Review
NFκB/p53 crosstalk—a promising new therapeutic target
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A B S T R A C T
The transcription factors p53 and NFκB determine cellular fate and are involved in the pathogenesis of most—if not all—cancers. The crosstalk between these transcription factors becomes increasingly appreciated as an important mechanism operating during all stages of tumorogenesis, metastasis, and immunological surveillance. In this review, we summarize molecular mechanisms regulating cross-signaling between p53 and NFκB proteins and how dysregulated interactions between p53 and NFκB family members contribute to oncogenesis. We furthermore analyze how such signaling modules represent targets for the design of novel intervention strategies using established compounds and powerful combination therapies.

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Abbreviations: AD, adaption domain; AKT, cellular homolog of murine thymoma virus v-akt oncogene, serine/threonine kinase B; AML, acute myeloid leukemia; AP-1, JUN/FOS transcription factor; ATM, ataxia telangiectasia mutated; ATR, ATM- and RAD3-related; ARD, ankyrin repeat domain; ARF, alternate reading frame of the INK4a/ARF locus; α5/11, integrin α5/11; BAFF, B-cell-activating factor; BAX, BCL-2-associated X protein; BCL, B cell lymphoma; BH3, BCL-2 homology domain 3; CDK, cyclin-dependent kinase; CHK1, checkpoint kinase 1; CHUK, checkpoint kinase 2; CHUK, conserves hox-luep-hoxiluous kinase; CK2, casein kinase 2; COP1, caspase recruitment domain family, member 12; CTD, C-terminal domain; DDR, DNA damage response; dsδ, double strand breaks; EGGG, epigallocatechin-3-gallate poly-phenol; EGF, epidermal growth factor receptor; EGFR, epidermal growth factor receptor; EGR1, early growth response 1; EMT, epithelial-mesenchymal transition; ERRK, extracellular-regulated related kinase; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-ligase; FAK, non-receptor focal adhesion kinase; FAS, TNF-receptor superfamily, member 6 (CD95, APO-1); FASL, FAS-ligand; GLUT, glucose transporter; HAT, histone acetyltransferase; HB-EGF, Heparin-binding EGF-like growth factor; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HDM2, human double minute ubiquitin ligase; HNSCC, head and neck squamous cell carcinoma; HU, hydroxyurea; ICAM, intracellular adhesion molecule; IκB, inhibitory proteins; IkK, bB kinase; IL-1, interleukin-1; JAK, Janus kinase; KAI1/CD82, cluster of differentiation 82; cluster of differentiation 82; LTβ, lymphotxin-β; LTA, lysophosphatidic acid; MCF, monocytone chemoattractant protein; MDM2, murine double minute ubiquitin ligase; MICA, Mitogen-activated protein kinase kinase; MMP, matrix metalloproteinase; Msnod, superoxide dismutase 2, mitochondrial; NEMO, NFκB essential modulator; NLS, nuclear localization signal; NFκB, nuclear factor ‘kappa-light-chain-enhancer’ of activated B-cells; NIK, NFκB-inducing kinase; p53; AIP1, p53-regulated Apoptosis-Inducing Protein 1; PAT, protein acetyltransferase; PDC, PDAC, protein deacetylase; PC, prostate carcinoma; PI3K, phosphatidylinositol 3-kinase inhibitor; PPase, phosphatase; PAK1, serinase regulator; PAMPs, pathogen-associated molecular pattern; PARP, poly(ADP-ribose)-polymerase; PDCG-A, platelet-derived growth factor; PIA, protein inhibitor y of activated STAT; PID, p53-induced protein with death domain; PHR2, p53-induced ubiquitin-protein ligase; PRD, proline rich domain; PUMA, p53 upregulated modulator of apoptosis; p21, cyclin-dependent kinase inhibitor 21 kDa; p53, tumor suppressor protein 53 kDa; p63, p53 homolog 63 kDa; p73, p53 homolog 73 kDa; RCP, RAB-coupling protein; RDI, ribosomal serine/threonine kinase; RIP, receptor interacting protein; RSK, ribosomal serine/threonine kinase; SIRT, silencing information regulator; SIRT, single strand breaks; SIRT, single strand breaks; SOX, reactive oxygen species; SGT, ubiquitin ligase complex of Skp1/Cul1/Rbx1/F-box protein-TRCP1; SHP, SH2 domain containing phosphatase; SUMO, small ubiquitin-like modifier; sβ, single strand breaks; SIRT, single strand breaks; SIRT, single strand breaks; TIGAR, TP53-induced glycolysis and apoptosis regulator; TNFα, tumor necrosis factor-α; TRAF, TNF receptor associated factor; TRIM24, Tripartite motif-containing 24 (TRIM24), transcriptional intermediary factor 1ε; ZEB1/ZEB2, zinc finger E-box binding transcription factors

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I. Introduction

Members of the nuclear factor-κB (NF-κB) and p53 protein families are pivotal for the maintenance of homeostasis and are frequently dysregulated in cancer. Intense investigations have demonstrated that both factors are linked to oncogenesis and all steps of tumorigenesis. Crosstalk of p53 and NF-κB occurs at multiple levels and has to be considered as a highly context-specific event. Generalizing this process as simply synergistic or antagonistic is hence misleading. Here, we focus on the crosstalk between the NF-κB family member p65 and p53 and we summarize possibilities to influence their interactions. Ongoing research will further clarify how p53 and NF-κB interact in specific cellular contexts and under specific stimuli. This knowledge will help to identify and develop strategies for tailored therapies.

2. Characteristics and activation of NF-κB proteins

2.1. NF-κB family members

The NF-κB transcription factor family crucially controls diverse biological processes, such as immune responses, development, cell survival, and growth. Therefore, it is not surprising that NF-κB signaling contributes to the development and progression of human diseases including cancer and aberrant immunological functions [1]. In mammals, five different NF-κB family members have been identified: RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). These proteins form hetero- or homodimers [2]. All NF-κB family members share an N-terminal stretch of approximately 300 amino acids. This Rel-homology domain (RHD) is responsible for DNA-binding, dimerization, and nuclear translocation. In resting cells, the majority of NF-κB dimers are associated with a family of ankyrin repeat domain (ARD) containing NF-κB inhibitory proteins. These inhibitors of NF-κB (IκBα, IκBβ, or IκBγ), mask the nuclear localization signal (NLS) present in the RHD and sequester NF-κB in the cytoplasm. Only RelA, RelB, and c-Rel possess the C-terminal transcriptional activation domain (TAD).

2.2. Classical and alternative pathways

Three major pathways regulate NF-κB proteins. These are the canonical, the alternative, and the atypical pathways. The canonical pathway is engaged in response to various inflammatory stimuli like the cytokines tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), and pathogen associated molecular patterns (PAMPs). Central for the canonical pathway is the IκB kinase (IKK) complex. The IKK signalosome consists of at least three core subunits, the two kinases IKKα (IKK1/CHUK) and IKKβ (IKK2) and the regulatory subunit IKKγ/NEMO [3,4]. Upon activation, the IKK complex phosphorylates IκBα which allows their ubiquitin-dependent proteasomal degradation. This proteolysis permits nuclear translocation of classical NF-κB (RelA/p50) (Fig. 1A).

Proteasomal degradation of IκBα involves the E3-ubiquitin ligase SCF (Skp1/Cul1/Rbx1), which uses the F-box proteins β-TrCP1 or β-TrCP2 as a serine S32/36-phosphorylated IκBα recognition subunit [5-7]. Classical NF-κB mediates survival under pro-inflammatory conditions.

The second, more recently described non-canonical or alternative pathway is for example triggered by B-cell-activating factor (BAFF), CD40 ligand, and lymphoxygen-1 (LTI). Such signals promote processing of the p52 precursor p100 [2]. This pathway is characterized by delayed kinetics with a dependency on de novo protein synthesis. Activation of non-canonical NF-κB signaling involves the NF-κB inducing kinase (NIK), which phosphorylates the T-loop series of IKKα [8,9]. Independent of IKKα and IKKγ, IKKξ phosphorylates S727 of p100 with subsequent ubiquitylation of p100 at lysine K63S. Proteasomal processing of the p100 C-terminal domain liberates p52 [10,11]. Afterwards, p52/RelB translocate to the nucleus and activate a subset of NF-κB target genes harboring IκB elements with high affinity for such dimers [12]. Under physiological conditions, the alternative pathway controls the development of secondary lymphoid organs, T- and B-cells.

2.3. Atypical pathways

Atypical NF-κB signaling refers to all pathways that do not fall in the two categories mentioned above [13,14]. This heterogeneous group of pathways is evoked by replicational or genotoxic stress (e.g. UV-light, γ-irradiation or chemotherapeutically active drugs inhibiting topoisomerases or the replication machinery) (Fig. 1B). Central to NF-κB activation by genotoxic stress is the regulatory IκK subunit IKKγ/NEMO [14,15]. Upon induction by genotoxic stress, NEMO accumulates in the nucleus and forms a complex with PIDD (p53-induced protein with death domain) and RIP1 (receptor interacting protein 1) [16,17]. In the presence of PIDD, the E3 SUMO-ligase PIASy (protein inhibitor of activated STAT) catalyzes covalent modification of NEMO with a small ubiquitin-like modifier (SUMO) [18]. Subsequently, NEMO is phosphorylated by the ataxia telangiectasia mutated (ATM) kinase at serine S85, which leads to desumoylation and K63-linked ubiquitylation (UbK63) of NEMO [17,19,20]. As a consequence, NEMO/ATM complexes locate to the cytoplasm and activate the IKK complex. Besides DNA-damage and cell cycle disturbances, other cellular stressors, like oxidative stress or heat shock, activate this NF-κB signaling pathway [21]. Interestingly, the ATM-related kinase ATR (ATM- and RAD3-related) blocks NEMO phosphorylation and NF-κB-dependent anti-apoptotic gene expression patterns [22]. Recent data demonstrate that the DNA-damage sensor poly(ADP-ribose)-polymerase-1 (PARP1) also contributes to NF-κB activation by genotoxic stress [23]. PARP1 generates poly(ADP-ribose) (PAR) chains that are transferred to glutamic acid residues of target proteins. After sensing single-strand DNA damage, PARP catalyzes its auto-PARylation. After dissociation from DNA-damage foci, PARylated PARP1 builds a scaffold to recruit NEMO, PIASy, and ATM, needed for subsequent NEMO sumoylation and NF-κB-dependent survival signaling [23]. In addition to
3. Characteristics and activation of p53

The p53 protein has been in the focus of research for more than 30 years. It is considered as a first line tumor suppressor regulating cell proliferation, senescence, (re-) differentiation, apoptosis, and metabolism [27,28]. Sophisticated regulatory circuits negatively regulate p53 in non-stressed cells and allow its rapid induction upon exposure to stressors (Fig. 2A).

p53 regulates cell cycle progression and cell death by transcription-dependent and -independent mechanisms. Low levels of stress induce p53 activating genes linked to cell cycle arrest, DNA repair, and senescence. Thus, p53 can promote repair and survival signaling, e.g., via transcriptional induction of the cyclin-dependent kinase inhibitor p21^{CDK}0. More intense stress stimuli enhance accumulation of p53 and activation of pro-apoptotic genes like PUMA, NOXA and BAX. These unlease caspases, which dismantle critical cellular proteins including those previously induced to permit repair and arrest. Consequently, damaged and potentially harmful cells can be eliminated [29–33] (Fig. 2B). Inactivation of anti-apoptotic mitochondrial BCL proteins by p53, control of autophagocytosis, regulation of miRNAs and the control of metabolic activity and kinase-dependent signaling are additional routes by which p53 affects cell fate decisions [34–37].

The p53 protein harbors seven functional domains and a fairly large set of enzymes catalyzes its posttranslational modifications [38,39]. These are phosphorylation, acetylation, methylation, glycosylation and the transfer of ubiquitin, SUMO, and NEDD (Fig. 2C). Each of these modifications can determine the stability and activity of p53 and the number of p53 regulators identified increases constantly. For example, dependent on the cell type and condition, several ubiquitin-ligases, COP1, PIRH2, TRIM24, β-TrCP, and HDM2/MMD2, target p53 for proteasomal degradation. These enzymes are functionally interconnected with each other and occur within ubiquitylinating complexes receiving input from numerous signaling pathways [40–44]. Likewise, a large set of serine/threonine kinases and lysine acetyltransferases, which are regulated by cell cycle progression, growth hormones and cytokines, direct phosphorylation and acetylation of p53 at different sites [45,46]. In addition, the p53 gene has several promoters and codes for different isoforms created by alternative splicing and translation initiation sites [47]. The p53 homologues p63 and p73 share this configuration at the gene and protein level. Nonetheless, this protein family exerts a multitude of agonistic and antagonistic functions creating multifaceted regulatory modules and networks [48–51].

Abolishing p53 functions accelerates oncogenic cascades at several levels. Genetic or structural inactivation of this protein occurs in over half of all human cancers [52]. Common p53 mutants carry “hot spot” mutations. These are e.g. the conformational mutants p53^{R172H} (Mus musculus)/p53^{R175H} (Homo sapiens) (p53^{R172H} is the Li–Fraumeni syndrome mutation of p53) and the DNA-contact mutants p53^{R270H}/p53^{R273H}. Either of these mutations is located within the DBD. Tumor-associated mutations in the TAD (p53^{S46}p53^{W26}), PRD (p53^{T4}p53^{D4}), and CTD (p53^{C6}p53^{S34}) are also found frequently [38]. As a result, p53 mutants often lack the ability to bind DNA sequences recognized by the wild type protein or have lost transcriptional activation capacity. Nevertheless, they functionally and physically interact with cancer-relevant transcription factors, including wild type p53 and NF-κB [53–56]. Such p53 mutants are hence able to affect signaling and chromatin modifications relevant for tumor maintenance, genetic instability, metastasis, immunological control, and chemotherapy.
resistance [57–59]. Compared to a loss of p53, mutant p53 even correlates with more aggressive tumor phenotypes and worse prognosis [28,60,61]. It should though be noted that biochemically different p53 mutants can be distinct functional units [54]. Biological outcomes of p53 mutation(s) may furthermore be dictated by the combination of mutant molecules and the levels of wild type p53. For example, co-expression of p53V274F/p53P223L alters p53 activity and promotes prostate cancer cell proliferation and resistance against FAS [62]. Another mutant, p53R175H, is structurally defective and confers pro-survival NF-κB activation in several cancer cell types [55,56]. These data prove that interactions of p53 with other (transcription) factors form additional layers controlling p53-dependent processes. Disturbances in such pathways might be a reason why mutations of p53 sometimes aggravate oncogenesis more than the loss of p53, e.g. from the perspective of stress responses (Fig. 2B). Below, we summarize examples of the functional interplay between p53 and NF-κB in particular contexts.

4. Crosstalk scenarios

Nuclear crosstalk between two transcription factors can occur at several levels. We would like to summarize and propose a matrix for models concerning transcriptional crosstalk phenomena. Therefore, transcription factor 1 and transcription factor 2 in Fig. 3 represent examples, e.g. NF-κB and p53. An indirect model explains crosstalk between transcription factors by induction of gene products. These could be receptors, cytosolic factors, or further DNA binding proteins (Fig. 3A). The exclusion model relies on one factor preventing binding of the other to target DNA. Transcription factor 2 could for example replace transcription factor 1 and shut down transcription (e.g. via recruitment of negative regulators) (Fig. 3B). Transcription factors can compete not only for space on chromatin, but also for cofactors. Depending on the levels, stability, localization, and posttranslational modifications these can recruit activators, and potentially also repressors from the other factor (Fig. 3C).

Activation in trans could be exerted by co-recruitment of positive transcriptional regulators by two transcription factors. Accordingly, trans-repression can be carried out via recruitment of repressors. Cis-activation relies on positive signals recruited by a transcription factor on chromatin. These can affect the other transcription factor directly or by altering the conformation of chromatin. Accordingly, suppressive effects in cis involve interactions of transcriptional repressors creating an overall negative effect (Fig. 3D).

The model termed clearance builds on the removal of deposited, inactive forms of a transcription factor. This complex re-set model e.g. suggests that a receptor activates two transcription factors and that induction of one of them removes inactive forms of the other from chromatin. Upon cognate stimulation, e.g. by a ligand-bound receptor, active transcription factors proceed to DNA and promote gene expression. This may involve posttranslational modifications, which can also be generated from within the cell. For productive transcriptional induction by both proteins, this model requires a delay in the activation of transcription factor 1 versus transcription factor 2. This could for example be achieved with receptor-mediated activation of one protein and time-delayed induction of the other in the nucleus. Chromatin cleared can then set for re-activation or can be target for further transcription factors entering from the cytosol. Such processes might create persistent target gene expression (Fig. 3E).

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Fig. 2. p53 signaling pathways and p53 structure. (A) The tumor suppressor p53 is activated by stimuli endangering the integrity of the genome and by oncopgenes. Thus, p53 serves as a first line barrier against tumorigenesis, even in the absence of intact gene DNA. While low levels of p53 promote gene expression for survival and repair, higher levels induce pro-apoptotic genes. The efficacy of a lot of chemotherapies relies on p53 induction. UV, ultraviolet light; γ, gamma-irradiation; HU, hydroxyurea; CPT, camptothecin; VP16, etoposide. (B) As p53 is a central modulator of cellular life/death decisions, it is not surprising that a very complex network controls and fine-tunes its functions tightly. The amplitude and persistence of stress stimuli determine accumulation of p53, with more intense stressors switching from arrest, repair, and survival signaling to pro-apoptotic gene expression patterns. The dose-dependent phenomenon characterized by low dose-induced prevention and regeneration on the one hand and damage caused by high doses on the other hand has been termed “homeostasis.” Changes of chromatin mediate such processes and crosstalk phenomena also dictate ON/OFF states ruling cellular survival or death. Active chromatin is marked with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks. Green, red, blue ovals are transcription factors or cofactors, which can be gene products of the stress response, e.g. with yellow dots representing positive transcriptional marks.
The oscillatory model argues further for permanent but transitory states. This model involves dynamic changes which require timed antagonistic activities, e.g., of HATs and HDACs, kinases and phosphatases. A second transcription factor could promote such exchanges leading to a continuous clearing and loading of chromatin. The upper row shows a rather complete reset of transcriptional complexes. The lower row considers that e.g., the activated transcription factor might persist. In both scenarios, cofactors cycle constantly and antagonistic enzymatic activities maintain fully processive rounds of oscillatory cycling (Fig. 3F). Such rather complex networks have already been found for signaling via the estrogen receptor and STAT proteins. In addition to alterations at the chromatin level, non-histone protein modifications are involved in these processes [46,63–69].

5. Processes regulated by the crosstalk between NFκB and p53

5.1. Cell cycle progression and apoptosis

The intricate regulation of cell survival or death is critical for normal and transformed cells. One cannot simply place p53 and NFκB family members into pro- or anti-survival categories. Instead, the molecular mechanisms of their interactions are complex and still to be elucidated fully. Identifying critical points where NFκB and p53 form functional units, up- and down-stream of each other, can be a critical step broadening our understanding of cancer biology.

A very surprising initial report on the interplay between p53 and NFκB reveals that, despite of its role as a tumor suppressor, NFκB becomes activated upon (re-)activation of p53. Even more so, p53-induced apoptosis requires NFκB. This mechanism of NFκB activation does not resemble TNFα-induced NFκB signaling, but is linked to activated MEK1 and RSK serine/threonine kinases [70]. Agents causing double-strand DNA breaks also induce RSK-dependent phosphorylation of nuclear p65, which lowers its affinity for IκBα. Consequently, the binding of NFκB to its cognate enhancers and NFκB activity increase [71]. Similar data are found with a dominant-positive p53 variant in glioma cells [72]. Thus, NFκB activation can be associated with apoptosis in cells with (hyper)active p53.

These findings argue that NFκB signaling linked to p53 can be a nuclear process and independent of cytosolic IκB degradation. Nevertheless, nuclear IκB can also be ubiquitinylated and processed, with p53 catalyzing this process [73]. In addition, replicational stress and DNA damage signaling evoke nuclear translocation of NFκB.
subunits [56,74], and functionally relevant degradation of IκBα upon DNA damage signaling has been reported [22,75]. Possible explanations for anti-survival NFκB signaling might be that NFκB is necessary to induce accumulation of the pro-apoptotic BH3-only proteins PUMA (p53 upregulated modulator of apoptosis), NOXA, and p53-AIP1 [76,77]. For example, TNFα-mediated upregulation of the p53 target gene PUMA at mRNA and protein levels depends on NFκB. Apoptosis of small intestinal epithelial cells, hepatocytes, and thymocytes, was consequently attenuated in PUMA-deficient mice treated with this cytokine [77]. TNFα stimulation of colon cancer cell lines carrying p53R273H (HT-29) also enhanced nuclear p53 levels and increased PUMA mRNA levels [78]. As this DNA-binding defective p53 mutant is recruited to this promoter via p65, it has retained or acquired an indirect ability to bind the PUMA promoter. Curiously, IL1β and IFNγ induce PUMA in pancreatic β-cells via NFκB but independent of p53 [79]. Also surprising is the finding that p53-dependent induction of the NOXA protein is blocked by a super-inhibitor of IκB although the mechanism responsible is independent of NFκB transfection. This finding suggests involvement of so far unknown pathways [76]. Such data argue that our view on p53 and NFκB target genes requires a novel definition.

Independent of the exact mechanisms, loss of NFκB p65 can confer resistance against stimuli that signal death through p53 activation. In contrast to a lack of p53, loss of p65 still cannot induce anchorage-independent growth or oncogene-induced tumorigenesis. Hence, p53 restricts tumor development by p65-dependent and -independent mechanisms [80]. Mouse embryonic fibroblasts (MEFs) from different p65 knockouts are morphologically heterogeneous with transformation linked to alterations in the p53 pathway. The fact that v-RAS+/p65− cells formed fewer colonies than control MEFs and kept high sensitivity to TNFα-induced apoptosis hints that p65 suppresses or restrains tumorigenesis depending on individual circumstances [81].

Albeit these findings argue for certain anti-tumor properties of NFκB, one has to keep in mind that p53 suppresses anti-apoptotic oncogenic NFκB in resting cells [56,82,83]. Dysregulation of this control mechanism can well be linked to cellular survival, e.g. as resistance towards chemotherapeutics or as adaptation to stresses. In particular, the p53R172H (corresponding to human p53R175H) mutant evokes strong NFκB signaling and protects murine pancreatic cells from apoptosis. [56]. These data are in agreement with a recent publication demonstrating that mutant p53 augments TNFα-induced NFκB activity and protects tumor cells from TNFα-induced apoptosis [55]. Therefore, p53 mutation rather than its loss can provide NFκB-dependent survival advantages to tumor cells of various origins.

Interestingly, a common feature of mutant p53-dependent gene signatures is an enrichment of genes associated with proliferation [53]. Since NFκB is linked to the regulation of cell cycle genes, it is interesting to investigate whether mutant p53 drives proliferation via p53/p65 complexes. In fact, mutant p53/NFκB cross signaling drives cell cycle progression indirectly, via activation of the mitogen-activated protein kinase kinase 3 (MAP2K3, upstream activator of the p38 MAPK). Here, it was demonstrated that mutant p53 binds together with NFκB and NF-Y to the MAP2K3 promoter and activates transcription of the gene. Furthermore, the MAP2K3 gene was shown to mediate proliferative functions of mutant p53 [84]. It has furthermore been found that p53 null lung cancer cells acquire a growth-advantage by producing soluble factors suppressing p53 in adjacent stromal cells [85].

Replicational stress, e.g. G1/S-phase arrest induced by dNTP depletion, also activates NFκB p65 and triggers its interaction with nuclear p53 [56]. Whereas ATM and CHK1 signaling and cytotoxic activation of NFκB p65/p50 are intact in p53 null cells, formation of a transcriptionally active complex requires nuclear p65 as well as p53. These findings, which were collected in diverse solid tumor cell lines and MEFs, argue for p53 being a licensing factor for NFκB target gene activation in cells arrested in S-phase. Since functionally important interactions of p53 and p65 also occurs after stimulation with the pro-inflammatory cytokine TNFα, the p53 licensing function equally operates upon canonical NFκB activation (Fig. 4). We speculate that this represents a cancer-protective mechanism facilitating elimination of p53 null cells. In agreement with this idea, a recent in vivo study assessing K-RAS driven lung tumorigenesis disclosed that NFκB suppression results in apoptosis of p53 null cancers [86]. It is moreover possible that p53/NFκB interactions operate during normal S-phase and meiosis. Furthermore, structurally mutant p53 reverts checking functions of wild type p53 on NFκB as it promotes anti-apoptotic gene expression patterns [55,56].

5.2. Metastasis

Metastasis is a fatal late-stage condition of cancer. This highly organ-specific process requires multiple steps and interactions between cancer cells and the host [87,88]. One mechanism of metastasis formation is the change from highly differentiated epithelial cell morphology to a mesenchymal phenotype. Epithelial-mesenchymal transition (EMT) involves alterations in gene expression patterns, e.g. a loss of E-cadherin and cytokeratins and an induction of N-cadherin, Vimentin, Fibronectin, metalloproteinases, and SNAIL [89]. During EMT, cancer cells acquire migratory capacity and invasiveness [90]. Identifying signaling pathways and the origin of pathologically transformed mesenchymal cells can give insights into valuable therapeutic interventions potentially targeting metastasis formation at early stages. Since wild type and mutant p53 as well as...
NFκB orchestrate gene expression associated with metastasis, they could be critical targets for such strategies [91,92].

Crosstalk between p53 and NFκB is associated with EMT [59]. For example, expression of wild type p53 in human soft tissue sarcoma cells and xenografts bearing mutant p53 decreased matrix metalloproteinase-9 (MMP-9) levels required for invasiveness. Of note, this cancer is highly lethal with metastasis determining survival. Interestingly, the effect of p53 on MMP-9 expression strictly depended on a κB binding site in the MMP-9 promoter, which indicates effects of p53 on transcriptionally active NFκB [93]. Additional metastasis regulators are regulated by p53 and NFκB. The tumor suppressor KAI1/CDB2 is a target gene of p53 and NFκB. Mutant p52R248Q, though cannot impair TNFα-mediated activation of KAI1/CDB2 in lung cancer cells [94,95]. In contrast, p53[227H] expression promotes cellular invasion and migration of endometrial cancer cells via dominant-negative effects on p53. This mutant p53 represses expression of the metastasis suppressors KAI1 and maspin, and the senescence regulator PA1H [96].

Critical functions of mutant p53 also occur at the level of signaling initiated by a membrane-bound receptor tyrosine kinase involved in oncogenesis. Mutant p53 stimulates metastatic patterns via constitutive activation of epidermal growth factor receptor (EGFR) and integrin α5/β1-signaling in various cell lines, mice, and primary tumors [60]. Mechanistically this relies on enhanced RAB-coupling protein (RCP)-dependent trafficking and recycling of these factors, with mutant p53 promoting interaction of α5/β1 and RAB indirectly. Activation of the serine/threonine kinase AKT by mutant p53 in cultured cells and human colorectal cancers proves EGFR (hyper) activation. Moreover, TGF-α-independent binding of p53[273H] and p53[227H] to p63 inactivates this transcription factor and promotes invasive migratory phenotypes [60].

Of note, certain breast cancers overexpressing EGFR demonstrate highly active NFκB. As both factors contribute to transformation of ER-negative human mammary epithelial cells [97], it will be interesting to determine how p53 is tied into this sequela. Other studies likewise report that p53[175H] expression correlates with cell migration, invasive capacities, and EGFR/ Akt activation, for example in endometrial cancer cells [98]. In prostate adenocarcinoma cells carrying intact wild type p53, p53 mutants (V143A; R175H; R249S; R273H) enhance expression of EGR1 (early growth response 1) at the mRNA and protein levels. EGR1 increases the expression of stimulatory EGFR ligands (HB-EGF), cytokines promoting angiogenesis (PDGF-A), TGF-β, and EGFR levels [99]. Secretion of such factors might hence impose p53 mutant properties to p53 positive cells in vivo. Senescence due to EGFR overexpression is equally suppressed by p53[175H] and by temperature-induced inactivation of p53[313A]. This finding, which was collected in telomerase-immortalized human esophageal cells, suggests that a loss of p53 functionality, but not EGFR signaling per se, expands a subpopulation of aberrantly regulated cells. A critical role for p53 in this system is further stressed by the finding that p53 controls p21 and the zinc finger E-box binding transcription factors ZEB1/ZEB2. These proteins belong to a senescence checkpoint counteracting EMT [100].

5.3. Immune surveillance

NFκB proteins regulate the differentiation, survival, and proliferation of immune cells. Accordingly, inflammatory cytokines and mediators, toxins, viruses, bacteria, and irradiation trigger beneficial NFκB during acute host responses. However, persistent NFκB signaling causes detrimental chronic inflammation, which is linked to genetic instability and carcinogenesis. Other key transcription factors for inflammation are e.g. STAT1 and STAT3. As for NFκB, a plethora of reports links their pro- and anti-apoptotic properties to p53 at multiple stages of immune responses [101–104]. Another aspect to be considered is that NFκB p53-dependently induces the FAS/FAS-ligand (FASL) system for immunological tumor surveillance [22,56]. Expression of this molecule can induce apoptosis of immune cells [105]. On the other hand, a lack of FASL expression in p53-negative cells could promote their elimination via immunologic surveillance. Neighboring p53-proficient cells could promote this by secreting the NFκB induced Interleukin-8 [56], a chemokine attracting immune cells. These examples show that p53 suppresses cancer via cell-autonomous as well as non-cell-autonomous activities.

Cells expressing p53 not only protect themselves by FASL secretion. Upon exposure to TNFα or stress, they induce the NFκB target gene MmSOD, an enzyme balancing ROS signaling [56,106]. Such complex interactions between p53 and NFκB likely are relevant for tumorigenesis associated with chronic inflammation and tissue damage [107–109]. The situation though seems complicated, as p65- and p53-dependent transcriptional activation of the MmSOD gene depends on the intensity of stress encountered. Transfection of low concentrations of p53 into p53 null prostate carcinoma cells increases, whereas high amounts suppress MmSOD levels. At the level of the MmSOD promoter, p53 binding does not affect its activity. Instead, the presence of an intrinsic-enhancer element harboring NFκB binding sites is necessary for positive effects of p53 on MmSOD expression [110]. The presence of NFκB at different genomic sites can therefore rule p53-NFκB crosstalks. Whether or not p53 and p65 interact and/or compete at such sites remains to be proven formally.

Above we summarized that DNA single (ssBs) and double (dsBs) strand breaks signal to p53 and NFκB. Similar lesions occur during T- and B-cell receptor recombination, normal S-phase and meiosis. Proper maturation of immunocompetent cells may hence be subject to control by p53-NFκB modules. Furthermore, the immune system serves as a barrier for neoplastic transformation and p53 contributes to the communication between cancer and immune cells [111]. Tumors carrying mutant p53 isoforms can e.g. protect themselves from attacks by the immune system via anti-apoptotic NFκB target gene induction [55,56,110]. Moreover, paracrine and autocrine stimuli secreted by cells with activated NFκB influence immunological reactions associated with inflammatory conditions ultimately leading to tumorigenesis. Cancer cells furthermore activate and signal reciprocally with infiltrating white blood cells, adjacent fibroblasts, the endothelial cell wall, and the extracellular matrix [112,113]. Therefore, targeting p53-NFκB modules might be a valuable strategy for therapies aiming at the tumor stroma and its dialog with the cellular environment.

5.4. Metabolic control

Tumor cells often prefer glycolysis and therefore generate ATP by metabolizing glucose to lactate. In contrast, oxidative phosphorylation occurs in normal tissues under conditions of sufficient oxygen supply. This observation was first described by Otto Warburg in the last century [114]. Recent work demonstrates that both, p53 and NFκB regulate metabolism in opposite manners, with p53 favoring oxidative phosphorylation and NFκB enhancing aerobic glycolysis [115]. p53 slows uptake of glucose by repressing the glucose transporters GLUT1 and GLUT4 [116], inhibits glycolysis by induction of the TIGAR (TP53-induced glycolysis and apoptosis regulator) gene [117], and favors oxidative phosphorylation by the synthesis of cytochrome c oxidase 2 gene encoding an important regulator of the cytochrome c oxidase complex [118]. Furthermore, loss of p53 promotes glycolysis dependent on p65, as p65 transcriptionally activates the GLUT2 gene [119]. High cellular glucose shunts through the hexosamine biosynthetic pathway to produce uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), the active substrate for O-GlcNAcylation (O-linked-N-acetylglucosamine, O-GlcNAc). Mechanistically, p53 deficiency permits O-GlcNAcylation of IKKβ on the inhibitory phosphorylation site S720 and increased activity of the enzyme [120].

Although these data provide functional insights into the crosstalk of p53 with NFκB at the level of metabolism, we may learn more about the therapeutic potential. For example, what are the roles of p53 and
p65 upon the treatment of cells with inhibitors of glycolysis? Is deletion of p65 in tumor cells reversing the Warburg effect? Furthermore, is the enzyme mediating O-GlcNAc-ylation, the O-GlcNAc transferase (OGT), therapeutically relevant and what are functions of p53 and p65 upon OGT inhibition?

Considering that p53 is more often mutated than deleted in tumors we also have to carefully consider mutual interactions of p53\textsuperscript{mut} and p65 with respect to metabolism. Interestingly, p53\textsuperscript{R172H} synergizes with oncogenic RAS to accelerate glycolysis and to induce GLUT3 p65-dependent [111]. Bearing in mind complex formation of p53\textsuperscript{R172H} with p65 in solution and on DNA [56], it is essential to investigate effects of this complex for metabolism. Deciphering the signaling pathways needed to build the complex should define novel targets for intervention with p53\textsuperscript{mut}/p65 cross-signaling.

6. Modulators of NF\textsubscript{κ}B/p53 cross-signaling

6.1. Proteins regulating the p53/NF\textsubscript{κ}B crosstalk

Collaborations between NF\textsubscript{κ}B and p53 govern stress- and inflammation-induced cancer and therapeutic resistance [55,56,70,71,82,110]. Which factors regulate their interactions?

A protein regulating both p53 and p65 is e.g. the tumor suppressor ARF. The \textit{ARF} gene (alternate reading frame INK4a) encodes p14\textsubscript{Homo sapiens} or p19\textsubscript{Mus musculus}. These proteins inhibit HDM2/MDM2, which augments p53 stability and suppress cell proliferation driven by the transcription factor E2F1 [43]. The p53 protein suppresses ARF transcription to maintain low levels of p53 in unstimulated cells. Oncogene activation triggers anti-apoptotic NF\textsubscript{κ}B p65 activity and ARF, which induces the checkpoint kinases ATR and CHK1. CHK1 phosphorylates p65 at threonine T505 and this modification inactivates its TAD. Since ATR induces and activates p53, ARF integrates p65 and p53 functions [121]. Furthermore, this kinase causes partial suppression of NF\textsubscript{κ}B induced by DNA breaks. ATR interacts with NEMO and disrupts its phosphorylation by ATM in cells with intact p53 signaling. Curiously, the established chemotherapeutic drug etoposide causes overall anti-apoptotic NF\textsubscript{κ}B expression, whereas another clinically relevant anti-cancer agent, hydroxyurea (HU), evokes pro-apoptotic gene expression [22]. Solving this issue further could explain why ATM-specific inhibition accentuates cytotoxicity evoked by chemotherapeutics inducing atypical NF\textsubscript{κ}B signals [122]. Experiments carried out with myeloblasts from myelodysplastic syndrome or acute myeloid leukemia (AML) patients already propose that pharmacological inhibition of ATM causally inhibits p65 and induces apoptosis [123]. The interesting question remains whether blocking ATM abrogates NF\textsubscript{κ}B signaling dependent or independent of p53.

A murine model of spontaneous melanoma, driven by H-RAS\textsuperscript{V12} and a lack of ARF revealed that IKK\textsubscript{β} also integrates p53 and NF\textsubscript{κ}B. In this system, IKK\textsubscript{β} impairs p53 expression and supports melanoma development. Genetic depletion accordingly unleashes p53 expression. Stabilized p53 carrying p-S\textsuperscript{15} decreases BCL2 and Survivin evokes apoptosis as well as cell cycle arrest, and attenuates cyclin-dependent and aurora kinases. Apparently, IKK\textsubscript{β} is central for NF\textsubscript{κ}B activity and equally for keeping p53 low during oncogene-induced melanocyte transformation [83].

Regulatory processes can also operate at the level of transcription factors beyond NF\textsubscript{κ}B and p53. For example, NF\textsubscript{κ}B and STAT3 cooperatively regulate genes implicated in survival and proliferation, mostly via transcriptional cooperation or via induction of cytokines releasing the other transcription factor from latency. Accordingly, NF\textsubscript{κ}B and STAT3 agonistically promote the development and progression of colon, gastric, and liver cancers [113]. The crosstalk between STAT3 and NF\textsubscript{κ}B therefore represents an attractive chemotherapeutic target. Since p53 and STAT3 affect each other antagonistically [124–126], activating p53 might reach this goal. This strategy could as well complement approaches targeting pro-inflammatory IKK-dependent NF\textsubscript{κ}B, for STAT3 delays NF\textsubscript{κ}B nuclear export in tumors and associated immune cells [127].

Contrary to wild type p53, mutant p53\textsuperscript{G199V} promotes expression of STAT3 in anaplastic thyroid cancer cells [128]. In head and neck squamous cell carcinomas (HNSCC), decreased p53 expression enhances cytokine-induced activation of NF\textsubscript{κ}B, STAT3, and induces higher expression of their target gene BCL-XL at low levels of BAX. Such observations further support inhibition of NF\textsubscript{κ}B and STAT3 together with restoration of p53 functions [126].

6.2. Pharmacological and physiological stimuli

Restoring wild type p53 leading to transcription-dependent and -independent control of tumor growth by this protein is clearly desired. Do we have drugs available that restore pro-apoptotic functions of p53 and NF\textsubscript{κ}B and on the other hand inhibit their cancer cell-protective properties? We would like to summarize some agents that target both factors (Fig. 5A) and which are promising for clinical use.

Fig. 5. (A) Targeting aberrant NF\textsubscript{κ}B and mutant p53 in cancer. (A) NF\textsubscript{κ}B, p53, their target genes, and regulators are structures for anti-cancer drug development. Shown are examples for agents currently used or tested in the clinic. (B) The interaction between p53 and NF\textsubscript{κ}B, especially with mutant p53 (p53\textsuperscript{mut}), is linked to anti-apoptotic NF\textsubscript{κ}B target gene expression. Blocking this crosstalk could be a valid therapeutic option for tumors with aberrant NF\textsubscript{κ}B signaling mediated by oncogenic p53 (red sphere: agent blocking p65 DBD; black sphere: agent blocking p65–p53 interaction surfaces).

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use. Optimal characteristics of such compounds are inhibition of anti-apoptotic NF-κB survival signaling and (re-)activation of p53. Both can eliminate transformed and malignant cells [52,129].

The NF-κB and p53 signaling pathways are regulated in a different manner by the proteasome. Whereas HDM2 and other E3 ubiquitin-ligases induce proteosomal degradation of p53, degradation of bHLH proteins is a pre-requisite for NF-κB activation. Nutlins specifically block interactions between HDM2/MDM2 and p53 allowing its accumulation inhibiting NF-κB. Simultaneous suppression of basal and induced NF-κB by this strategy promoted tumor cell death and suppressed metastasis-associated gene expression in lung and pancreatic cancers [130,131]. For example, Nutlin-3 attenuates blocked interactions between HDM2/MDM2 and p53 allowing its suppressed metastasis-associated gene expression in lung and pancreatic cancers [130,131]. For example, Nutlin-3 attenuates interactions between HDM2/MDM2 and p53 allowing its suppressed metastasis-associated gene expression in lung and pancreatic cancers [130,131]. For example, Nutlin-3 attenuates interactions between HDM2/MDM2 and p53 allowing its suppressed metastasis-associated gene expression in lung and pancreatic cancers [130,131]. For example, Nutlin-3 attenuates interactions between HDM2/MDM2 and p53 allowing its suppressed metastasis-associated gene expression in lung and pancreatic cancers [130,131]. For example, Nutlin-3 att

Anti-oxidative polyphenols such as theaflavins (TF) and deriva-
tives thereof are formed during the enzymatic oxidation of tea leaves. In breast cancer cells, TF inhibit NF-κB-mediated cell migration, putatively by attenuating expression of the metastatic proteins metalloproteinase (MMP)-2 and MMP-9. This inhibitory effect on NF-κB requires p53-phosphorylation via p38 MAP-kinase and the formation of reactive oxygen species (ROS), i.e. inhibition of NF-κB via a p38–p53–ROS crosstalk is a pre-requisite for theaflavins to accomplish the anti-migratory effect in breast cancer cells [133].

Other small molecule compounds, CP-31398, PRIMA-1, the cytotoxic plant alkaloid ellipticine, and its derivative NSC-176327 correct certain mutants of p53 towards the p53 wild type conforma-
tion. Ellipticine can for example restore activation of p53-responsive genes by nine different p53 mutants (e.g. p53R175H) in cultured tumor cells and xenografts. Such properties permit selective elimination of tumors expressing mutant p53 [134,135]. Reconstituting p53 function is hence feasible for certain mutants [136]. The fact that cancers with mutant p53 might be targeted with therapies directed against EGF and integrin signaling [60] furthermore opens a new avenue for (co-)targeting approaches.

Several agents correcting proteosomal dysfunctions are currently tested in clinical trials [40–44,137,138]. Bortezomib (PS-341) is the first proteasome inhibitor approved for the therapy of multiple myeloma [139]. These agents and other proteosomal inhibitors, e.g. S-2209, lactacystin, and MG-132, suppress NF-κB DNA binding and target gene expression by stabilizing IκB [73,140]. In addition, such compounds enhance p53 levels, alter the coexpressor/coactivator balance in the cell, and disturb cell cycle regulation [141,142]. Interestingly, proteosomal inhibitors were also found to kill tumor cells devoid of p53 [143]. This observation is promising regarding the insufficient p53 pathways in most tumors and the unleashed NF-κB activation in p53-negative cells [56,82]. An inhibitor acting at the most apical step of ubiquitinylation targets ubiquitin-activating enzyme (E1), which transfers activated ubiquitin to E2 ubiquitin-conjugases. PYR-41 is the first such agent identified. This compound blocks ubiquitinylation and cytokine-induced NF-κB, probably via impaired ubiquitinylation of IκBα due to inhibition of the TRAF6-IKK module. Remarkably, induction of p53 and its target genes by PYR-41 correlates with the successful elimination of tumor-derived cells [144].

NF-κB protects cells from lethal effects of TNFx, which limit usage of this cytokine in clinical settings. Optimized chemotherapy might rely on preservation of pro- and inhibition of anti-apoptotic NF-κB functions. Inhibitors of histone deacetylases (HDACi, e.g. LBH-588; SAHA; VPA) can activate genes by loosening tight chromatin structures formed by hypoacetylated histones and DNA. However, certain genes are repressed by hyperacetylation. The induction of non-histone protein acetylation together with secondary effects can likewise antagonize cellular signaling. Indeed, HDACi turned out to be inhibitors of NF-κB-activated pathways [145–147]. At the molecular level, HDACi e.g. induce IκB, loss of TNFR, and acetylation of STAT1.

These alterations, which counteract NF-κB and facilitate apoptosis induction, likely are the reason for enhanced killing when cells undergo treatment with HDACi and TNFx [141,142]. So far, wild type p53 could not be linked to cellular sensitivity towards HDACi. Induction of p21 in HDACi-treated cells rather is due to an unspecific permissiveness of chromatin [141,142]. Even reduced levels of p53 were found in HDACi-sensitive AML cells [148]. Remarkably, HDACi also attenuate mutant p53 and they kill cells with mutant p53 more efficiently than p53 null cells. Whether non-specific restoration of p53-like functions [149], the inhibition of NF-κB [53–56], or other effects caused by HDACi explain their preferential cytotoxicity to cells with mutant p53 needs to be resolved. Reduced survival signaling due to different effects on these transcription factors may be a reason for potent anti-tumor activities of HDACi and genotoxic drugs when given combined to patients suffering from breast or lung cancers [150–152].

Like other phytochemicals, e.g. resveratrol (Vitis vinifera), sibirilin (Silybum marianum), catechins (Camellia sinensis; Theobroma cacao), derivatives of diferuloylmethane (curcumin, the primary bioactive compound of Curcuna longa) suppress NF-κB activation associated with the expression of genes implicated in cellular growth, transformation, angiogenesis, metastasis, and inflammation [153]. At the molecular level, curcumin was found to attenuate the expression of NF-κB target genes, such as cyclin D1 and HDM2, to induce wild type p53 and its target gene p21, and to inhibit STAT3 [154]. The safety and efficacy of this poly-phenol in cell culture and animal models prompted phase I clinical trials for its use as an anti-cancer agent [155]. Resveratrol is an antioxidant counteracting inflammation, metastasis, and angiogenesis by inhibition of NF-κB and AP-1 [JUN/FOS]. This agent furthermore activates pro-apoptotic p53 sparing normal cells [156]. Since p53 and NF-κB are regulated by the class III HDAC sirt1 (silencing information regulator 1), a potential mecha-

A synthetic derivative of the Draacaen benzoferian lignan (benfur) also shows anti-tumor activities. It represses MDM2, inhibits NF-κB, and provokes apoptosis of p53-positive and -negative leukemic cells [157]. The sesquiterpene α-bisabolol promotes accumulation of nuclear wild type p53 and NF-κB subunits. Nevertheless, this substance equally attenuates NF-κB-mediated transcription and causes apoptosis of cell lines derived from solid tumors [158]. Another poly-phenol, epigallo-
catechin-3-gallate (EGCG) from green tea (Camellia sinensis), and the EGFR tyrosine kinase inhibitor erlotinib synergistically inhibit the growth of head and neck squamous cell carcinomas (HNSCC) in vitro and in murine xenograft models. Treatment with EGCG and erlotinib strongly inhibits erlotinib-induced expression of the CDK inhibitors p21 and p27 [159], which restrict apoptosis in other systems and upon other stimuli [33,160,161]. Moreover, EGFR inhibition and ECGG lowers p53 protein levels and promotes expression of p53, which was found required for growth inhibition by these agents [159]. It seems that natural products and derivatives thereof might serve as drugs complementing the arsenal of chemicals and biologicals for the fight against cancer and unbalanced inflammation.

7. Novel models and considerations for therapies

7.1. Possible chemotherapeutic caveats

Although p53 is induced by most chemotherapeutics, it should be kept in mind that p53 mutation occurs in over half of all human tumors. Thus, any p53-based strategy may trigger mutant p53 worsening disease [52]. Moreover, tumors harboring p53 may have lost cell-cycle arrest and apoptosis sensitivity by further mutations. While depletion of mutant p53 represents an extremely attractive target, one may consider that even wild type p53 is able to promote
undesired survival of cancer cells exposed to chemotherapeutics [28,56,162]. Up to ten p53 variants and various mutants furthermore make it difficult to judge whether a unique p53-based optimal cancer therapy might arise [27,47,49]. Crosstalk of p53 with other cellular signaling molecules and transcription factors might equally be subject to the ratio of its isoforms and the abundance of its interaction partners. IKKs are at the heart of inducible NF-κB activities [3,163]. Targeting these kinases, e.g., with BMS-345541, therefore appears as very attractive targets for pharmacological interference strategies. However, NF-κB is able to suppress liver carcinogenesis in vivo. The mechanism underlying is IKKα-dependent inhibition of hepatocyte death associated with compensatory proliferation and hepatocellular carcinoma (HCC). At the molecular level, IKKκ- deficiency leads to increased ROS levels, inhibition of phosphatases, and STAT3 phosphorylation. Hepatocyte-specific STAT3 ablation prevented cancer development and progression [164], which supports the intense search for inhibitors of STAT3, e.g., STA-21 [165] (Fig. 5A).

Targeted approaches could also aim for target genes of NF-κB. Examples are the Survivin gene, which can be repressed with YM-155 [166], or the inactivation of BCL proteins by BH3 mimetics such as ABT-737 [167] (Fig. 5A). Similar as IKK blockade, inactivation of BCL family members has recently been called into question as a therapeutically valid approach. Independent labs noted that γ-irradiation evoked PUMA-driven apoptotic leukocyte death followed by lymphomagenesis [168,169]. Bim which is not a target gene of p53 or p65 is still not linked to tumorigenesis [168,169]. Therefore, agents supporting induction of this BH3-only protein might circumvent undesired effects of puma induction. As in the HCC model [164], reduced proliferation as well as less replication stress-associated DNA damage in the puma null hematopoietic stem/progenitor cells protects mice from irradiation-induced tumorigenesis [168,169]. The high risk of translocations upon T- and B-cell receptor recombination may accelerate lymphomagenesis in this model. Nonetheless, the fact that IL1β and IFNγ induce PUMA in pancreatic β-cells illustrates that similar processes operate in non-hematopoietic cells [79].

Co-activation of p53 with NF-κB in tumors treated with drugs targeting replication or DNA stability might be the reason for therapeutic failures due to pro-survival NF-κB signaling [55,56,70,71,82,110]. On the other hand, the above mentioned results attest that NF-κB can prevent excessive repopulation via mutants from the stem cell niche. Perhaps, a life/death balance evolved during centuries of evolution and this Yin/Yang is far more delicate and intertwined than previously assumed. At present, we are also unable to answer if adaptive responses tie into this complicated scenario. Whether sole induction of p53 or mere suppression of NF-κB is sufficient for therapy correspondingly requires caution reflection. Furthermore, p53 activation allows DNA repair responses as well as apoptosis. Even more, p53 and NF-κB target genes can play unexpected pro- or anti-tumorigenic roles and examining p53 and NF-κB target genes without considering possible crosstalks and interactions seems insufficient. Tumors treated with p53 inducers furthermore represent heterogeneous mixtures of cells. Stochastic distribution of p53 regulation might allow oscillatory responses [32] and error-prone repair can promote oncogenesis [170]. Therefore, precise targeting of cancers is required, also to avoid secondary tumor formation. Whereas depletion of p53 or p65 might have unforeseeable tumor-promoting effects, depletion of oncogenic p53 mutants clearly represents an attractive pharmacological target. Such agents could interfere with the DNA binding capacity of NF-κB or disrupt the surface connecting p65 and p53 (Fig. 5B).

7.2. New model systems

Many of the ideas concerning the molecular crosstalk between p53 and p65 come from valid cell-based in vitro models. However, the rapid progress of genetic engineering in the mouse and the development of defined mouse models for most relevant cancers [171], now allows investigating the impact of p53/p65 interactions for the initiation and progression of tumors in vivo. Furthermore, many mouse lines to manipulate expression and function of p53 (see http://www.p53.free.fr/p53_info/Mouse_model/p53_mouse_models.html) [172] or p65 [173,174] are available. They offer the opportunity to generate simple (e.g. murine low-passage genetically engineered tumor cell lines [56]) or complex cell-based ex vivo models (e.g. tumor cells where fixed alleles can be manipulated by tamoxifen in a CreloxP-dependent manner [86]). The magnitude of this experimental approach was recently demonstrated in a mouse model of lung adenocarcinoma. In MEFs the conditional expression of the K-RASG12D oncogene from the endogenous promoter (lox-stop-lox (LSL)-K-RASG12D) induces nuclear translocation of p65 only in the case of the simultaneous deletion of p53. The molecular mechanism by which p53 loss activates p65 upon expression of K-RASG12D is though still unclear. However, critical functions of p53/ p65 cross signaling are demonstrated elegantly with this experiment and the impact of NF-κB signaling towards tumor initiation was revealed with a dominant-negative IκBx “super-inhibitor” [86]. Consistently, adeno-CRE-dependent activation of K-RASG12D in the lung induces NF-κB and conditional deletion of p65 reduces tumor formation [175]. Although this work shows that deletion of p65 affects tumor formation irrespectively of p53 proficiency or deficiency, the observed effects were less dramatic than with dominant-negative IκBx, arguing that other NF-κB family members, like c-Rel [86], may (co-)contribute to lung tumor formation. More importantly, inhibiting NF-κB signaling by targeting IκBx in established lung tumors, leads to a profound tumor regression in vivo, arguing that blocking NF-κB is a therapeutic approach for K-RAS mutant tumors. Interestingly, only lung cancer cells with the K-RASG12D/p53-negative phenotype are addicted to p65 ex vivo and respond with an apoptotic program upon inhibition of canonical NF-κB signaling [86]. Whether NF-κB is indeed the therapeutic “Achilles heel” of K-RAS and p53 mutated lung tumors in vivo, compared to K-RAS mutated wild type p53 tumors, awaits further clarification. Nevertheless, the model described demonstrates ways to tailor therapies.

A novel murine K-RAS-induced melanocyte transformation model reveals that IKKβ attenuates p53 and allows transformation. Hence, melanoma patients with highly active NF-κB and wild type p53 could benefit from inhibition of the IKKβ integrator [83]. The observation that NF-κB inhibition though sufficient to eliminate p53 negative lung cancers [86] contrasts the need for p53 in melanoma development [83]. However, this discrepancy may reflect differences between lung and skin cancers or technical variations - suppression of IKKβ versus blocking NF-κB nuclear import. The latter can alter the NF-κB/p53 complex ratio, as direct interaction between the p65 N-terminal protein (1–285) and p53 is possible [55,176]. Moreover, DNA damage caused by adriamycin enhances formation of p65–p53–IKKα and p53–IKKβ complexes [176]. Thus, given that p65 and p53 can associate, retention of one factor in a cellular compartment might affect the other (e.g. the cytosolic functions of p53 or the duration of nuclear retention of p65), and altered posttranslational modifications and quenching effects will possibly take place. Whether p53–NF-κB interactions are the reason for an unexpected activation of their target genes or a lack thereof [177,178] is equally possible though remains to be proven formally.

8. Concluding remarks

Inventive new therapeutics could rely on the knowledge about the hallmarks of cancer. Up to now we perceive them as independence from external growth signals, the ability to override growth inhibitors, reduced response to hormones and chemotherapeutic agents, evasion of apoptosis, unlimited replication, tissue invasion, metastasis, sustained angiogenesis, manipulation of the tumor microenvironment, subversion of adaptive immunity, cancer-related inflammation, genetic instability, and accumulation of random genetic alterations.
Cellular functions of p53 and NF-κB in health and disease depend on their quantity and qualitative factors. Either is governed by posttranslational modifications. These structural alterations create novel interaction surfaces with other proteins, regulate intracellular localization, and affect DNA-binding and gene expression. Thus, the activation state of regulators, PAT/PDAC, kinase/PPase, transferase/deconjugase, etc., is able to determine the outcome of crosstalk between transcription factors in a cell-type- and stimulus-dependent manner. A multiplicity of dose-dependent or time-related responses of biological systems to stressors has already been described, but the molecular understanding of these phenomena often awaits detailed clarification. Perhaps, NFκB-p53 crosstalks affect the decision when p53 ceases repair functions and activates pro-apoptotic gene expression patterns. It is plausible that dynamic alterations ruling these transcriptional regulators direct chromatin accessibility and cell fate decisions.

The fact that replicational stops, genotoxic noxes and other potentially harmful stressors activate cytoprotective NFκB and p53 at repair genes argues for initial cellular decisions towards repair. At present, we are not aware of how these mechanisms can prime cells for adaption or elimination. Future research will explore the signaling topology of stress responses and how NFκB and p53 reactions control ambivalent reaction patterns of cells and organisms to stress time- and dose-dependently. An improved understanding of the molecular signatures of stress responses may also promote ongoing efforts for the effective use of the organism’s preventive and regenerative potentials. Preconditioning effects of stressors are of ongoing efforts for the effective use of the organism’s preventive and regenerative potentials. Preconditioning effects of stressors are of dynamic alterations ruling these transcriptional regulators direct chromatin accessibility and cell fate decisions.

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