Guardian of the Genome:

An insight into the response dynamics of tumour suppressor protein 53 following DNA damage and hyperproliferative signals

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Tumour suppressor protein 53 (p53), also canonically referred to as the guardian of the genome, is a crucial cellular stress sensor involved in coordinating responses to cellular insults such as hypoxia, ribonucleotide depletion, telomere attrition, oncogene activation, oxidative damage and hyperproliferation, amongst other factors such as UV light and γ-irradiation. The types of response to cellular damage include activation of cell cycle arrest, DNA repair pathways, senescence, and in the most severe cases of stress, apoptosis. Loss of p53 is highly associated with cancer development due to the absence of the vital genome protection that p53 is renowned for, thus an enhanced understanding of p53 function is vital to facilitate cancer detection, prognosis and treatment. However, the exact mechanisms of cellular protection are yet to be fully elucidated. Within this article, the most well-defined mechanisms regarding p53 regulation and function are discussed.

Brief introduction to p53

Cancer is defined by a population of cells proliferating and behaving in an uncontrolled manner (Feroz and Sheikh, 2020). Throughout time, cells have evolved many mechanisms to challenge stress in order to protect the genome (Feroz & Sheikh, 2020). Without these mechanisms, mutations and chromosomal abnormalities associated with oncogene activation or tumour suppressor repression, transmitted to daughter cells during mitosis, may initiate development of cancer (Feroz & Sheikh, 2020). Located on chromosome 17 the tumour suppressor 53 (TP53) gene, encoding transcription factor p53, is a highly studied gene involved in orchestrating many responses to genomic stress, resulting in its canonical name: “the guardian of the genome” (Feroz & Sheikh, 2020). In fact, the significance of p53 within prevention of tumorigenesis is undeniable, given that p53 activity is altered significantly in over 50% of all sporadic human cancers, either by mutation at the TP53 locus or by oncogenic events that reduce the functionality of wild type p53, for example upregulation of p53 repressors and downregulation of p53 activators (Sullivan *et al.*, 2018). This makes p53 the most frequently mutated protein in human cancer. Similarly, people with Li-Fraumeni syndrome, characterised by inheritance of a mutant TP53 allele, have an increased predisposition to cancer (Bieging, Mello & Attardi, 2014). Clearly, inactivation of p53 is pivotal to carcinogenesis. Here, we discuss the most well-defined mechanisms by which p53 is known to protect the cell.

Regulation of p53

The maintenance of genomic integrity is regulated by distinct signalling mechanisms (Giaccia & Kastan, 1998). Activation of p53 is initiated following a host of diverse cellular insults, such as oncogene activation, telomere attrition, ribonucleotide depletion, hyper-proliferation, oxidative damage, in addition to environmental factors, including γ-irradiation, UV light and chemotherapeutics (Giaccia & Kastan, 1998). Under normal physiological conditions, ideally parading a scarcity of cellular stressors, p53 levels are minimal as a result of the critical p53 regulator, mouse double minute 2 homolog (MDM2) (Chen, 2012). This protein negatively regulates p53 activity and keeps p53 levels low in the absence of DNA damage signals by antagonising the N-terminal trans-activation domain of p53, a region critical for activation of p53 (Kruse & Gu, 2009). Therefore, MDM2 represses p53 transactivation function. Moreover, MDM2 exhibits ubiquitin E3 ligase activity through binding and poly-ubiquitinating lysine residues in the p53 C-terminus in preparation for proteasome-mediated degradation of p53 (Zhang & Xiong, 2001). This process involves covalently attaching a repeating chain of the small regulatory protein called ubiquitin to a lysine residue on the substrate protein, which ultimately stabilises the substrate protein and marks it as a target for degradation by the 26S proteosome (Callis, 2014). Interestingly, MDM2 is also a p53 responsive gene, meaning that upon transcription of p53, MDM2 transcription is simultaneously activated resulting in increased MDM2 protein levels (Aubrey *et al.*, 2018). This generates a negative feedback loop responsible for maintaining low p53 levels, vulnerable to disruption only when cellular stress signals are high (Aubrey *et al.*, 2018).

Perhaps the most extensively defined signalling pathways leading to p53 activation are the ataxia telangiectasia mutated (ATM)-dependent response to acute DNA damage and the ADP-ribosylation factor (ARF)-dependent response to hyperproliferative signals (Feroz & Sheikh, 2020). Regarding response to DNA damage, specifically DNA double-strand breaks, ATM and ataxia telangiectasia

and Rad3 related (ATR) protein kinases are recruited for phosphorylation and thus activation of checkpoint kinases CHK1 and CHK2 (Feroz & Sheikh, 2020). These kinases phosphorylate p53 on serine 20 whilst ATM and ATR phosphorylate p53 on serine 15 (Sakaguchi *et al.*, 1998). Each of these post-translational modifications disrupt MDM2 binding to p53, preventing negative regulation and allowing p53 to perform its various DNA damage response activities (Chen *et al.*, 2005). Similarly, hyperproliferative signals induce the ARF-dependent response (Cheng & Chen, 2010). Here, unregulated cell division results in amplified liberation of the E2F transcription factor, which plays a role in stimulating ARF transcription, an important tumour suppressor (Cheng & Chen, 2010). ARF forms stable complexes with MDM2, sequestering MDM2 within nucleoli and inhibiting MDM2 E3 ubiquitin ligase activity, dissociation of the MDM2-p53 complex again permitting the transcriptional endeavours of p53 (Kruse & Gu, 2009).

Role in tumour suppression

Stabilisation of p53 by ATM, ATR, and ARF in response to cellular stress allows induction of manifold downstream transcriptional targets, encoding genes that ultimately share the same goal: preserving genomic integrity (Bieging, Mello & Attardi, 2014). A broad range of target genes are activated by p53, mainly implicated in cell cycle arrest, DNA repair, senescence and apoptosis (Bieging, Mello & Attardi, 2014). An overview of the role of p53 in tumour suppression is detailed in Figure 1.

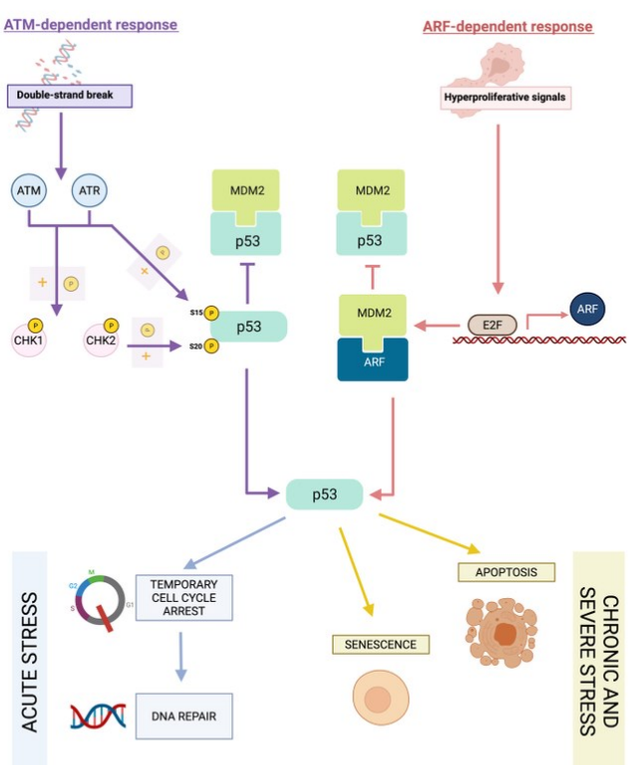

Figure 1. A schematic overview of the activation of p53 by the ATM-dependent and ARF-dependent pathways following cellular stresses such as DNA damage and hyperproliferative signals, respectively, involving uncoupling of p53 from its negative regulator, MDM2. This leads to p53 functional responses, those being temporary cell cycle arrest and DNA repair regarding transient cellular stresses, and senescence or apoptosis regarding prolonged and severe cellular stresses. Created with BioRender.com

To achieve cell cycle arrest, p53 prompts transcriptional activation of CDKN1A, encoding p21, a protein responsible for binding to cyclin E/cyclin-dependent kinase 2 (CDK2) and cyclin D/cyclin-dependent kinase 4 (CDK4) complexes (Hafner *et al.*, 2019). In normal circumstances, these complexes would phosphorylate retinoblastoma protein (pRb) complexed with E2 transcriptional factor (E2F), subsequently triggering conformational changes that liberate E2F from the complex in order to initiate expression of genes important for DNA replication and G1/S transition in the cell cycle (Burke *et al.*, 2010). Yet, p21 binds these cyclin/CDK complexes, preventing phosphorylation of pRb protein, arresting the cell cycle at the G1/S regulation point (Slebos *et al.*, 1994). Similarly, p53 stabilisation obstructs cells at the G2/M phase by repressing 14-3-3s promoters, which usually encode proteins that sequester cell division cycle 25C (CDC25C) within the cytoplasm, a process needed to activate cyclin/CDK complexes (Hermeking *et al.*, 1997). This temporary cell cycle arrest provides essential cell-cycle checkpoints which grant the cell with enough time to repair possible genomic lesions before the cell begins cycling again and DNA replication begins (Chen, 2016). This enhances survival of damaged cells and prevents propagation of DNA aberrations to progeny cells from which malignancies might arise (Chen, 2016). To take advantage of the quiescent cellular states implemented by p53, specialised DNA repair machineries within the cell pursue damage removal (Bieging, Mello & Attardi, 2014). Examples of such repair pathways include nucleotide excision repair (NER), base excision repair (BER) and non-homologous end-joining (NHEJ) (Bieging, Mello & Attardi, 2014). Often, p53 also directly plays a direct role in these pathways, both through modulation by transcriptional activation of target genes and participation in the pathway itself (Bieging, Mello & Attardi, 2014).

Despite the best efforts of the cellular machinery to repair damaged DNA, sometimes it is simply not possible to counteract the more severe and prolonged cellular stressors (Amaral *et al.*, 2010). In these circumstances, to prevent further proliferation and propagation of genetic defects possessing the potential to generate neoplasia, p53 may induce permanent cell cycle arrest, termed senescence, as opposed to the temporary cell cycle arrest induced by transient stimuli (Mijit *et al.*, 2020). Senescence is defined as irreversible cell cycle arrest by which the cell remains functional but further replication is inhibited (Kruiswijk, Labuschagne & Vousden, 2015). p53 achieves this by sustained transcriptional activation of p21 (Qian & Chen, 2013). Moreover, should the severity and duration of stress become extreme, p53 will even induce cell death by apoptosis via transcriptional activation of pro-apoptotic B-cell lymphoma 2 (BCL-2) family proteins such as BCL-2 homologous antagonist killer (BAK1), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1) and p53 upregulated modulator of apoptosis (PUMA) (Hafner *et al.*, 2019). Evidently, p53 plays a central role in evaluating the fate of cells; as such, p53 has even been referred to as “a lifeguard with a licence to kill” (Kruiswijk, Labuschagne & Vousden, 2015).

Conclusion and future considerations for p53 research

In conclusion, p53 is a highly relevant tumour suppressor which under normal circumstances is coupled with its negative regulator MDM2 (Kruiswijk, Labuschagne & Vousden, 2015). In the presence of various stress stimuli, p53 is released from the MDM2-p53 complex by either the ATM-dependent response or the ARF-dependent response to permit coordination of an adaptive gene expression programme resulting in either growth arrest or cell death, depending on the transience of the stress stimuli (Kruiswijk, Labuschagne & Vousden, 2015). Only the most extensively defined mechanisms have been described in this article. Despite several decades of research, the comprehensive role of p53 in tumour suppression is currently unclear and has yet to be fully dissected, owing to its complex dynamics and multifaceted functions. It is even likely that additional functions of p53 are yet to be discovered. For example, it was recently discovered that p53 may also modulate invasion and tumour-stromal cell cross talk within the tumour microenvironment (Bieging, Mello & Attardi, 2014). Moreover, current knowledge of p53 activity is founded on experimental data obtained from mouse models or cell culture studies which often do not consider important variables such as age, sex and ethnicity (Sullivan *et al.*, 2018). With novel technologies, addressing these concerns can be made possible through isolation of various cell lineages *ex-vivo* to determine potential differences in p53 chromatin binding, activation and regulation (Sullivan *et al.*, 2018). Likewise, the question of how exactly p53 tumour suppressor function can be heightened for cancer treatment has yet to be fully answered. Currently, many MDM2 inhibitors, which mechanistically act by preventing the negative regulation of p53, are deemed ineffective as monotherapies and induce significant haematological toxicity following long term treatment regimens (Tisato *et al.*, 2017). Nevertheless, it has been suggested that therapeutic combinatorial approaches may prove effective through modifying the function of vital p53 cofactors or target genes, with hopes that synergistic activity will reduce toxicity and strengthen the tumour suppressive activity of MDM2 inhibitors (Sullivan *et al.*, 2018). Therefore, future directions for p53 research include systematic biochemical and cytological studies to decipher the specific details of p53-mediated tumour suppression and answer the many open questions still existing within p53 research. Not only will this refine comprehension of p53 function, but enhanced understanding of the components involved in p53-mediated tumour suppression will also facilitate cancer detection and prognosis, in addition to increased flexibility during identification of potential therapeutic targets for cancer treatment.

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