radiation and ceramide-induced apoptosis. *Oncogene* 2003, 22(37): 5897-5906.
197. Wang Y, Meng A, Lang H, Brown SA, Konopa JL, Kindy MS*, et al.* Activation of nuclear factor kappaB In vivo selectively protects the murine small intestine against ionizing radiation-induced damage. *Cancer Res* 2004, 64(17): 6240-6246.
198. Stone HB, Moulder JE, Coleman CN, Ang KK, Anscher MS, Barcellos-Hoff MH*, et al.* Models for evaluating agents intended for the prophylaxis, mitigation and treatment of radiation injuries. Report of an NCI Workshop, December 3-4, 2003. *Radiat Res* 2004, 162(6): 711-728.
199. Pellmar TC, Rockwell S. Priority list of research areas for radiological nuclear threat countermeasures. *Radiat Res* 2005, 163(1): 115-123.
200. Romano MF, Lamberti A, Bisogni R, Garbi C, Pagnano AM, Auletta P*, et al.* Amifostine inhibits hematopoietic progenitor cell apoptosis by activating NF-kappaB/Rel transcription factors. *Blood* 1999, 94(12): 4060-4066.
201. Jakob U, Reichmann D. *Oxidative Stress and Redox Regulation*. Springer, 2013.
202. Copp RR, Fahl WE, Peebles DD. Amino thiol compounds and compositions for use in conjunction with cancer therapy. Google Patents; 2005.
203. Weiss JF, Hoover RL, Kumar KS. Selenium pretreatment enhances the radioprotective effect and reduces the lethal toxicity of WR-2721. *Free Radic Res Commun* 1987, 3(1-5): 33-38.
204. Kretz-Remy C, Arrigo AP. Selenium: a key element that controls NF-kappa B activation and I kappa B alpha half life. *Biofactors* 2001, 14(1-4): 117-125.
205. Bell SC, Maniar M. FORMULATION OF RADIOPROTECTIVE α, β UNSATURATED ARYL SULFONES. Google Patents; 2007.
206. Maniar M, Bell SC. FORMULATIONS OF RADIOPROTECTIVE α, β UNSATURATED ARYL SULFONES. Google Patents; 2008.
207. Ghosh SP, Perkins MW, Hieber K, Kulkarni S, Kao TC, Reddy EP*, et al.* Radiation protection by a new chemical entity, Ex-Rad: efficacy and mechanisms. *Radiat Res* 2009, 171(2): 173-179.
208. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D*, et al.* An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science* 2008, 320(5873): 226-230.
209. Burdelya LG, Brackett CM, Kojouharov B, Gitlin, II, Leonova KI, Gleiberman AS*, et al.* Central role of liver in anticancer and radioprotective activities of Toll-like receptor 5 agonist. *Proc Natl Acad Sci U S A* 2013, 110(20): E1857-1866.

210. Shakhov AN, Gudkov A. Methods of protecting against apoptosis using lipopeptides. Google Patents; 2007.
211. Shakhov AN, Singh VK, Bone F, Cheney A, Kononov Y, Krasnov P*, et al.* Prevention and mitigation of acute radiation syndrome in mice by synthetic lipopeptide agonists of Toll-like receptor 2 (TLR2). *PLoS One* 2012, 7(3): e33044.
212. Singh VK, Ducey EJ, Fatanmi OO, Singh PK, Brown DS, Purmal A*, et al.* CBLB613: a TLR 2/6 agonist, natural lipopeptide of Mycoplasma arginini , as a novel radiation countermeasure. *Radiat Res* 2012, 177(5): 628-642.
213. Whitnall MH, Villa V, Seed TM, Benjack J, Miner V, Lewbart ML*, et al.* Molecular specificity of 5-androstenediol as a systemic radioprotectant in mice. *Immunopharmacol Immunotoxicol* 2005, 27(1): 15-32.
214. Stickney DR, Dowding C, Authier S, Garsd A, Onizuka-Handa N, Reading C*, et al.* 5-androstenediol improves survival in clinically unsupported rhesus monkeys with radiation-induced myelosuppression. *Int Immunopharmacol* 2007, 7(4): 500-505.
215. Whitnall MH, Wilhelmsen CL, McKinney L, Miner V, Seed TM, Jackson WE, 3rd. Radioprotective efficacy and acute toxicity of 5-androstenediol after subcutaneous or oral administration in mice. *Immunopharmacol Immunotoxicol* 2002, 24(4): 595-626.
216. Kiang JG, Smith JT, Agravante NG. Geldanamycin analog 17-DMAG inhibits iNOS and caspases in gamma-irradiated human T cells. *Radiat Res* 2009, 172(3): 321-330.
217. Gorbunov NV, Kiang JG. Up-regulation of autophagy in small intestine Paneth cells in response to total-body gamma-irradiation. *J Pathol* 2009, 219(2): 242-252.
218. Mettler FA, Jr., Voelz GL. Major radiation exposure--what to expect and how to respond. *N Engl J Med* 2002, 346(20): 1554-1561.
219. Gandhi NM, Nair CK. Radiation protection by diethyldithiocarbamate: protection of membrane and DNA in vitro and in vivo against gamma-radiation. *J Radiat Res* 2004, 45(2): 175-180.
220. Neta R. Role of cytokines in radioprotection. *Pharmacol Ther* 1988, 39(1-3): 261-266.
221. Neta R, Monroy R, MacVittie TJ. Utility of interleukin-1 in therapy of radiation injury as studied in small and large animal models. *Biotherapy* 1989, 1(4): 301-311.
222. Okunieff P, Wu T, Huang K, Ding I. Differential radioprotection of three mouse strains by basic or acidic fibroblast growth factor. *Br J Cancer Suppl* 1996, 27: S105-108.
223. Zhang M, Qian J, Xing X, Kong FM, Zhao L, Chen M*, et al.* Inhibition of the tumor necrosis factor-alpha pathway is radioprotective for the lung. *Clin Cancer Res* 2008, 14(6): 1868-1876.
224. Mickelson AB, Horoho PD, Institute B. *Medical Consequences of Radiological and Nuclear Weapons*. U.S. Government Printing Office, 2013.
225. Gupta D, Arora R, Mohan RR, Almasan AA, Gudkov AV, Macklis RM. Novel Strategies for protecting mitochondria (the cellular powerhouse) against Low-LET radiation: A review. In: Arora R (ed). *Herbal Radiomodulators: Application in Medicine, Homeland Defence & Space*. CAB International, UK: Oxfordshire, 2008, pp 215-226.
226. Gupta D, Arora R, Garg AP, Goel HC. Radiation protection of HepG2 cells by Podophyllum hexandrum Royale. *Molecular and cellular biochemistry* 2003, 250(1-2): 27-40.
227. Chawla R, Arora R, Kumar R, Sharma A, Prasad J, Singh S*, et al.* Antioxidant activity of fractionated extracts of rhizomes of high-altitude Podophyllum hexandrum: role in radiation protection. *Mol Cell Biochem* 2005, 273(1-2): 193-208.
228. Mittal A, Pathania V, Agrawala PK, Prasad J, Singh S, Goel HC. Influence of Podophyllum hexandrum on endogenous antioxidant defence system in mice: possible role in radioprotection. *J Ethnopharmacol* 2001, 76(3): 253-262.
229. Sajikumar S, Goel HC. Podophyllum hexandrum prevents radiation-induced neuronal damage in postnatal rats exposed in utero. *Phytother Res* 2003, 17(7): 761-766.
230. Gupta D, Arora R, Garg AP, Bala M, Goel HC. Modification of radiation damage to mitochondrial system in vivo by Podophyllum hexandrum: mechanistic aspects. *Mol Cell Biochem* 2004, 266(1-2): 65-77.
231. Goel HC, Gupta D, Gupta S, Garg AP, Bala M. Protection of mitochondrial system by Hippophae rhamnoides L. against radiation-induced oxidative damage in mice. *The Journal of pharmacy and pharmacology* 2005, 57(1): 135-143.
232. Goel HC, Prasad J, Singh S, Sagar RK, Kumar IP, Sinha AK. Radioprotection by a herbal preparation of Hippophae rhamnoides, RH-3, against whole body lethal irradiation in mice. *Phytomedicine* 2002, 9(1): 15-25.
233. Goel HC, Salin CA, Prakash H. Protection of jejunal crypts by RH-3 (a preparation of Hippophae rhamnoides) against lethal whole body gamma irradiation. *Phytother Res* 2003, 17(3): 222-226.
234. Prasad NR, Menon VP, Vasudev V, Pugalendi KV. Radioprotective effect of sesamol on gamma-radiation induced DNA damage, lipid peroxidation and antioxidants levels in cultured human lymphocytes. *Toxicology* 2005, 209(3): 225-235.

235. Grotz KA, Henneicke-von Zepelin HH, Kohnen R, al-Nawas B, Bockisch A, Kutzner J*, et al.* [Prospective double-blind study of prophylaxis of radioxerostomia with Coumarin/Troxerutine in patients with head and neck cancer]. *Strahlenther Onkol* 1999, 175(8): 397-403; discussion 404.
236. Maurya DK, Salvi VP, Krishnan Nair CK. Radioprotection of normal tissues in tumor-bearing mice by troxerutin. *J Radiat Res* 2004, 45(2): 221-228.
237. Epperly M, Jin S, Nie S, Cao S, Zhang X, Franicola D*, et al.* Ethyl pyruvate, a potentially effective mitigator of damage after total-body irradiation. *Radiat Res* 2007, 168(5): 552-559.
238. Epperly MW, Greenberger JS, Mitchell PF. Radioprotective agents. Google Patents; 2007.
239. Liang X, Chavez AR, Schapiro NE, Loughran P, Thorne SH, Amoscato AA*, et al.* Ethyl pyruvate administration inhibits hepatic tumor growth. *J Leukoc Biol* 2009, 86(3): 599-607.
240. Herodin F, Drouet M. Cytokine-based treatment of accidentally irradiated victims and new approaches. *Exp Hematol* 2005, 33(10): 1071-1080.
241. Singh VK, Newman VL, Romaine PL, Wise SY, Seed TM. Radiation countermeasure agents: an update (2011-2014). *Expert Opin Ther Pat* 2014, 24(11): 1229-1255.
242. Glisson B, Komaki R, Lee JS, Shin DM, Fossella F, Murphy WK*, et al.* Integration of filgrastim into chemoradiation for limited small cell lung cancer: a Phase I study. *Int J Radiat Oncol Biol Phys* 1998, 40(2): 331-336.
243. Gava A, Bertossi L, Ferrarese F, Coghetto F, Marazzato G, Andrulli AD*, et al.* [Use of filgrastim, granulocyte colony stimulating factor (G-CSF), in radiotherapy to reduce drop-outs because of radiogenic leukopenia]. *Radiol Med* 1998, 95(3): 232-236.
244. Davis TA, Clarke TK, Mog SR, Landauer MR. Subcutaneous administration of genistein prior to lethal irradiation supports multilineage, hematopoietic progenitor cell recovery and survival. *Int J Radiat Biol* 2007, 83(3): 141-151.
245. Day RM, Barshishat-Kupper M, Mog SR, McCart EA, Prasanna PG, Davis TA*, et al.* Genistein protects against biomarkers of delayed lung sequelae in mice surviving high-dose total body irradiation. *J Radiat Res* 2008, 49(4): 361-372.
246. Singh VK, Grace MB, Parekh VI, Whitnall MH, Landauer MR. Effects of genistein administration on cytokine induction in whole-body gamma irradiated mice. *Int Immunopharmacol* 2009, 9(12): 1401-1410.
247. Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NF-kappaB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. *BMC Cancer* 2006, 6: 107.
248. El-Missiry A, Othman AI, Alabdan A. *Melatonin for Protection Against Ionizing Radiation*. INTECH Open Access Publisher, 2012.
249. Mihandoost E, Shirazi A, Mahdavi SR, Aliasgharzadeh A. Can melatonin help us in radiation oncology treatments? *Biomed Res Int* 2014, 2014: 578137.
250. Carsten RE, Bachand AM, Bailey SM, Ullrich RL. Resveratrol reduces radiation-induced chromosome aberration frequencies in mouse bone marrow cells. *Radiat Res* 2008, 169(6): 633-638.
251. Fu Y, Wang Y, Du L, Xu C, Cao J, Fan T*, et al.* Resveratrol inhibits ionising irradiation-induced inflammation in MSCs by activating SIRT1 and limiting NLRP-3 inflammasome activation. *Int J Mol Sci* 2013, 14(7): 14105-14118.
252. Vorotnikova E, Rosenthal RA, Tries M, Doctrow SR, Braunhut SJ. Novel synthetic SOD/catalase mimetics can mitigate capillary endothelial cell apoptosis caused by ionizing radiation. *Radiat Res* 2010, 173(6): 748-759.
253. Doctrow SR, Lopez A, Schock AM, Duncan NE, Jourdan MM, Olasz EB*, et al.* A synthetic superoxide dismutase/catalase mimetic EUK-207 mitigates radiation dermatitis and promotes wound healing in irradiated rat skin. *J Invest Dermatol* 2013, 133(4): 1088-1096.
254. Prasad KN. *Radiation Injury Prevention and Mitigation in Humans*. Taylor & Francis, 2012.
255. Guney Y, Turkcu UO, Hicsonmez A, Andrieu MN, Guney HZ, Bilgihan A*, et al.* Carnosine may reduce lung injury caused by radiation therapy. *Med Hypotheses* 2006, 66(5): 957-959.
256. Tanaka RA, Ramos FM, Almeida SM, Vizioli MR, Boscolo FN. Evaluation of radioprotective effect of carnosine (beta- alanyl-1- histidine) on the wound healing in rats. *J Appl Oral Sci* 2005, 13(3): 253-258.
257. Chen T, Burke KA, Zhan Y, Wang X, Shibata D, Zhao Y. IL-12 facilitates both the recovery of endogenous hematopoiesis and the engraftment of stem cells after ionizing radiation. *Exp Hematol* 2007, 35(2): 203-213.
258. Basile L, Gallaher TK, Ellefson DD. Il-12 for radiation protection and radiation-induced toxicity mitigation. Google Patents; 2013.
259. Tse K, Horner AA. Update on toll-like receptor-directed therapies for human disease. *Ann Rheum Dis* 2007, 66 Suppl 3: iii77-80.
260. Tallant T, Deb A, Kar N, Lupica J, de Veer MJ, DiDonato JA. Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-kappa B and proinflammatory gene program

activation in intestinal epithelial cells. *BMC Microbiol* 2004, 4: 33.
261. Burdelya LG, Gleiberman AS, Toshkov I, Aygun-Sunar S, Bapardekar M, Manderscheid-Kern P*, et al.* Toll-like receptor 5 agonist protects mice from dermatitis and oral mucositis caused by local radiation: implications for head-and-neck cancer radiotherapy. *Int J Radiat Oncol Biol Phys* 2012, 83(1): 228-234.
262. Singh VK, Ducey EJ, Fatanmi OO, Singh PK, Brown DS, Purmal A*, et al.* CBLB613: A TLR 2/6 Agonist, Natural Lipopeptide of Mycoplasma arginini , as a Novel Radiation Countermeasure. *Radiat Res* 2011.
263. Huang WH, Lee AR, Chien PY, Chou TC. Synthesis of baicalein derivatives as potential anti-aggregatory and anti-inflammatory agents. *J Pharm Pharmacol* 2005, 57(2): 219-225.
264. Hwang JM, Tseng TH, Tsai YY, Lee HJ, Chou FP, Wang CJ*, et al.* Protective effects of baicalein on tert-butyl hydroperoxide-induced hepatic toxicity in rat hepatocytes. *J Biomed Sci* 2005, 12(2): 389-397.
265. Peng CY, Pan SL, Huang YW, Guh JH, Chang YL, Teng CM. Baicalein attenuates intimal hyperplasia after rat carotid balloon injury through arresting cell-cycle progression and inhibiting ERK, Akt, and NF-kappaB activity in vascular smooth-muscle cells. *Naunyn Schmiedebergs Arch Pharmacol* 2008, 378(6): 579-588.
266. Ha YM, Chung SW, Kim JM, Kim DH, Kim JY, Lee EK*, et al.* Molecular activation of NF-kappaB, pro-inflammatory mediators, and signal pathways in gamma-irradiated mice. *Biotechnol Lett* 2010, 32(3): 373-378.
267. von Holzen U, Pataer A, Raju U, Bocangel D, Vorburger SA, Liu Y*, et al.* The double-stranded RNA-activated protein kinase mediates radiation resistance in mouse embryo fibroblasts through nuclear factor kappaB and Akt activation. *Clin Cancer Res* 2007, 13(20): 6032-6039.
268. Bucur O, Stancu A, Muraru M, Melet A, Petrescu S, Khosravi-Far R. PLK1 is a binding partner and a negative regulator of FOXO3 tumor suppressor. *Discoveries* 2014, 2(2): e15.
269. Wang YY, Chen SM, Li H. Hydrogen peroxide stress stimulates phosphorylation of FoxO1 in rat aortic endothelial cells. *Acta Pharmacol Sin* 2010, 31(2): 160-164.
270. Lee EK, Kim JM, Choi J, Jung KJ, Kim DH, Chung SW*, et al.* Modulation of NF-kappaB and FOXOs by baicalein attenuates the radiation-induced inflammatory process in mouse kidney. *Free Radic Res* 2011, 45(5): 507-517.
271. Habraken Y, Piette J. NF-kappaB activation by double-strand breaks. *Biochem Pharmacol* 2006, 72(9): 1132-1141.
272. Lin Y, Ma W, Benchimol S. Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis. *Nat Genet* 2000, 26(1): 122-127.
273. Shen ZQ, Dong ZJ, Peng H, Liu JK. Modulation of PAI-1 and tPA activity and thrombolytic effects of corilagin. *Planta Med* 2003, 69(12): 1109-1112.
274. Duan W, Yu Y, Zhang L. Antiatherogenic effects of phyllanthus emblica associated with corilagin and its analogue. *Yakugaku Zasshi* 2005, 125(7): 587-591.
275. Kinoshita S, Inoue Y, Nakama S, Ichiba T, Aniya Y. Antioxidant and hepatoprotective actions of medicinal herb, Terminalia catappa L. from Okinawa Island and its tannin corilagin. *Phytomedicine* 2007, 14(11): 755-762.
276. Cheng JT, Lin TC, Hsu FL. Antihypertensive effect of corilagin in the rat. *Can J Physiol Pharmacol* 1995, 73(10): 1425-1429.
277. Zhao L, Zhang SL, Tao JY, Pang R, Jin F, Guo YJ*, et al.* Preliminary exploration on anti-inflammatory mechanism of Corilagin (beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose) in vitro. *Int Immunopharmacol* 2008, 8(7): 1059-1064.
278. Dong XR, Luo M, Fan L, Zhang T, Liu L, Dong JH*, et al.* Corilagin inhibits the double strand break-triggered NF-kappaB pathway in irradiated microglial cells. *Int J Mol Med* 2010, 25(4): 531-536.
279. Ghosh SP, Kulkarni S, Perkins MW, Hieber K, Pessu RL, Gambles K*, et al.* Amelioration of radiation-induced hematopoietic and gastrointestinal damage by Ex-RAD(R) in mice. *J Radiat Res* 2012, 53(4): 526-536.