

Crosstalk between stress-induced NF- κ B, p53 and HSF1 signaling pathways – review.

Piotr Widlak*, Michalina Gramatyka** and Marek Kimmel***

* *Center of Oncology – Maria Skłodowska-Curie Memorial Institute, Gliwice, 44-101, Poland (e-mail: widlak@io.gliwice.pl)*

** *Foundation of Cardiac Surgery Development, Zabrze, 41-800, Poland (e-mail: michalina.gramatyka@gmail.com)*

*** *Rice University, Houston, TX-77005, USA, and Silesian University of Technologies, Gliwice, 44-100, Poland (e-mail: kimmel@rice.edu)*

Abstract: The signaling pathways depending on NF- κ B, p53 and HSF1 transcription factors are components of the cellular response to stress. NF- κ B transcription factor regulates genes responsible for inflammation, immune response and cell survival. p53 either activates cell cycle arrest and DNA repair or induces apoptosis in cells with DNA damage. HSF1 activates under stress conditions and induces the heat shock response. All these pathways are essential for cancer and other human diseases. Here we review biological data on these signal transduction pathways and introduce complex crosstalk between these regulatory circuits as an apparent challenge for mathematical modeling.

Keywords: biosystems; experimental biology; modeling; stress response; transcriptional regulation;

1. INTRODUCTION

Divergent signal transduction pathways are activated in the cell as the result of stress conditions. Different stress factors (e.g. increased temperature, DNA damage, cytokines) trigger different signal transduction cascades leading to activation of genes and mechanisms responsible for survival, and the final cell response is the resultant of all activated pathways. The three major pathways determining cellular response to stress are regulated and executed by NF- κ B, p53 and HSF1 transcription factors. The signaling dependent on NF- κ B, p53 and HSF1 provides an adaptive mechanism for stress tolerance, and deregulation of these signaling pathways can influence the cell growth and survival. As a consequence, these pathways are critically important for development and response to the treatment of cancer and other human diseases. While the interactions that occur within each of these pathways and their general importance for cancer cells are extensively described, much less is known about the crosstalk between them.

2. NF- κ B PATHWAY

Dimeric protein complex NF- κ B serves as transcription factor regulating cell response to different types of stimuli. Its primary function is regulation of immune response and inflammation (e.g. expression of cytokines). Other genes, whose transcription depends on the NF- κ B factor, include those coding proteins involved in apoptosis (mainly anti-apoptotic proteins), activation of cell cycle progression (cyclin D1 and c-myc factor), angiogenesis and metastasis (adhesion molecules). In general, the κ B responsive element

could be found in regulatory regions of several hundred of genes. Up-regulation of NF- κ B pathway is frequently observed in cancer cells, which may contribute to their resistance to the anticancer treatment.

NF- κ B transcription factors are dimers formed by members of the multigene NF κ B/Rel family, which include five proteins. RelA (p65), RelB and c-Rel require heterodimerization for transcriptional activity. Other two proteins: p100 and p105 are precursor proteins for p50 and p52. In some cases homodimers of p50 or p52 can lead to inhibition of gene expression. The RelA/p50 heterodimer is the major regulator involved in so called canonical NF- κ B pathway (e.g. involved in inflammation and response to cytokines).

Generally, in resting cells NF- κ B dimers are sequestered in the cytoplasm by association with inhibitory proteins called I κ B. I κ B mask the nuclear localization signals (NLS) of NF- κ B and prevent binding of NF- κ B to DNA. Expression of I κ B (the major inhibitor of NF- κ B) is controlled by a highly NF- κ B-responsive promoter, which together with activation of other NF- κ B-dependent target *TNIP2* gene encoding for A20 protein generates the major internal circuits of autoregulation of NF- κ B signaling. Pro-inflammatory extracellular signals or cellular stress can induce activation of IKK kinase, which in turn phosphorylates I κ B protein. Phosphorylation of I κ B causes its ubiquitination and degradation in proteasome, which allows the translocation of NF- κ B to nucleus and its binding to the κ B DNA regulatory elements (see Figure 1). (reviewed in: Hayden et al., 2004;

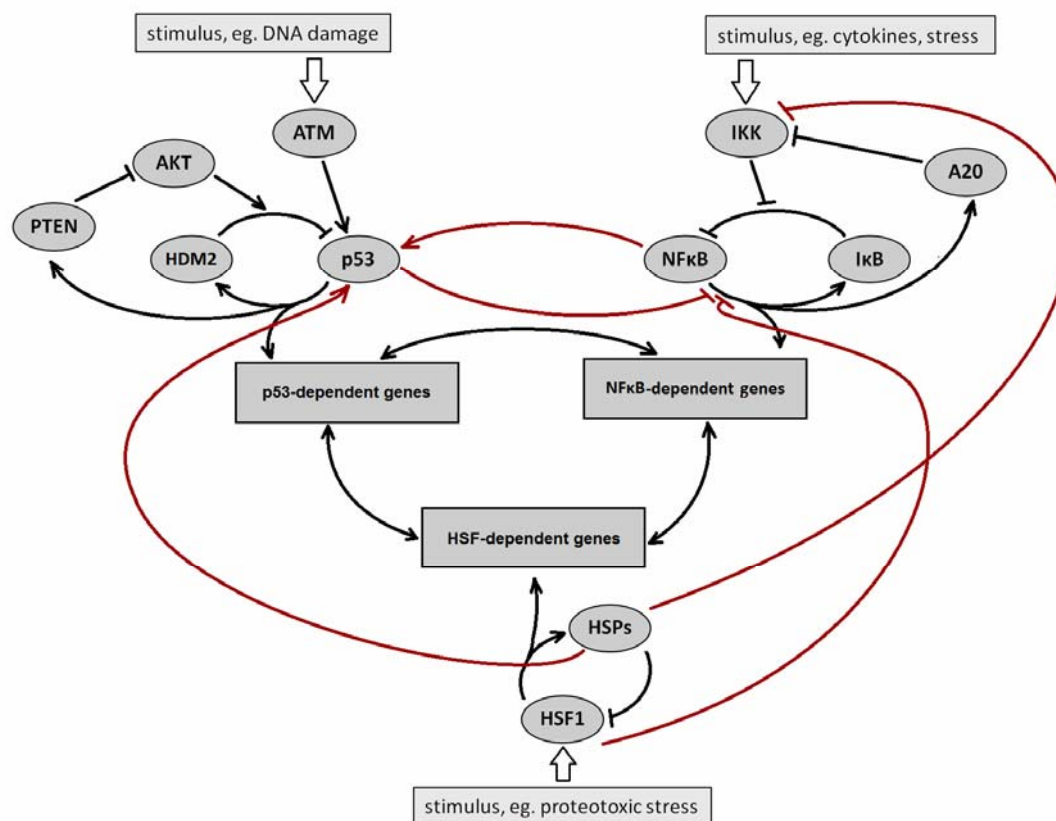


Figure 1. Schematic diagram of interactions between NF- κ B, p53 and HSF1 pathways. Diagram shows major elements of regulatory circuits controlling each pathway (connected by solid lines; see the text for details). Dotted lines correspond to examples of crosstalk elements between the pathways (see the text for details).

Perkins, 2004; Schumm et al., 2006; Tergaonkar et al., 2002; Tian et al., 2003).

3. p53 PATHWAY

“The guardian of the genome”, p53 protein, is a transcription factor encoded by *TP53* gene. Main function of p53 is regulation of gene expression in response to DNA damage (e.g. ionizing radiation-induced double strand breaks; DSBs): it activates cell cycle arrest (enabling DNA repair) or apoptosis, if DNA damage exceeds certain “repairable” threshold. p53 is also responsible for cell senescence, angiogenesis, redox regulation and metastasis. When damage-activated p53 binds to DNA it induces the expression of several genes responsible for inhibition of the cell cycle (eg. CDK inhibitor p21) or activation of apoptosis (eg. BAX). However, the p53-responsive elements could be found in regulatory regions of several hundred of genes. p53 protein plays important role as a suppressor of tumorigenesis. Inactivation of this protein due to *TP53* gene mutation is one of the most common genome disturbances in human cancers – it is detected in about 50% of all cases.

The concentration of p53 protein in the cell is regulated by ubiquitin ligase MDM2 (in human HDM2). Expression of p53 stimulates *MDM2* gene transcription and H/MDM2 protein synthesis, which in turn mediate p53 degradation in

proteasome – this constitutes the major negative feedback loop regulating p53. The proteins that can stimulate the activity of p53 protein in response to stress factors are mainly kinases involved in the genome integrity checkpoint (e.g. ATR, ATM, CHK1, CHK2). Phosphorylation of p53 prevents its binding to H/MDM2 and allows binding to DNA (see Figure 1). (reviewed in: Efeyan et al., 2007; Shangary et al., 2008; Tergaonkar et al., 2002)

4. HSF1 PATHWAY

Cellular response to several types of suboptimal conditions that typically lead to denaturation of proteins involves elevated expression of heat shock proteins (HSPs), and is usually called the heat shock response (HSR). HSPs are the major molecular chaperones: during synthesis HSPs assist in proper protein folding, and during proteotoxic stress HSPs protect stress-labile proteins and contribute to proteolysis of damaged proteins. In mammalian cells there are several types of HSPs, classified according to their molecular weight: HSPH (Hsp110), HSPC (Hsp90), HSPA (Hsp70), DNAJ (Hsp40) and HSPB (small HSPs). Beside inducible HSPs, which are exemplified by the most abundant HSPA1, other members of the HSP family are expressed constitutively in the absence of stress. Constitutively and inducibly expressed HSPs exhibit distinct physiological functions for cellular

maintenance and adaptation to stress, respectively. Heat shock transcription factor 1 (HSF1) is the primary transcription factor responsible for stress-induced activation of *HSP* genes.

In addition to activation of *HSP* genes, HSF1 is involved in regulation of many other genes associated with multiple cellular processes including cell signaling, maintenance of cell integrity, development, growth and fertility. HSF1 also regulates genes involved in activation of processes such as apoptosis, RNA splicing and ubiquitination of proteins. Its binding to DNA can stimulate cell migration, angiogenesis, autophagy, aneuploidy and anchorage-independent as well as mitogen-independent growth of cell. Overexpression of HSF1 was observed in several tumors and cancer cell lines. Moreover increased expression of HSF1 (and HSP proteins) can lead to cancer chemo- and radio-resistance. It has been reported that HSF1 can support tumor growth and neoplastic transformation. Knockout of HSF1 reduces the risk of neoplastic transformation induced by overexpression of HER2 or mutant p53.

Under physiological conditions HSF1 monomers exist in cytoplasm in complexes with different HSP proteins. Proteotoxic stress conditions cause an accumulation of denatured proteins and a subsequent release of HSPs from such complexes. Released HSPs serve as molecular chaperones for miss-folded proteins, while unbound HSF1 monomers create trimers and translocate to the nucleus. In nucleus HSF1 trimers are phosphorylated and then bind to HSE sequences in DNA, which regulate the HSP genes expression. HSF1 activity results in accumulation of HSPs that in turn rebind HSF1 into inactive complexes, completing the negative feedback loop (see Figure 1). (reviewed in: Ankar & Sistonen, 2011; Dai et al., 2007; Janus et al., 2011; Santoro, 2000; Voellmy, 2004; Zyllicz et al., 2001)

5. CROSSTALK BETWEEN NF- κ B AND p53 PATHWAYS

The major signal transduction pathways involved in the cellular response to stress do not function separately – several interactions between them exist, which influence the final response. There are stimuli (eg. ionizing radiation) that at the same time can activate both p53 and NF- κ B pathways. The function of these pathways, however, is different: activation of p53 can cause cell cycle arrest and apoptosis, while activation of NF- κ B can cause resistance to apoptosis and stimulate proliferation. Both p53 and NF- κ B transcription factors can interact with the other pathway. Moreover, different p53-dependent genes respond differently to stimulation with NF- κ B, and different members of NF- κ B family have different effects on p53 pathways. This fact further complicates the crosstalk between the NF- κ B and p53 pathways.

The *TP53* gene promoter contains a putative κ B-binding element and binding of NF- κ B to this promoter activates the transcription of p53. It is also supposed that NF- κ B enhances expression of M/HDM2, and in consequence negatively regulates p53. On the other hand p53 induce phosphorylation of RelA and its transport to nucleus, where it can serve as a

transcription factor. The p300/CBP complex (with histone acetyltransferase activity) is a transcription co-activator essential for expression of genes activated by both p53 and NF- κ B. Because of this both transcription factors compete for binding with it. In result NF- κ B binding to p300/CBP can suppress expression of genes dependent on p53, while binding of active p53 to p300/CBP results in a loss of NF- κ B activity. IKK kinase regulates transcription factor binding to p300/CBP because CBP phosphorylated by IKK preferentially binds to NF- κ B. (reviewed in: Webster et al., 1999; Tergaonkar et al., 2002; Perkins, 2004)

It is generally assumed NF- κ B activity is antagonistic to the activity of p53. In some cases, however, both factors can act synergistically. For example NF- κ B can induce inhibition of tumor growth. The homodimer of p52 protein is involved in regulation of the gene encoding cyclin D1 (protein responsible for cell proliferation), and p53 protein can regulate the activity of this dimer. The presence of p53 induces transformation of the activation complex p52/Bcl-3 into inhibitory complex, which consists of p52 dimer and the histone deacetylase HDAC1 (Schumm et al., 2006).

6. CROSSTALK BETWEEN NF- κ B AND HSF1 PATHWAYS

The Heat Shock Response (HSR) and NF- κ B pathway interfere with each other in a number of ways. Heat shocked cells do not exhibit cytokine-induced degradation of I κ B, nuclear translocation of NF- κ B and activation of NF- κ B-dependent genes. Several lines of evidence indicate that the stress-induced aggregation and inactivation of IKK kinases is primarily responsible for blocking of the NF- κ B cascade. Furthermore, it was shown that HSPA1 and HSPB1 could bind to IKK and inhibit its activity. On the other hand, however, it has been reported that HSPA1 over-expressed in the absence of the heat shock did not suppress activation of the NF- κ B pathway by TNF α and facilitate renaturation of IKK after heat shock. (reviewed in: Santoro, 2000; Janus et al., 2011).

HSF1 can co-regulate transcription of several NF- κ B-dependent genes, and some of these genes contain HSEs in their regulatory regions. Binding of HSF1 to these elements is crucial for downregulation of *TNFA* and upregulation of *NOS2* genes. HSF1 can also modulate chromatin structure of the *IL6* gene promoter, which facilitates the binding of other activators or repressors to the *IL6* gene. Using functional genomics approach (combination of gene expression microarrays and ChIP-seq) a signature of thirteen NF- κ B-activated genes was identified (namely *SLC12A7IL8*, *EPB41L2*, *CCL2*, *CD83*, *FOSB*, *EGR1*, *ATF3*, *ZFP36*, *PPP1R15A*, *GADD45B*, *RRAD*, *IFNGR2*, *TNF*), in which TNF α induced expression was suppressed upon direct binding of heat-activated HSF1 (Janus & Widlak, unpublished). Furthermore, stress-induced HSF1 can suppress the activity of some NF- κ B-dependent genes (e.g., *IL6*) indirectly through activation of ATF3, a negative transcriptional regulator of pro-inflammatory genes. Additionally, HSF1 can interact with proteins, which expression is regulated by NF- κ B; this includes STAT-1 and

C/EBP β proteins involved in regulation of immune response. On the other hand, several *HSP* genes contain binding elements for NF- κ B-regulated proteins like STAT-1 and NF-IL6; binding of these proteins co-stimulate HSF1-dependent expression of HSPs. (reviewed in: Singh et al., 2000; Santoro, 2000; Janus et al., 2011).

7. CROSSTALK BETWEEN p53 AND HSF1 PATHWAYS

The proper functioning of p53 protein is generally supported by HSPs and HSF1-regulated pathways affect the balance between p53 synthesis, degradation and its nuclear translocation. The binding of Hsp90 to p53 stabilizes this complex – the p53 becomes resistant to ubiquitination and degradation associated with H/MDM2 action, which leads to accumulation of p53 in cytoplasm. Hsp90 can also influence the binding of p53 to the p21/WAF1 gene promoter by stabilizing the p53-DNA complex (under heat shock condition also Hsp70 and Hsp40 are involved in stabilization of p53-DNA complexes). (reviewed in: Walerych et al., 2009; Zyllicz et al., 2001).

Translocation of p53 to nucleus depends on microtubules, which polymerization is regulated by HSF1. Proper HSF1 activity is also required for phosphorylation and activation of p53 by ATR and CHK1 kinases in response to DNA damage. In response to stress associated with DNA damage HSF1 can promote cell cycle arrest in G₂/M phase, and apoptosis by enhancing transcription of p53-dependent genes. On the

other hand tumorigenic transformation dependent on mutated p53 could be supported by HSF1 activity. In cells with impaired function of p53 the overexpression of HSF1 can lead to aneuploidy and genomic instability. In some cases DNA damage can induce cellular senescence, where specific crosstalk between all three pathways could be observed. In such cells p53 down-regulates ELAVL1(HuR)/SIRT1 pathway resulting in HSF1 suppression and enhancement of the MAPK/NF- κ B signaling, which supports chronic inflammation and senescence phenotype (Kim et al., 2012).

8. MODELING OF CROSSTALK BETWEEN STRESS-INDUCED PATHWAYS

The simple computational model that described the temporal control of NF- κ B activation by the coordinated degradation and synthesis of I κ B proteins and subsequent translocation of released NF- κ B to nucleus was first proposed by Hoffman and co-workers (2002); comprehensive review of this problem can be found in (Cheong et al., 2008). More complex model of NF- κ B regulatory module that included participation IKK and A20 was proposed by Lipniacki and co-workers (2004) The mathematical model that described regulation of p53 based on oscillations in p53/MDM2 positive and negative feedbacks was proposed by Lev Bar-Or and co-workers (2000) and refined by Ciliberto and co-workers (2005). The oscillations of the p53/MDM2 module were proven experimentally at the level of individual living cell (Lahav et al., 2004). The more complex model of

Table 1. Characteristics of numerical models of described pathways.

Pathway	Components	Key features	Reference
NF- κ B	NF- κ B, I κ B α	responsive to IKK stimulus; inducible I κ B α ; stable I κ B β and I κ B γ ; I κ B α negative feedback loop; oscillations of nuclear NF- κ B;	Hoffman et al., 2002
NF- κ B	IKK, NF- κ B, I κ B α , A20	three form of IKK assumed (neutral, active, inactive); (inducible) A20 negative feedback loop; oscillations of cytoplasmic I κ B α ;	Lipniacki et al., 2004
p53	p53, MDM2	inducible p53, inducible MDM2; MDM2 negative feedback loop; oscillations of p53 and MDM2;	Lev bar-Or et al., 2000
p53	DSB, p53, MDM2	responsive to DSB; cytoplasmic/nuclear translocation of MDM2; ubiquitination of p53; phosphorylation of MDM2;	Ciliberto et al., 2005
p53	DSB, ATM, p53, MDM2	activation of ATM by DSB; ATM-mediated phosphorylation of p53 and MDM2;	Qi et al., 2007
NF- κ B and p53	DSB, AKT, p53, MDM2, PTEN, PIP3, IKK, NF- κ B, I κ B α , A20	NF- κ B upregulates transcription of p53; p53 attenuates transcription of I κ B α and A20; PTEN, PIP3 and AKT-mediate feedback control of MDM2; different time sequence of activation of p53 (by DSB) and NF- κ B (by TNF α);	Puszynski et al., 2009
NF- κ B and HSP	HSP70i, IKK, NF- κ B, I κ B α , A20	HSP attenuates activation of IKK; HSP reduces steady-state level of RelA(p65);	Sheppard et al., 2014

p53/MDM2 regulation that included ATM module activated by ionizing radiation-induced double strand breaks was proposed afterward by Qi and co-workers (2007). The model describing crosstalk between p53/MDM2 and NF- κ B/I κ B was proposed by Puszynski and co-workers (2009). In this model earlier models of p53 and NF- κ B regulation were combined assuming that NF- κ B up-regulated the transcription of p53, whereas p53 attenuated transcription of I κ B and A20. The authors demonstrated a hypothetical possibility that activation of NF- κ B could have either anti- or pro-apoptotic role: NF- κ B activation preceding p53 activation made cells more resistant to DNA damage-related death while NF- κ B activation preceded by DNA damage increased probability of cell death. This work suggested that diverse roles of NF- κ B in apoptosis and cancer depended on the dynamic context of p53 and NF- κ B pathways activation. Of note, we showed experimental evidences that activation of NF- κ B before irradiation of cells could reduce p53-dependent apoptosis in such cells (Szoltysek et al., 2008). Most recently numerical model describing crosstalk between HSF1/HSP and NF- κ B/I κ B pathways was presented by Sheppard and coworkers (2014). The model assumes complex influence of HSP70 on activity of IKK and levels of both RelA(p65) and I κ B. Brief characteristics of above-mentioned models are presented in Table 1.

9. CONCLUSIONS

The three major signaling pathways described above regulate the cell cycle, apoptosis, DNA repair, inflammation and senescence, hence the crosstalk between them determines the fate of the cell subjected to stress. Complex regulation of this pathways and their multi-stage cross-interactions make them not only a real challenge for experimental biology but also the interesting subject for mathematical modeling.

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