



The IGF-1R/AKT pathway determines cell fate in response to p53

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Abstract: p53 that is activated in response to DNA-damaging stress can induce apoptosis or either transient or permanent cell cycle arrests. Apoptosis and permanent cell cycle arrest (senescence) are bona-fide tumor suppressor mechanisms through which p53 inhibits cancer cell survival. In contrast, transient cell cycle arrests induced by p53 can increase survival by allowing cells time to repair their DNA before proceeding with cell division. Mechanisms that control the choice of response to p53 (apoptosis *vs.* arrest) are not fully understood. There is abundant crosstalk between p53 and the IGF-1R/AKT/mTORC1 signaling pathway. Recent studies indicate this crosstalk can determine the choice of response to p53. These findings have clear implications for the potential use of IGF-1R pathway inhibitors against p53 wild-type or p53-null or mutant cancers.

Keywords: p53; IGF-1R/AKT pathway; mTORC1; apoptosis; senescence

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P53 is stabilized and activated in response to DNA-damaging stress

Wild-type p53 is a stress responsive transcription factor and potent tumor suppressor. Under normal conditions, p53 is expressed at low levels and inactive due to MDM2, an E3 ubiquitin ligase that binds the N-terminus of p53 and promotes its ubiquitination and degradation (1,2). However, the p53 protein is stabilized in response to DNA damage, aberrant oncogene signaling, and other stresses that could potentially drive a normal cell towards tumorigenesis (3,4). In the case of DNA damaging stress, multiple damage-induced kinases such as ATM/ATR and Chk1/Chk2 promote phosphorylations in the p53 N-terminus, including sites within or near the MDM2-binding domain. These phosphorylations can have two effects: first, phosphorylation at sites like S15, S20, and S37 can disrupt or weaken MDM2-p53 binding, causing the p53 protein to be stabilized (5,6). Second, these phosphorylations (e.g.,

at S15) can also promote recruitment of acetyl-transferases such as p300, CBP, and pCAF (7-9). These acetyl-transferases promote acetylation of lysine residues in p53s C-terminus. For example, pCAF promotes acetylation at lysine 320 (K320) and p300/CBP can promote acetylation at multiple lysines including K370, K371, K372, K381, and K382. Acetylation at these C-terminal lysines can increase p53s ability to bind DNA and can also promote recruitment of coactivators and histone-modifying enzymes to increase p53s transcriptional activity (9-11). The findings support a model in which the stabilization and activation of p53 following DNA damage occurs through N-terminal phosphorylations followed by C-terminal acetylation.

The effect of stabilizing and activating p53 can vary and may depend on cell-type, the level of DNA damage, and the ability of cells to undergo DNA repair (12-14). For example, in response to transient or low levels of DNA damage p53 can trigger reversible arrests in the G1 and G2-phases of the cell cycle (15). The G1 arrest is mediated by

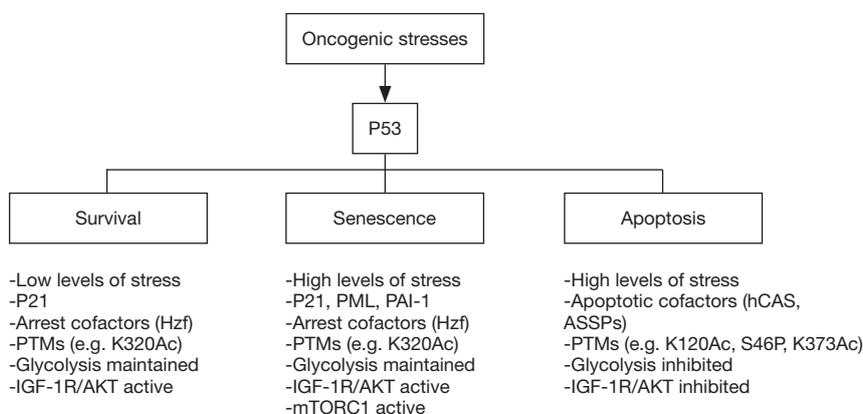


Figure 1 p53 induced by stress can promote survival, senescence, or apoptosis. Wild-type p53 is expressed at low levels and inactive in most cells. However, p53 is stabilized and activated in response to DNA damage, aberrant oncogene signaling, and other stresses that could potentially drive a normal cell towards tumorigenesis. p53 that is stabilized and activated in response to these stresses can promote survival, senescence, or apoptosis. Factors that can determine the choice of response to p53 are listed.

p21, a p53-responsive gene product that arrests cells in G1-phase by binding to and inhibiting the activity of G1-phase cyclin-cdk complexes (16-18). p53 is not required to initiate the G2 arrest after DNA damage but functions to maintain the arrest. G2-arrest maintenance by p53 may result from down-regulation of *Cyclin B1*, *CDC2*, and other genes, or by increased expression of 14-3-3 σ , which can sequester and inhibit cyclin B-CDC2 complexes (19-21). Notably, the reversible G1 and G2 arrests mediated by p53 can increase survival in response to radiation or chemotherapeutic drug treatment by allowing cells time to repair their DNA before proceeding with either replicative DNA synthesis or mitosis (22-25). In contrast, when DNA damage is prolonged or excessive, activated p53 can trigger either a permanent, senescent arrest that is also dependent on p21 (26-29) or apoptotic death by inducing expression of factors like Puma, Noxa, and Bax that disrupt the mitochondrial membrane and promote release of factors like cytochrome-C that activate caspases to initiate apoptosis (29,30). The molecular factors and/or pathways that control the choice of response to p53 (e.g., survival, senescence, or apoptosis) are not fully understood (Figure 1). Understanding how this choice is made could reveal strategies to increase p53-mediated cancer cell killing.

How the choice of response to p53 is made

Some cell types are more susceptible to apoptosis in response to p53 activation than others. For example, most hematologic cancer cells that express wild-type p53 undergo

apoptosis as their primary response to p53 activation (31-34), while normal fibroblasts and most non-hematologic cancers (sarcomas, carcinomas) undergo cell cycle arrest with minimal apoptosis (35,36). One possibility is that p53-responsive apoptotic genes are in a more accessible conformation in hematologic cells or apoptosis-inducing cofactors are more highly expressed and therefore these cells are more prone to p53-mediated apoptosis. The presence or absence of cofactors may also determine the choice of response to p53. For example, Hzf is a transcription cofactor that binds and functions with p53 to increase cell cycle arrest genes but not apoptosis inducing genes (37). In contrast, ASPP and hCAS are factors that can bind and/or cooperate with p53 to induce apoptotic genes but not cell cycle arrest genes (38-40). Thus the choice of response to p53 may depend, in part, on the relative levels of cofactors like Hzf, ASPP, and hCAS. Certain p53-responsive factors, such as PML and PAI-1, contribute to p53-dependent senescence. PML is a scaffold protein that localizes in nuclear foci termed PML-bodies. The *PML* gene is transcriptionally activated by p53 (41). PML, in turn, can activate p53 by recruiting it into PML bodies along with CBP, which then acetylates p53 to increase its activity. Evidence PML is important for p53-mediated senescence includes studies from Pandolfi and colleagues in which it was reported PML $^{-/-}$ MEFs were resistant to p53-dependent senescence in response to oncogenic Ras expression (42). PAI-1 is a factor that can antagonize growth factor signaling and is transcriptionally activated by p53. Kortlever *et al.* [2006] reported PAI-1 is required for

replicative senescence in MEFs that is p53-dependent (43). Thus, high levels and/or induced expression of PML or PAI-1 may increase p53-dependent senescence. Metabolic differences can also influence the response to p53. For example, p53 can inhibit glycolysis by causing repression of multiple glycolytic enzyme genes. Studies by our lab and others indicated that maintaining glycolysis could protect cells from p53-dependent apoptosis induced by small-molecule MDM2 antagonists (44,45). It was suggested that glycolysis inhibits p53-mediated apoptosis by in some way maintaining pro-survival autophagy (44). Finally, certain factors may determine whether cell cycle arrest induced by p53 is transient (reversible) or permanent (senescent). For example, mTORC1 is a kinase complex that senses and responds to nutrient and energy levels and, when activated, promotes protein translation and cell growth. Korotchkina and colleagues reported that inhibiting mTORC1 could convert a permanent, p53-dependent senescent arrest into a reversible, quiescent arrest (46). The results indicated that mTORC1 activity promotes senescence in p53-arrested cells. The mechanism by which mTORC1 activity promotes senescence is unknown. However, it was suggested that continued mTORC1 dependent cell growth under conditions where cell proliferation (the cell cycle) is inhibited by p53 may present conflicting signals to the cell, and the cell responds to these conflicting signals by undergoing senescence (47).

Post-translational modifications (PTMs) and the level of stress can regulate p53 promoter selectivity and therefore play an important role in the choice of response to p53. In early studies, Oda *et al.* examined the response of p53 wild-type cells to increasing doses of UV radiation (13). P53 was phosphorylated at S46 in response to high UV doses but not low UV doses, and the cells underwent apoptosis. S46 phosphorylation increased the ability of p53 to bind and induce expression of the gene encoding the apoptotic inducing factor AIP1. These results supported a model in which specific stress-induced PTMs promote p53-dependent apoptosis in response to high levels of stress. Later studies showed S46 can be phosphorylated by multiple stress-induced kinases, including HIPK2 and p38 (48-50). Studies by Mayo *et al.* reported that S46 phosphorylation not only increases the apoptotic function of p53 but can also increase p53 protein stability (51). Specifically, the Mayo study reported that S46 phosphorylated p53 had an increased affinity to bind and activate the *PTEN* gene promoter but a reduced affinity to bind and activate the *MDM2* promoter. It was suggested

PTEN can stabilize p53 by blocking AKT-mediated activation of MDM2. Thus, these studies suggested the same PTM (S46P) can increase p53s apoptotic function and also contribute to the stabilization of p53 by increasing *PTEN* expression and reducing AKT and MDM2-mediated p53 degradation. P53 can also be acetylated at K320 and K373 in response to DNA damaging stress. Knights *et al.* [2006] reported that acetylation at K320 increased p53s ability to promote p21 expression, cell cycle arrest, and survival, whereas K373 acetylation increased p53s ability to bind and activate apoptotic genes including *AIP1* (52). These results support the idea that specific PTMs can regulate promoter selectivity and cell fate in response to p53. Acetylation of p53 at K120 is yet another PTM that can regulate p53s apoptotic function. Gu and others reported K120 is acetylated by TIP60 and hMOF, and that this acetylation increases p53 binding to the *PUMA* gene promoter in response to DNA damage and is required for p53-dependent apoptosis (30,53). Later studies by Charvet *et al* reported that PI3K signaling can regulate p53-dependent apoptosis by modulating GSK3 β activity, TIP60 activity, and p53 K120 acetylation (54). Charvet *et al.* reported that GSK3 β activates TIP60 by phosphorylating it at position S86. This phosphorylation increases TIP60 dependent K120 acetylation and thus p53-dependent induction of PUMA and apoptosis. GSK3 β is inhibited by AKT downstream of PI3K. Thus, in the Charvet study PI3K inhibitors increased GSK3 β and TIP60 activity and increased p53-dependent PUMA induction (54). The results suggested PI3K/AKT signaling activated downstream of growth factors can reduce p53 K120 acetylation and apoptotic function to facilitate survival and proliferation.

The IGF-1R/AKT/mTORC1 pathway

The IGF-1R/AKT/mTORC1 pathway is aberrantly activated in multiple cancers and promotes proliferation, survival, growth, and metabolism. There is abundant crosstalk between the IGF-1R/AKT/mTORC1 pathway and p53 (*Figure 2*). Recent studies indicate IGF-1R/AKT/mTORC1 pathway activation can determine the choice of response to p53 following chemotherapeutic drug treatment. The following sections will discuss crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway, how this crosstalk affects cell fate in response to p53 activation, and the implications of these findings for the therapeutic use of IGF-1R pathway inhibitors.

The IGF-1R/AKT pathway is activated by ligands IGF1

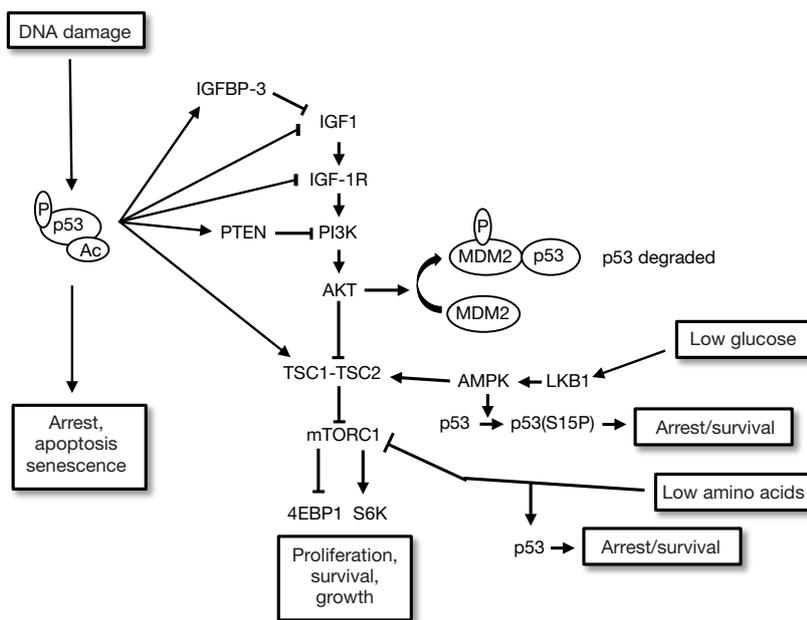


Figure 2 Negative crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway. p53 activated by DNA damage can inhibit the IGF-1R/AKT/mTORC1 pathway at multiple points to block proliferation, growth, and survival. AKT-mediated phosphorylation of MDM2 can increase the ability of MDM2 to degrade p53. mTORC1-dependent cell growth is inhibited in response to low energy or nutrient levels. Low glucose levels activate AMPK which then phosphorylates and activates TSC2, leading to inhibition of mTORC1. AMPK also promotes p53 phosphorylation at S15, resulting in p53-mediated cell cycle arrest. Amino acid depletion can inhibit mTORC1 from the lysosome. Amino acid depletion can also activate p53 resulting in p53-dependent cell cycle arrest. AMPK, AMP-activated kinase.

and IGF2 that bind the receptor IGF-1R stimulating its auto-phosphorylation on tyrosines [pathway reviewed in (55)]. This leads to recruitment and activation of adaptor proteins, Shc and IRS1 and IRS2. Shc activation stimulates the Ras-Raf-Mek-ERK pathway to promote proliferation. IRS1/2 activation promotes recruitment of PI3K, which phosphorylates lipids in the plasma membrane. This results in an increased local concentration of PIP3, which promotes recruitment of PDK1 and mTORC2. PDK1 and mTORC2 activate AKT by phosphorylation at two sites: serine 473 (S473) is phosphorylated by mTORC2 and threonine 308 (T308) is phosphorylated by PDK1. PI3K-AKT signaling is counteracted by PTEN, a lipid phosphatase and tumor suppressor that reduces PIP3 levels. Activated AKT can promote survival by inhibiting and/or promoting the activity of various pro/anti apoptotic factors (56-59). In addition, AKT can also inhibit and promote the degradation of p27 (60,61), a cyclin dependent kinase inhibitor that like p21 can arrest cells in G1-phase by binding and inhibiting G1-phase cyclin-cdk complexes (62). AKT also phosphorylates and inhibits TSC2, a protein that in conjunction with

TSC1 normally inhibits the mTORC1 kinase complex. Thus, AKT activates mTORC1 by inhibiting the TSC1/TSC2 complex. Activated mTORC1 promotes protein translation and cell growth by phosphorylation of substrates (e.g., S6K and 4EBP-1). Notably, activated S6K (pS6K) can also inhibit signaling from IGF-1R to AKT by promoting the degradation of IRS1 (63,64).

mTORC1 also responds to changes in nutrient and energy levels and adjusts cell growth accordingly (65). For example, glucose deprivation reduces energy (ATP) levels and increases the intracellular AMP/ATP ratio. This, in turn, activates AMP-activated kinase (AMPK) in an LKB1-dependent manner (66). AMPK phosphorylates and activates TSC2, which then inhibits mTORC1 (67). This insures that cell growth via mTORC1 is inhibited when energy levels are low. Deprivation of amino acids (e.g., serine and glutamine) also inhibits mTORC1. Current models suggest the mTORC1 complex localizes at the lysosome where it carries out amino acid sensing (68,69). Upon amino acid deprivation mTORC1 is released from the lysosome and rendered inactive. Thus mTORC1-dependent cell growth

is inhibited when amino acids are lacking. Importantly, p53 is activated and blocks proliferation in response to the same nutrient and energy stresses that inhibit mTORC1. For example, AMPK activated by glucose deprivation has been reported to phosphorylate p53 at S15 to trigger a p53-dependent cell cycle arrest (70), and amino acid deprivation has also been reported to cause p53-dependent cell cycle arrest (71,72). It would be inappropriate and potentially catastrophic for the cell cycle and cell division to continue if nutrient and energy levels are too low to support it. Thus, the ability of glucose and amino acid deprivation to inhibit mTORC1 and activate p53 insures that cell growth and cell division are coordinately inhibited when energy or nutrient levels are limiting.

Negative crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway

The IGF-1R/AKT/mTORC1 pathway promotes proliferation, survival, and cell growth. In contrast, p53 inhibits proliferation and can reduce survival. Given these opposing effects, it is perhaps not surprising that p53 can inhibit the IGF-1R/AKT/mTORC1 pathway and, conversely, that IGF-1R/AKT/mTORC1 pathway activation can inhibit p53 (Figure 2). Evidence IGF-1R/AKT activation can inhibit p53 includes studies from Mayo and colleagues in which it was found AKT activated by exogenous IGF1 promoted the phosphorylation of MDM2 and this phosphorylation increased the ability of MDM2 to degrade p53 (73). There are several points at which p53 can inhibit the IGF-1R/AKT/mTORC1 pathway. For example, p53 can repress expression of the *IGF-1R* and *IGF1* genes (74,75) and induce expression of IGF-BP3, a factor that can sequester and inhibit IGF1 (76). MDM2, which is transcriptionally activated by p53, can bind and promote the degradation of IGF-1R (77-79). Thus, MDM2-mediated IGF-1R degradation is another mechanism by which p53 may inhibit the IGF-1R pathway. P53 has also been reported to promote PTEN expression (80), thus blocking AKT activation downstream of PI3K. In addition, p53 can promote expression of AMPK β 1 and TSC2 to inhibit mTORC1 (81,82), and p53 can also induce expression of sestrin 1 and sestrin 2, which promote AMPK activation to inhibit mTORC1 (83). The ability of p53 to inhibit mTORC1 is logical. It would be inappropriate for a cell to continue to grow in response to stresses like DNA damage that activate p53 to inhibit cell cycle progression. The ability of p53 to inhibit IGF-1R/AKT/mTORC1 signaling

at multiple points insures that cell proliferation (cell cycle progression) and cell growth are coordinately inhibited in response to DNA damage and other p53-activating stresses.

Positive crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway

In addition to reducing survival through apoptosis or senescence, activated p53 can also increase survival in response to DNA damage and other stresses. This survival function of p53 results, at least in part, from its ability to promote reversible G1 and G2-phase cell cycle arrests (15,22-24). These arrests allow time for the stress to be resolved before proceeding with cell division (15,22). In addition to the negative crosstalk described above, there is also evidence for positive crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathways which could contribute to cell survival (Figure 3). For example, Murray *et al.* [2003] reported that exogenous IGF1 stabilized wild-type p53 in different cancer cell lines and that the stabilized p53 protected the cells from UV-radiation induced death (84). The stabilization of p53 was blocked by a PI3K inhibitor and by expression of a dominant-negative mutant of AKT. The results suggested AKT could stabilize and activate p53 downstream of IGF1. Later the same group showed that IGF1 increases p53 acetylation and the arrest-promoting activity of p53 by downregulating expression of the deacetylase SIRT1 (85). A mechanism by which AKT can stabilize p53 comes from studies by Blattner and colleagues. In these studies it was found that GSK3 β can bind and phosphorylate MDM2, and this phosphorylation increases MDM2s ability to promote p53 degradation (86,87). In this model, AKT stabilizes p53 by phosphorylating and inhibiting GSK3 β . Indeed, this group reported that AKT activated by DNA-PK is required for p53 to be stabilized in response to ionizing radiation (IR), probably by blocking GSK3 β and MDM2-dependent p53 degradation (88). There is also evidence that mTORC1 can promote p53 protein synthesis. For example, Lee *et al.* [2007] reported that TSC2 $-/-$ cells have elevated mTORC1 activity and express elevated levels of p53 (89). mTORC1 inhibition reduced p53 levels in these cells, supporting the idea that mTORC1 promotes p53 synthesis. Finally, Xiong *et al.* [2007] reported that IGF-1R $-/-$ MEFs express low levels of p53 due to decreased synthesis. The authors concluded that IGF-1R can promote p53 synthesis though, in this case, the increased synthesis appeared to be independent of mTORC1 (90).

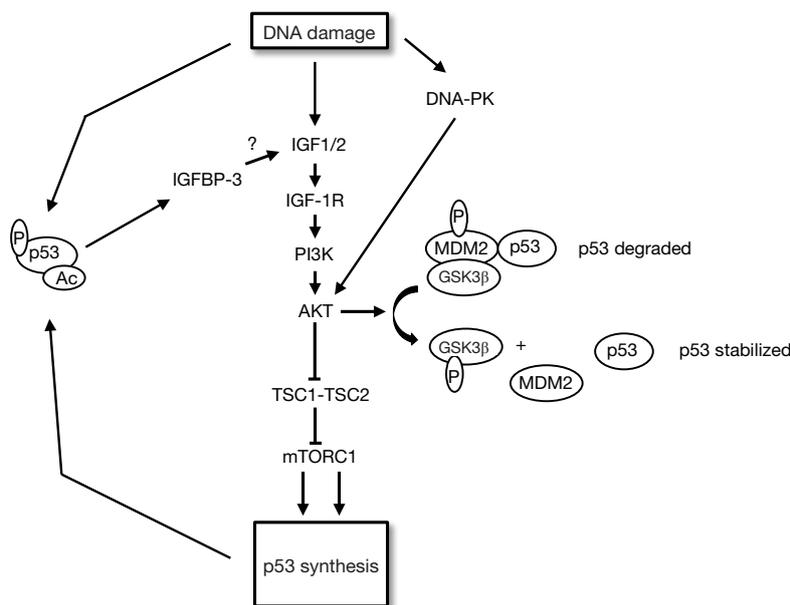


Figure 3 Positive crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway. The IGF-1R/AKT/mTORC1 pathway can be activated in response to DNA damaging stress. The IGF-1R/AKT/mTORC1 pathway was less activated in cells where p53 was depleted by shRNA, indicating p53 can contribute to IGF-1R/AKT/mTORC1 activation. One way in which this might occur is through p53-dependent induction of IGF1/2, which can both inhibit and potentiate IGF1 signaling. AKT activated in response to DNA damage can stabilize p53 by phosphorylating GSK3b and inhibiting p53 degradation mediated by the MDM2 and GSK3b. mTORC1 can promote p53 protein synthesis.

IGF-1R/AKT signaling determines the choice of response to p53

In our recent studies we reported that cisplatin (CP) activated the IGF-1R/AKT/mTORC1 pathway and stabilized p53 in osteosarcoma (OS) cells (91,92). p53 knockdown reduced IGF-1R/AKT/mTORC1 activation by CP, and IGF-1R, AKT, and mTORC1 inhibitors reduced the accumulation of p53. These data demonstrated positive crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway in response to CP. mTORC1 inhibition reduced p53 synthesis in the CP-treated cells, indicating IGF-1R/AKT/mTORC1 activation promotes p53 accumulation in part through mTORC1-dependent p53 synthesis (91). The mechanism by which p53 contributed to IGF-1R/AKT/mTORC1 activation was not clarified. However, the ability of p53 to increase IGF-1R/AKT/mTORC1 activation did not appear to result from increased expression of ligands IGF1 or IGF2 or from p53-mediated inhibition of mTORC1 (91). IGF-BP3 is a p53-responsive factor that, as mentioned earlier, can bind and sequester IGF1 to reduce IGF-1R pathway signaling. Recent reports showed that IGF1/2 can also potentiate

the mitogenic effects of IGF1 (93,94). One possibility is that p53 contributed to IGF-1R/AKT/mTORC1 activation by inducing IGF1/2, which then potentiated IGF1-dependent activation of this pathway.

How does crosstalk between p53 and the IGF-1R/AKT/mTORC1 pathway affect cell fate in therapy (CP) treated cells? To address this question, control, p53 knockdown, and p53-null OS cells were treated with CP alone or CP in combination with an IGF-1R inhibitor (OSI-906) or AKT inhibitor (MK2206) (91,92). Apoptosis, senescence, and long-term survival (colony formation) was then assessed. A number of interesting findings emerged: First, p53 knockdown increased apoptosis and reduced colony formation in OS cells treated with CP. These findings indicated that p53 promotes survival in OS cells in response to CP. This survival function of p53 could result from transient arrests that allow DNA repair. However, because the IGF-1R/AKT pathway was *less* activated in p53 knockdown cells, we speculate p53 can also reduce apoptosis and increase survival by maintaining or contributing to IGF-1R/AKT activation. Second, IGF-1R and AKT inhibition reduced p53 protein levels and p53-dependent

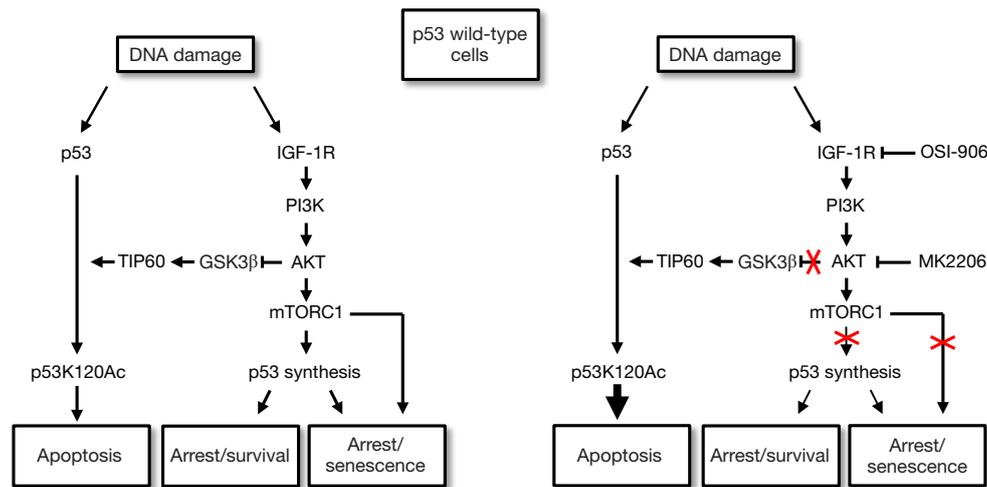


Figure 4 IGF-1R/AKT/mTORC1 signaling inhibits p53-dependent apoptosis and promotes p53-dependent senescence. (Left) DNA damaging stress stabilizes p53 and activates the IGF-1R pathway. AKT inhibits p53-dependent apoptosis by inhibiting GSK3 β and reducing TIP60-dependent acetylation of p53 at K120. At the same time, mTORC1 activated downstream of AKT promotes p53 protein synthesis. mTORC1 activity contributes to p53-mediated senescence. (Right) IGF-1R and AKT inhibitors increase p53-dependent apoptosis by increasing GSK3 β activity, which increases TIP60-dependent acetylation of p53 at K120. At the same time, IGF-1R and AKT inhibitors block mTORC1 activity and mTORC1-dependent p53 synthesis, thus reducing p53-mediated senescence.

senescence, but increased p53s apoptotic function. This was evidenced by the finding that IGF-1R/AKT inhibition increased p53-dependent apoptotic gene expression (*PUMA*, *NOXA*) and p53-dependent death after CP. The results indicated that IGF-1R/AKT signaling maintains p53 protein levels but inhibits its apoptotic function. Finally, we speculated IGF-1R/AKT inhibition might increase p53s apoptotic function by inhibiting GSK3 β and increasing K120 acetylation of p53. Consistent with this possibility, IGF-1R inhibition reduced pAKT (S473) and pGSK3 β (S9) levels in CP treated cells but increased in the relative amount of K120-acetylated p53.

Based on our findings the following model is proposed (Figure 4). In this model, the ability of p53 to induce cell cycle arrest is dependent on p53 levels, while the ability of p53 to induce apoptosis is dependent on specific PTMs including K120 acetylation. IGF-1R/AKT/mTORC1 pathway activation maintains p53 protein levels through mTORC1 dependent p53 synthesis and thus promotes p53-dependent arrest. At the same time, IGF-1R/AKT signaling inhibits p53s apoptotic function by inhibiting GSK3 β and TIP60-dependent acetylation of p53 at K120. IGF-1R/AKT inhibition reduces p53 protein levels and p53-dependent arrest/senescence, but increases p53 K120 acetylation and p53-dependent apoptosis.

The case in p53-null cells

We also examined how IGF-1R/AKT inhibition would affect CP-sensitivity in cells that lack p53 expression. Surprisingly, IGF-1R and AKT inhibition reduced apoptosis and increased colony formation in p53 knockdown cells and p53-null cells treated with CP (91,92). We found this effect was dependent on p27, a cyclin-cdk inhibitor that like p21 can arrest cells in G1-phase. AKT activated downstream of IGF-1R can phosphorylate p27, leading to its degradation and cytoplasmic sequestration (60,61). In our studies we found that p27 levels were decreased in CP-treated OS cells and this effect was blocked by IGF-1R and AKT inhibitors. This result indicates p27 reduction in CP-treated cells most likely results from AKT-mediated p27 degradation. We found that IGF-1R/AKT inhibition maintained p27 levels in CP-treated cells and induced a G1 arrest/delay that was p27-dependent. Most importantly, IGF-1R/AKT inhibition reduced apoptosis and increased colony formation in p53 knockdown/null cells, and these effects were reversed by p27 knockdown. These findings indicate that IGF-1R and AKT inhibition can reduce apoptosis and increase long-term survival in p53 knockdown or null cells in a p27-dependent manner. The most likely scenario is that IGF-1R inhibition blocks AKT-dependent degradation of p27, and stabilized p27 then mediates cytoprotective arrest or delay

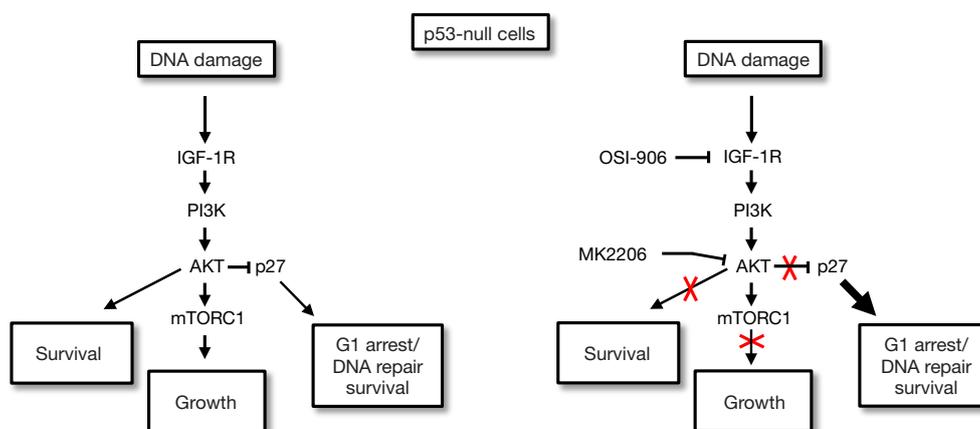


Figure 5 In p53-null cells, IGF-1R/AKT inhibition stabilizes p27 which then mediates a protective G1-arrest that promotes repair and survival. (Left) DNA damaging stress activates the IGF-1R pathway in p53-null cells. Activated AKT promotes survival but also inhibits p27, which could otherwise promote a protective G1-arrest and survival. (Right) IGF-1R and AKT inhibition reduces AKT-mediated survival, but also restores p27, which then mediates a G1-arrest that allows DNA repair to promote survival.

in G1-phase that allows DNA repair and survival (*Figure 5*).

Clinical implications

Multiple IGF-1R pathway inhibitors have been developed as potential therapeutics. However, while these inhibitors have shown promise in pre-clinical studies they have failed to increase patient survival when given alone or in combination with chemotherapy agents. The reason(s) for the disappointing clinical effect of these inhibitors is not fully understood. Our studies suggest the ability of IGF-1R/AKT inhibitors to increase cancer therapy responses is dependent on p53-status and the extent to which cells undergo apoptosis and senescence. In p53 wild-type cells treated with CP, our studies showed that IGF-1R/AKT inhibition increased p53-dependent apoptosis but reduced p53-dependent senescence, and had no effect on long-term survival (91,92). Based on these results we predict IGF-1R/AKT inhibitors may fail to enhance therapy responses in p53 wild-type cancers due to the opposing effects of reducing senescence while increasing apoptosis. In contrast, in p53-null and p53-knockdown cells, IGF-1R/AKT inhibition reduced apoptosis in response to CP and increased long term survival (91,92). Our results showed these effects were dependent on p27. CP treatment alone caused a pronounced depletion of p27 in p53-null cells, most likely through p27 protein degradation. IGF-1R/AKT inhibition maintained p27 levels in CP treated cells, and p27 then mediated a G1 arrest that

reduced apoptosis and increased long-term survival. Based on these results we predict IGF-1R/AKT inhibitors may reduce the effectiveness of chemotherapy against cancers that lack wild-type p53 by stabilizing p27 and thus causing p27-dependent cancer cell survival.

A final question is if we can use this information to increase the effectiveness of IGF-1R/AKT inhibitors. Our results suggest IGF-1R/AKT inhibitors may fail to enhance CP-induced killing in p53-null cancer cells due to stabilization of p27, which then mediates a protective G1 arrest. Based on this, we considered a schedule-dependent approach that reduced p27 levels prior to IGF-1R/AKT inhibition might maximize cancer cell killing by CP. Specifically, we considered that if AKT inhibitor was added 24 h after CP treatment it would inhibit AKT-mediated survival pathways but fail to trigger a protective G1 arrest because p27 would already be depleted/degraded. To test this possibility, we treated two different p53-null OS cell lines (MG63 and SAOS) with CP alone or CP plus the AKT inhibitor MK2206 (92). In these experiments, MK2206 was either given at the same time as CP or given 24 h after CP treatment (sequential treatment). Importantly, when the cells were treated with MK2206 24 h after treatment with CP (sequential treatment), p27 levels were not restored, the cells failed to arrest in G1 phase, and colony formation was completely inhibited. The results suggest combination chemotherapy plus IGF-1R/AKT inhibitors could increase killing in cancers that lack wild-type p53 but only if the inhibitors

are given in a schedule-dependent manner (e.g., 24 h after chemotherapy treatment).

In p53 wild-type cells the effectiveness of IGF-1R/AKT inhibitors was limited by a reduction in p53 levels and a corresponding reduction in p53-dependent senescence. Therefore, approaches that maintain p53 levels might increase the effectiveness of IGF-1R/AKT inhibitors. A schedule-dependent approach similar to that described above might work in this regard. For example, the reduction in protein levels caused by IGF-1R/AKT inhibitors results, at least in part, from reduced p53 synthesis (91). p53 is stabilized and accumulates in therapy treated cells. If IGF-1R/AKT inhibitors are given 24 h after therapy treatment, then the p53 protein that is already stabilized may be converted to an apoptotic form (e.g., K120 acetylated) while being refractory to any reduction caused by IGF-1R/AKT inhibition. It will be important to ask if a schedule-dependent approach or other approaches can increase the effectiveness of IGF-1R pathway inhibitors in cancer cells with or without wild-type p53, and if these approaches can then be translated into clinical practice.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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