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Tumor Suppressor p53 and Estrogen Receptors in Nuclear–Mitochondrial Communication

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Abstract

Several gene transcription regulators considered solely localized within the nuclear compartment are being reported to be present in the mitochondria as well. There is growing interest in the role of mitochondria in regulating cellular metabolism in normal and disease states. Various findings demonstrate the importance of crosstalk between nuclear and mitochondrial genome, transcriptome, and proteome in regulating cellular functions. Both tumor suppressor p53 and estrogen receptor (ER) were originally characterized as nuclear transcription factors. In addition to their individual roles as regulators of various genes, these two proteins interact resulting in major cellular consequences. In addition to its nuclear role, p53 has been localized to the mitochondria where it executes various transcription-independent functions. Likewise, ERs are reported to be present in mitochondria; however their functional roles remain to be clearly defined. In this review, we provide an integrated view of the current knowledge of nuclear and mitochondrial p53 and ERs and how it relates to normal and pathological physiology.

Keywords

Estrogen receptor; p53; tumor suppressor; breast cancer; transcription; aging; glycolysis; OXPHOS; apoptosis; metabolism

1. Introduction

Communication between genomes of the nucleus and mitochondria is required for the coordinate expression of proteins required for the regulation of mitochondrial biogenesis and function, which is essential in maintaining the fate of the cell (Leigh-Brown et al., 2010). Regulation of these processes involves well-orchestrated actions of both mitochondrial DNA (mtDNA) and nuclear-encoded gene products. With the exception of handful of proteins, mitochondrial proteome is mainly composed of proteins that are transcribed from the nuclear genome (Scarpulla, 2002; Szczepanek et al., 2012). Over the years substantial effort has

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been dedicated to study the nuclear factors that regulate mtDNA replication and transcription as well as the expression and transport of nuclear-encoded mitochondrial proteins. For years scientists have speculated the presence of nuclear encoded transcription factors (TFs) within the mitochondria. While biological actions of many of these nuclear TFs in the mitochondrial compartment are being elucidated, functions of some remain ambiguous. Among these TFs, tumor suppressor protein p53 and estrogen receptors (ERs) ER α and ER β have been shown to have distinct mitochondrial roles. Accumulating evidence suggests that other than their nuclear location, both p53 and ERs can be localized to the mitochondria (Chipuk and Green, 2006; Marchenko et al., 2000; Pedram et al., 2006). A pool of p53 translocates to mitochondria in response to apoptotic stimuli, resulting in a cascade of events such as the loss of mitochondrial membrane potential, generation of reactive oxygen species (ROS), cytochrome c release, and caspase activation (Marchenko et al., 2000; Mihara and Moll, 2003; Zhao et al., 2005). Recent findings on the mitochondrial localization of ERs raise questions on the role of these receptors within the mitochondria. Accumulating evidence on the nuclear interaction between these two proteins and the crosstalk between the respective signaling pathways coupled with the reports on localization of both of them in the mitochondria heighten the importance of investigating role of these proteins in coordinating the nuclear-mitochondrial functions.

2. p53 and Estrogen Receptors: Nuclear Proteins With Opposing Functions

p53 is a key tumor suppressor protein that serves as a sensor of cellular stress, and by integrating various signaling pathways, plays a central role in cellular processes such as cell cycle arrest, apoptosis, senescence, and differentiation. Since its discovery in 1979, evidence has been continuously emerging on multiple functions of p53 in normal and cancer cells. In addition to its ability to initiate cell-cycle arrest and apoptosis, it has been shown to regulate metabolism, autophagy and oxidative status of the cell (Bensaad et al., 2006; Bensaad and Vousden, 2007; Cheung and Vousden, 2010; Matoba et al., 2006). Of note, p53 elicits biological protective, pro-survival responses to maintain genome integrity and viability in cells that sustain limited, reversible damage. These various responses rely on the ability of p53 to function as a transcriptional activator of an increasing array of target genes as well as on its transcription-independent activities including those that occur in the cytosol and mitochondria.

ERs, on the other hand, are nuclear hormone receptors that act as transcription factors to regulate genes involved in growth, development and differentiation of secondary sex characteristics, homeostasis, and metabolism and play a fundamental role in proliferation of breast cancer cells (Ali and Coombes, 2002; Osborne and Schiff, 2005; Pearce and Jordan, 2004; Shao and Brown, 2004). Estrogen receptor α (ER α) plays an important role in the onset and progression of breast cancer, whereas p53 functions as a major tumor suppressor.

Investigators have documented the delicate relationship of estrogen signaling and ER α with p53. Das and coworkers have reported that ER α binds to p53 and represses its nuclear function in mammary epithelial stem/progenitor cells, breast cancer cells, and in xenograft tumors (Konduri et al., 2010; Liu et al., 2009; Liu et al., 2006; Sayeed et al., 2007) suggesting a potential role for the ER α –p53 interaction in mammary tissue homeostasis and

oncogenesis. Genetic support for this idea comes from the longstanding clinical observation that ER α -positive breast cancers express wild type p53 whereas ER α -negative ones harbor mutant p53 (Cattoretti et al., 1988; Miller et al., 2005). These observations suggest that functional suppression of nuclear p53 is an important step in breast oncogenesis. In addition to the functional regulation by protein-protein interaction, ER α and p53 regulate each other at the transcriptional level as well. p53 has been shown to be recruited to the ER α gene promoter resulting in increased transcription of ER α (Angeloni et al., 2004; Shirley et al., 2009). On the other hand, ER α was reported to activate p53 transcription by binding to ERE half-sites within the promoter and knockdown of ER α decreases expression of p53 and its downstream targets (Berger et al., 2012). Together, these observations suggest the existence of a feedback loop between ER α and p53.

3. Mitochondrial p53

3.1. Mechanisms of p53 Delivery to the Mitochondria

It has become evident that in response to stress signals, cytoplasmic p53 rapidly translocates to the mitochondrial outer membrane (Marchenko et al., 2000). This phenomena is observed under many types of DNA damage, hypoxia, oncogene deregulation, oxidative damage in human and mouse cell types both in primary as well as immortal and malignant cell types (Arima et al., 2005; Moll et al., 2006; Stommel et al., 1999). While nuclear p53 export is a slow process requiring 3–8 hours (Stommel et al., 1999), stress-induced mitochondrial p53 translocation is reported to be a rapid process visible within 30 min and summing within 2 hours (Marchenko et al., 2000). Additionally, when expressed in numerous p53-null cancer cell lines, a mitochondrially targeted p53 fusion protein is able to bypass the nucleus and is able to promote apoptosis and long-standing growth retardation as competently as the endogenous wild type p53 (wt-p53) (Marchenko et al., 2000; Mihara et al., 2003). In addition to localization at the surface of the mitochondria, a pool of p53 localizes in the mitochondrial matrix and complexes with mitochondrial chaperones mtHsp70 and mtHsp60 operating within mitochondria (Dumont et al., 2003; Dupont et al., 2009; Marchenko et al., 2000). Then again, it is now becoming evident that endogenous p53 can be located at mitochondria even in the absence of exogenous stress, indicating that p53 has functions in the normal physiology of this organelle (De et al., 2012; Ferecatu et al., 2009; Mahyar-Roemer et al., 2004). Nevertheless, mechanism(s) underlying mitochondrial p53 translocation remains ambiguous. In majority of proteins targeted to the mitochondria, a mitochondrial targeting signal characterized by a stretch of hydrophobic and positively charged residues is present either at the N-terminus or at the interior regions of the protein (Hahne et al., 1994; Roise et al., 1988). For proteins targeted to the mitochondrial matrix, TCA cycle proteins, and other proteins associated with mitochondrial metabolism, the N-terminal signal is cleaved off after the protein enters the matrix compartment. However, for several proteins targeted to mitochondrial inner membrane and intermembrane space, N-terminal or internal uncleaved signals have also been reported (Folsch et al., 1996). The outer mitochondrial membrane proteins and most mitochondrial intermembrane space proteins lack canonical N-terminal targeting signals (Mihara, 2000; Pfanner et al., 2004; Rapaport, 2003).

Comprehensive proteomic analyses of mitochondria from different cells and tissues suggest that almost half of nuclear-encoded proteins linked with mammalian mitochondria lack canonical N-terminal targeting signals. Consequently, the nature of the signals or the mechanism responsible for the mitochondrial translocation of proteins such as p53 containing noncanonical signals remains unclear. Using the Fusspro algorithm from EMBL database Mootha et al., identified a serine protease-like processing site followed by a cryptic mitochondrial targeting signal in p53 (Mootha et al., 2003; Taylor et al., 2003). The cytoplasmic processing of p53 to a 42 kDa product and mitochondrial protein import requires an inducible cytoplasmic endoprotease for activation of this cryptic mitochondrial targeting signal.

According to some reports, a translocation motif is not found in p53 and phosphorylation/ acetylation alterations are not responsible for the mitochondrial targeting of p53 under stress conditions (Marchenko et al., 2007; Nemajero et al., 2005). Convincing evidence has been offered to demonstrate that the source of mitochondrially translocated p53 is a distinct pool of stress stabilized cytoplasmic p53 (Marchenko et al., 2007). Monoubiquitinated p53, generated by basal levels of Mdm2-type E3 ligases, provides a trafficking signal that redirects the stress-stabilized pool of cytoplasmic p53 to mitochondria. In the presence of a stress stimuli, multi-monoubiquitinated p53 intermediate is rapidly stabilized by stress-induced disruption of the p53–Mdm2 complex and diverted to mitochondria. Upon entry to mitochondria, p53 undergoes prompt deubiquitylation by mitochondrial HAUSP (herpesvirus-associated ubiquitin-specific protease) via a stress-induced p53–HAUSP complex to generate apoptotically active non-ubiquitinated p53. Whether p53 translocation is regulated by Mdm2 is still not clear. This question becomes critical in light of a study that demonstrated the two common polymorphic variants of human p53 (Arg72 and Pro72) differing greatly in their apoptotic ability have different subcellular localization. Arg72, which is stronger in inducing cell death localized more efficiently to mitochondria and was also more ubiquitinated compared to the Pro72 (Dumont et al., 2003). It is not clear how Arg72 is protected from degradation or de-ubiquitinated in the cytoplasm. A feasible scenario would be that Mdm2 is physically escorting p53 to mitochondria, but neither Mdm2 nor Mdm2-p53 complexes are present in the mitochondria. Other Mdm2-like proteins have been found to regulate p53 functions, in particular MdmX (also known as Mdm4) (Parant et al., 2001; Shvarts et al., 1996; Wade et al., 2013). Interestingly, while both MdmX and Mdm2 have the capacity to bind to p53 and block its transactivation, MdmX is unable to facilitate the nuclear export of p53 or its degradation. This suggests that MdmX is in fact playing a protective role towards p53 from Mdm2-mediated suppression (Jackson and Berberich, 2000). Mancini and colleagues observed that a fraction of Mdm4 stably localizes to the mitochondria under lethal stress conditions, promotes mitochondrial localization of p53 phosphorylated at Ser46 and facilitates its binding to BCL2 leading to cytochrome C release and apoptosis (Mancini et al., 2009). These observations indicate that MdmX enhances p53-targeting to the mitochondria; however, it is not clear whether changes in p53 ubiquitylation status is involved in MdmX-dependent mitochondrial p53 translocation. A p53 missense mutant (K351N in the tetramerization domain) identified in a cisplatin-resistant ovarian carcinoma cell line (A2780 CIS) was found to be deficient in nuclear export, monoubiquitination, and cisplatin-induced translocation to mitochondria, Bax oligomerization,

and mitochondrial membrane polymerization (Muscolini et al., 2011). This observation identified a particular amino acid in p53 that is targeted for monoubiquitination followed by translocation to the mitochondria. Tumor suppressor Tid1, a mitochondrial DnaJ-like chaperone (Lu et al., 2006) is another protein that regulates mitochondrial translocation of p53 (Lu et al., 2006; Trinh et al., 2010). Tid1 binds to p53 under hypoxic conditions and directs p53 translocation to the mitochondria followed by intrinsic apoptosis.

The oligomerization state of mitochondrial p53 is another unsettled controversy. Roemer and colleagues showed that in comparison to tetrameric nuclear p53, mitochondrial p53 is mostly monomeric (Heyne et al., 2008). However, Murphy and coworkers observed in their cross-linking studies that most mitochondrial p53 is present in dimers or higher-order multimers. Furthermore, mutations of the p53 oligomerization domain significantly affected its ability to stimulate the oligomerization of BAK. Their cross linking studies indicate that the bulk of p53 localized to the mitochondria is in dimeric or higher-order oligomeric form (Pietsch et al., 2008).

Another intriguing aspect is the presence of mutant p53 in the mitochondria. Moll and colleagues have reported that certain tumors possess high levels of mutant p53 protein that is constitutively localized to the mitochondria and bound to BAK (Mihara et al., 2003). They showed that in contrast to wt-p53, four naturally occurring in p53 mutants (R175H, L194F, R273H, R280K) are constitutively present in mitochondria in unstressed breast cancer cells.

3.2. p53: Role in Mitochondrial Genome Integrity and Function

There is substantial evidence for p53's role in mitochondrial DNA maintenance and DNA-damage repair. A small portion of p53 protein was detected in the inner mitochondrial membrane sub-fraction containing components of the mtDNA base excision repair (mtBER) complex and was found to play a role in mtBER, via yet unknown pathway (Chen et al., 2006). One of the critical players involved in the maintenance of mitochondrial genomic stability is the mtDNA polymerase γ (mtPol γ), which is the singular DNA polymerase in mitochondria and plays a vital role in mtDNA replication and repair (Copeland et al., 2003; Kaguni, 2004). A physical interaction between p53 and mtPol γ *in vivo* has been identified in HCT116 cells where p53 enhances its replication function and interacts with the mitochondrial genome (Achanta et al., 2005). This binding of p53 to the mitochondrial genome was induced by a DNA-damage response but was not dependent on DNA-damage. Study conducted to clarify the functional collaboration between p53 and mtPol γ during DNA synthesis in mitochondria showed that the presence of p53 in mitochondria, provided by exogenous recombinant p53 or endogenous p53, is capable of proofreading function during mitochondrial DNA replication (Bakhanashvili et al., 2008). In an *in vitro* system derived from the mitochondria of mouse liver devoid of nuclear contaminants, gap-filling function of mtPol γ was less efficient in p53 knockout extracts, but the activity was regained when complemented with recombinant p53 (Achanta et al., 2005; de Souza-Pinto et al., 2004). Intriguingly, defects in mitochondria arising from mitochondrial oxidative phosphorylation (mtOXPHOS) has been shown to induce cell cycle arrest potentially involving nuclear genes required for DNA damage checkpoint response, although direct role of p53 in this phenomenon is yet to be shown (Kulawiec et al., 2009). p53 is also reportedly

involved in mtDNA transcription and integrity maintenance by binding and regulating the activity of the mitochondrial transcription factor A (TFAM), a multi-functional protein involved in various aspects of mtDNA replication, nucleoid formation, DNA protection, DNA repair and damage sensing. *In vitro* binding studies show that TFAM interacts with N-terminal transactivation domain of p53, whereas the C-terminal regulatory domain of p53 provides a secondary binding site for TFAM (Wong et al., 2009). In human KB epidermoid cancer cells and in HCT116 adenocarcinoma cells, p53 physically interacts with TFAM and binding of TFAM to cisplatin-modified DNA was significantly enhanced by p53, whereas binding to oxidized DNA was inhibited implying both TFAM and p53 show preferential binding to distorted DNA (Yoshida et al., 2003). Roemer's group has identified a putative p53 binding sequence within the human mitochondrial genome (Heyne et al., 2004). Regardless of strong evidence of p53 in the interior of the mitochondria and association with mitochondrial DNA, thus far there is no clear indication of p53's ability to regulate transcription from the mitochondrial genome.

3.3. p53-Mediated Cell Death

The role of nuclear p53 in triggering apoptotic cell has been intensely studied, and involves both the transactivation of proapoptotic Bcl-2 family members (e.g., Bax, Noxa, Puma) as well as the transcriptional repression of antiapoptotic factors (such as Bcl-2). Caspase activation requires the release of various proteins from the intermembrane space. Mitochondrial outer membrane permeabilization (MOMP) is often required for the release of proteins responsible for the activation of the caspase proteases. Under a range of cell death-inducing stresses cytoplasmic p53 rapidly translocates to mitochondria, where it interacts with mitochondrial membrane proteins from the Bcl-2 family to activate MOMP in a transcription-independent manner. Once at the mitochondrion, direct action of p53 induces MOMP and subsequent apoptosis by activating the release of pro-apoptotic machinery of caspases from the mitochondrial intermembrane space. Intensive work performed on p53 function indicates that the pro-apoptotic effects of cytoplasmic p53 are not dependent on transcription. Nonetheless, capability of nuclear p53 to regulate transcription of pro-apoptotic mitochondrial proteins comes into play to coordinate mitochondrial apoptotic response.

Most studies suggest that p53 may induce apoptosis by forming complexes with mitochondrial proapoptotic proteins located in the outer membrane of mitochondria. However, Clair and coworkers have shown that p53 translocated not only to the outer membrane of mitochondria but also into the matrix and physically interacted with the primary antioxidant enzyme, manganese superoxide dismutase (MnSOD). Their investigation revealed the relationship between p53 translocation to mitochondria and p53-induced transcription of mitochondria-anchored proapoptotic gene Bax that result in subsequent decrease of mitochondrial membrane potential (Zhao et al., 2005). Both wild type and mutant p53 was reported to interact with caspase-3 associated with both the inner and outer mitochondrial membranes; however mutant p53 likely interfere with the ability of pro-caspase-3 to become proteolytically activated by caspase-9 (Frank et al., 2011). Of note, it has been reported that p53 undergoes caspase-dependent cleavage resulting in the generation of four fragments, two of which lack a nuclear localization signal and

consequently localized to mitochondria. These mitochondrial p53 fragments induce mitochondrial membrane depolarization in the absence of transcriptional activity and may induce MOMP (Sayan et al., 2006). Importantly, Mancini et al., reported that under lethal stress a fraction of MdmX protein stably resides at the mitochondria promoting the recruitment of the p53 phosphorylated on Ser46, that in turn, binds Bcl-2 to promote MOMP and cytochrome c release. This observation raises the intriguing question if MdmX by itself or in association with MDM2 is an important player in regulating mitochondrial translocation of p53.

While roles of p53 in cell death by promoting apoptosis and autophagy are well documented, it remained unclear whether p53 also contributes in the regulation of necrosis. Recently, Vaseva et al. identified a new role of p53 in activating necrosis by inducing the opening of the permeability transition pore (PTP), which resides in the inner mitochondrial membrane (IMM) (Vaseva et al., 2012). In this study, incubation of mitochondria with purified p53 induced opening of the PTP and when cells were treated with necrosis inducer hydrogen peroxide (H₂O₂), endogenous p53 specifically interacted with the key regulator of PTP, cyclophilin D (CYPD). This study, for the first time, provided definitive proof that p53 induces necrosis due to its transcription-independent local action within the mitochondria in response to oxidative stress by triggering the opening of the PTP in a CYPD-dependent manner.

4. The Role of p53 in Cellular Metabolism

Differentiated cells typically use mitochondrial oxidative phosphorylation as their main way to generate energy (Bayley and Devilee, 2012; Vander Heiden et al., 2009). However, most tumor cells use glycolysis even when oxygen is accessible. This phenomenon has been coined as the “Warburg effect” (Moreno-Sanchez et al., 2007; Warburg, 1956). It is becoming increasingly clear that this metabolic transformation plays a role in tumor progression and that p53 is key player in the regulation of energy metabolism. Loss of p53 function in cells is associated with significant deficiency in mitochondrial biogenesis, decrease in oxygen consumption, and stimulated glycolysis manifested with increased lactate generation indicating a role of p53 in promoting oxidative phosphorylation (Ibrahim et al., 1998; Matoba et al., 2006). p53 can avert metabolic transformation by restraining the glycolytic pathway thereby exerting oncosuppressive functions to counteract the Warburg effect (Bensaad and Vousden, 2007; Cheung and Vousden, 2010; Galluzzi et al., 2008; Vousden, 2009; Wang et al., 2012). p53 prevents the uptake of glucose, the substrate for glycolysis, by blocking the expression of glucose transporters GLUT1 and GLUT4 (Schwartzberg-Bar-Yoseph et al., 2004). Additionally, p53 is able to inhibit insulin receptor (INSR) overexpressed when p53 is incapacitated in cancer (Webster et al., 1996). Moreover, a p53-inducible gene, TP53-induced glycolysis and apoptosis regulator (TIGAR) was found to lower the fructose-2,6-bisphosphate (Fru-2,6-P₂) levels in cells, resulting in inhibition of glycolysis (Bensaad et al., 2006; Li and Jogl, 2009). p53-mediated overexpression of TIGAR results in the block of glycolysis in the redirection of glucose catabolism towards the pentose phosphate pathway (PPP). Inhibition of glycolysis and stimulation of PPP by p53 increases synthesis of nucleotides required for DNA repair and accumulate reduced nicotinamide adenine dinucleotide phosphate (NADPH) to increase

antioxidant defenses. Consistent with antiglycolytic role of wild type p53, it has been reported that mutant p53 activates transcription of type II hexokinase (HK II) (Mathupala et al., 1997). HK II, a protein bound to the outer mitochondrial membrane via the voltage-dependent anion channel (VDAC), is expressed at high levels in cancer cells enabling it to bind incoming glucose leading to activation of glycolysis (Mathupala et al., 2006; Robey and Hay, 2006). However, in certain contexts when p53's tumor suppressor activities are not properly regulated, it might elicit opposite effects (Olovnikov et al., 2009; Vousden and Prives, 2009). Nevertheless, it is largely accepted that p53 frequently obstructs glycolysis, sustains mitochondrial homeostasis and regulates mitochondrial respiration.

Several lines of evidence have shown that the preservation of wild type p53 is linked with the maintenance of oxidative phosphorylation. These pro-respiratory roles of p53 are presumably regulated by direct transcriptional activation of various nuclear genes. Loss of p53 in human cancer cells was correlated with diminished mitochondrial cytochrome c oxidase II (COXII) activity attributed to decreased protein levels of the COXII subunit encoded by the mitochondrial genome indicating that p53 regulates COXII protein levels by post-transcriptional regulation of the COXII subunit (Zhou et al., 2003). Later it was revealed that p53 balances the usage of the respiratory and glycolytic pathways through its transcriptional target SCO2 which is essential to assemble mitochondrial cytochrome c oxidase (complex IV in the electron transport chain) encoded by the mitochondrial genome. While decreased SCO2 expression can promote the switch to aerobic glycolysis in p53-deficient cells, it can be reversed by exogenous SCO2 (Matoba et al., 2006). Recently, mitochondrial phosphate-activated glutaminase (GLS2) was identified as a target of p53. Activation of GLS2 transcription by p53 shifts the energy production from the glycolytic pathway to mitochondrial respiration and glutaminolysis (Hu et al., 2010; Suzuki et al., 2010; Vousden, 2010). Importantly, impaired mitochondrial electron transport can induce p53. Khutornenko et al. found that interruption of electron transfer to complex III and resulting pyrimidine deficiency elicited accumulation of p53, followed by apoptosis (Khutornenko et al., 2010; Tolstonog and Deppert, 2010). Moreover, loss of complex I biogenesis in the absence of MWFE subunit reduced the steady state levels of p53 (Compton et al., 2011). A consequence of mitochondrial respiration is the production of reactive oxygen species (ROS). While increasing mitochondrial activity, p53 balances the deleterious effect of ROS by activating transcription of nuclear genes encoding mitochondrial antioxidant enzymes such as aldehyde dehydrogenase (*ALDH4*) and Manganese-superoxide dismutase (*SOD2*) (Olovnikov et al., 2009).

Genes encoding apoptosis-inducing factor (AIF) (Stambolsky et al., 2006) and ribonucleotide reductase subunit (p53R2) which has distinct functional roles in mitochondria are also transcriptional targets of p53. AIF is crucial in regulating various cell death pathways as an oxidoreductase to maintain the stability and integrity of complex I in the electron transport chain. p53 controlled p53R2 regulates mitochondria homeostasis and function (Bourdon et al., 2007). In human and murine cells depletion of cytoplasmic polyadenylation element-binding protein (CPEB) is associated with abnormal p53 mRNA short poly(A) tail and a reduced translational competence, resulting in an ~50% decrease in p53 protein levels associated with fewer mitochondria than wild-type cells, reduced

respiration and enhanced rates of glycolysis. On the other hand ~50% reduction of p53 levels in cells containing normal levels of CPEB is correlated with a reduced mitochondrial mass and change in energy metabolism due at least in part to dysfunctional p53 mRNA polyadenylation and translation (Burns and Richter, 2008). Interestingly, SCO2 levels were reduced in cells in which either CPEB or p53 were knocked down, suggesting that the impact of CPEB on cellular respiration occurs via p53 mRNA translation and SCO2. This study highlights the importance of steady state p53 protein levels under normal conditions operating to regulate energy metabolism in the mitochondria. Figure 1 shows a schematic depiction of p53's role in glucose metabolism, mitochondrial respiration, mitochondrial homeostasis, and cell death.

5. Estrogen Receptors

Elmwood Jensen first described the ER in 1958, and after almost two decades later this receptor was identified as a member of nuclear receptor superfamily (Jensen, 1962). Years later a second ER was discovered (Kuiper et al., 1996; Ogawa et al., 1998). The “Jensen” receptor was then named ER-alpha and the new receptor ER-beta (ER β) and since then the two forms of the ER have been extensively investigated.

ER α and ER β are encoded by separate genes, *ESR1* and *ESR2*, respectively, and multiple splice variants exist for these receptors. ER α and ER β are highly conserved in the DNA-binding domain (DBD) with a high similarity in amino acid sequence (96%) and 53% homology for the ligand-binding domain and least homology in the transactivational domain (30%) (Delaunay et al., 2000; Kuiper et al., 1996). Each receptor has distinct tissue expression patterns, post-translational modifications, and cellular localization in normal and disease states. ER α is expressed in various human tissues such as brain, breast, bone, urogenital tract, uterus, prostate, cardiovascular system, and liver, whereas ER β is present in brain, breast, bone, urogenital tract, prostate, cardiovascular system, and lungs (Pearce and Jordan, 2004). When both ER α and ER β coexist, the proliferative function of estrogens mediated via ER α can be opposed by ER β where ER α promotes cell proliferation while ER β has proapoptotic and cell differentiation function (Chang et al., 2008; Jensen et al., 2010; Pearce and Jordan, 2004). However, there are models where ER β expression has been shown to be associated with proliferation (Jensen et al., 2001; Skliris et al., 2006) contradicting the proposed tumor suppressor role of ER β . Notably, increased growth inhibition and induction of apoptosis was observed in ER β -knockdown non-small cell lung cancer (NSCLC) cells in a ligand-independent manner (Zhang et al., 2010a). Moreover, ER β from the mitochondrial fraction physically interacted with the proapoptotic protein Bad: the DNA-binding domain along with the hinge domain of ER β and the BH3 domain of Bad are necessary for this interaction.

The classical mechanism of action of ER as a transcription factor involves binding to a ligand, which results in receptor dimerization with a second ER followed by recruitment to the promoter regions of target genes directly through binding to estrogen response elements (EREs) or indirectly through other DNA-binding factors (McDonnell, 2004; Osborne and Schiff, 2005). ER α and ER β bind to estrogens, notably 17- β estradiol (E2), the most potent endogenous ligand, at comparable affinity (Harris et al., 2002; Kuiper et al., 1997; Kuiper et

al., 1998). The selective ER modulators (SERMs) tamoxifen and raloxifene bind both ERs with different affinities and their effects on transcription are often ER subtype specific. *In vitro* studies have demonstrated that tamoxifen acts as a partial agonist on ER α , but has a pure antagonist effect on ER β (Barkhem et al., 1998). ER α status is a key predictor of the prospect that breast tumors will respond to endocrine therapy, such as treatment with the anti-estrogen tamoxifen. On the other hand, a large pool of ERs has been described in the cytoplasm of various target cells, but the precise cytoplasmic localization and functions are unclear. In transfected cells, ER β localizes to the nucleus (Matsuda et al., 2002), whereas the nuclear localization of ER β in nontransfected cells is rarely reported. Interestingly, some reports suggest that ER β is a poor transcriptional factor (Cowley et al., 1997; Cowley and Parker, 1999; Pettersson et al., 1997; Yi et al., 2002).

6. Mitochondrial Localization of ERs

Evidence for the presence of both ERs in mitochondria in various mammalian tissues and cell lines has been obtained from various techniques including distribution and binding of radio labeled ligands, immunoblotting of mitochondrial preparations, immunocytochemistry, and mass spectrometry (Cammarata et al., 2004; Klinge, 2008; Psarra and Sekeris, 2008; Yager and Chen, 2007). These studies suggest that ER β is localized in mitochondria in a variety of tissues, including rabbit ovaries and uterus, human lens epithelial cells, spermatocytes, primary cerebral cortical and cerebral cortical and hippocampal neurons, primary cardiomyocytes, as well as HepG2, SaOS-2, and MCF-7 cell lines. While ER β is thought to be the predominant receptor in mitochondria, ER α has also been reported to be in mitochondria of a few tissues, *e.g.*, rabbit uterus (Monje and Boland, 2001) and in MCF-7 cells (Chen et al., 2009). More recently, high-throughput *in vivo* DNaseI footprinting of the mitochondrial genome of 143B cells at single-nucleotide resolution revealed EREs strongly suggesting the presence of ER(s) in the mitochondria (Mercer et al., 2011). Some cells are reported to have both receptors in mitochondria; in particular, Pedram et al. have described the presence of both ERs in the mitochondria of MCF-7 breast cancer cells (Pedram et al., 2006). By examining the primary amino acid sequence of ER β using the TargetP program (Emanuelsson et al., 2000), Chen and colleagues were the first to report the presence of a mitochondrial targeting peptide signal (mTP) in the internal amino acid sequence (a.a 220–270) of ER β (Chen et al., 2004a), whereas no such targeting signal was found in ER α . Their study also demonstrates the presence of both ERs within the mitochondria of MCF7 cells and enhanced ER α and ER β mitochondrial localization in MCF-7 cells after E2 treatment. In contrast, Studies conducted by Srinivas and co-workers on mitochondrial ER β role in non-small-cell lung cancer (NSCLC) cells have demonstrated a ligand-independent role of ER β in regulating apoptosis, establishing a novel function for ER β in the mitochondria (Zhang et al., 2010b). Although the localization of ERs to the mitochondria has been demonstrated by various techniques, the issue still remains unsettled (Klinge, 2008; Psarra and Sekeris, 2008). First, there are many challenges in studying the molecular mechanism of action of ER β due to the lack of immortalized cell lines expressing high levels of endogenous ER β . The literature is still unreliable regarding which cell lines expresses endogenous ER β mRNA and more significantly the protein. For example, some studies have reported MCF-7 breast cancer cells to be ER β -negative; however, several other studies

including own unpublished studies have shown that MCF-7 cells express endogenous ER β . Bulk of molecular studies on ER β have been conducted using cell lines engineered to express exogenous ER β , and fewer studies have used cell lines expressing the endogenous protein. Studies that rely on the endogenous ER β suffer from the deficiency of commercially available antibodies with high specificity against ER β . The currently available antibodies of ER β will detect many protein bands of different molecular weight and some of them tend to cross-react with ER α . An important question yet to be resolved is whether the difficulty in detecting endogenous ER β is due to poor quality of antibodies or it is caused by the highly labile nature of the protein and/or varying expression of its multiple isoforms.

Yang et al. have reported presence of ER β in human heart mitochondria based on MALDI-TOF-MS method to compare observed peptide masses generated by trypsin digestion of mitochondrial proteins with calculated peptide mass lists generated from protein sequences known from the genome sequencing projects (Yang et al., 2004). However, Gustafsson and coworkers contested this study based on the argument that MALDI-TOF-MS analysis performed on crude mitochondria was founded on comparison of peptide mass lists and the fact that amino acid fragments with different amino acid components can lead to the same peptide mass/charge ratios (Schwend and Gustafsson, 2006). Yang et al. disagreed pointing out that the negative results obtained by Gustafsson group could be attributed to the low concentration of ER β in the mouse liver preparations assayed (Yang et al., 2006). Intriguingly, a recent protein mass spectrometry study conducted to characterize mitochondrial proteins in 14 mouse tissues did not reveal either ER α or ER β in any tissue, with an estimated 85% of proteins identified and a false discovery rate of 10% (Pagliarini et al., 2008). Thus, the localization of ERs in the mitochondria remains controversial, and this issue should be explored and scrutinized in authentic pure mitochondria using variety of unbiased independent techniques.

Numerous findings on the roles of nuclear proteins such as ER and p53 as important regulators of communication between the mitochondrial and nuclear genome and proteome in different cells and tissues highlight the importance of these proteins in metabolism, cell survival, and cell death of normal and cancer cells. Importance of such research underscores the necessity of ascertaining the quality of isolated “pure” mitochondria used in various studies. Although a huge number of studies have corroborated the notion of ER translocation to the mitochondria, recognizing and highlighting the limitations of ascertaining the quality of mitochondria isolated by different protocols require careful consideration. Most of the methods are limited to isolating crude mitochondria fraction that does not permit an accurate analysis of the composition of ‘real’ mitochondria and its associated membranes, thus leading to misrepresentative inferences. In many studies, the descriptions of mitochondria “pure” or experiments performed “isolated mitochondria” are ambiguous. Investigators as well as reviewers need to carefully consider methods to obtain highly purified mitochondria and verify the isolated mitochondria are devoid of endoplasmic reticulum (ER), nuclear, and other cytosolic proteins. The quality and the purity of the preparation should be verified by techniques such as western blot analysis of different markers for nuclear, cytosolic, and mitochondrial compartments. We have successfully used the protocol developed by Wieckowski and colleagues to obtain highly purified mitochondria-associated membranes

(MAM) and mitochondria from tissues and cultured cells without contamination from other organelles (Wieckowski et al., 2009).

7. Role of ERs in Transcriptional Regulation of Nuclear Genes Encoding Mitochondrial Proteins

E2 is able to exercise direct and indirect effects on mitochondrial function in a variety of tissues. The ability of E2 to regulate both nuclear DNA- and mitochondrial DNA (mtDNA)-encoded genes in MCF-7 cells has been established (Gavrilova-Jordan and Price, 2007). However, whether E2 exerts a direct role in regulating mitochondrial function by facilitating binding of mtERs to mtDNA leading to changes in mitochondrial gene transcription is not understood. Klinge and coworkers have reported E2- and nuclear ER α -dependent transcriptional activation of respiratory factor-1 (NRF-1) resulting in mitochondrial biogenesis in MCF-7 human breast cancer cells (Mattingly et al., 2008). NRF-1 is a nuclear transcription factor that regulates transcription of mtDNA transcription factors and plays a critical role in integrating nuclear-mitochondrial communications by activating transcription of nuclear-encoded mtDNA-specific transcription factors including TFAM, which is reported to interact with p53 (discussed in section 3.4). As observed by Klinge and coworkers, E2 treatment of MCF-7 cells increased the nuclear-encoded TFAM transcription and two mitochondrial-encoded mRNAs regulated by TFAM (Complex IV, Cytochrome *c* oxidase subunit I; and NADH dehydrogenase subunit 1) thereby inducing mitochondrial biogenesis, and oxidative phosphorylation. This was inhibited by ER α antagonist fulvestrant (ICI 182,780) indicating ER α dependence. Conversely, siRNA knockdown of NRF-1 inhibited the E2-induced increase in mtDNA and thus mitochondrial biogenesis, indicating that the E2-induced increase in mitochondrial biogenesis is mediated by NRF-1. Of note, binding of recombinant ER β to mitochondrial DNA of MCF-7 cells has also been documented by electrophoretic mobility shift (EMSA) analysis (Chen et al., 2004a). These authors also reported that E2 treatment contributed to significant increase in oxygen consumption in MCF-7 cells, but not in MDA-MB-231 ER α -negative breast cancer cells. Taken together, a definitive proof of ER in the mitochondria will provide a potential mechanism to rationalize ability of E2 to synchronize the expression of mtDNA and nuclear-encoded mitochondrial protein genes. If ER is present in the mitochondria, its capability to send signals from mitochondria to the nucleus remains unknown. A clue to synchronized regulation of expression of both mitochondrial and nuclear encoded genes by E2 came from studies on changes in the proteome of mitochondria isolated from the brains of ovariectomized female rats treated for 24 h with E2 followed by 2d-gel analysis and liquid chromatography-tandem mass spectrometry protein identification. The mitoproteome displayed an increased expression of many proteins that couple glucose utilization to the TCA cycle as well as electron transport chain (ETC) proteins requiring communication between mitochondrial- and nuclear encoded gene transcription. This ability of E2 to shift the mitochondrial proteome to a profile consistent with a glycolytic driven TCA cycle strongly suggests the presence of ER in both the mitochondria and nucleus (Nilsen et al., 2007). Thus, localization of ER α and ER β in both nuclear and mitochondrial compartments will provide a feasible mechanism for the coordination of nuclear and mitochondrial gene expression and function.

8. Regulation of Metabolism by ERs

The genomic activity of E2 is mediated by ER α and ER β . In breast cancers, a main response to estrogen is an upturn in cellular proliferation. Some studies have suggested that there may be a response mediated by nonnuclear ER located in the cell membrane or cytosol.

Supporting this theory, using breast cancer cell lines, Ko and colleagues have presented data suggesting an increase in the uptake of the ^{18}F FDG after estrogen stimulation is mediated by nonnuclear ER (Ko et al., 2010). An estrogen that has access to the cell membrane, but not the nucleus, was able to mimic similar effects to those of E2 on ^{18}F -FDG uptake.

Membrane-initiated E₂ action via PI3K-Akt pathway stimulated hexokinase activity and glycolysis resulting in increased ^{18}F -FDG uptake. Interestingly, the increase in ^{18}F -FDG was independent of change in glucose transporter type 1 (GLUT1) expression.

It is becoming increasingly apparent that E2 is involved in various mechanisms that regulate cellular physiology and disease. The generation of ER α /ER β dual knockout mice has been an invaluable tool in analyzing roles of each receptor function in cellular metabolism.

Glucose transporter 4 (GLUT4) once docked on the plasma membrane, permits the transport of glucose into the cell. Upon binding to its receptors, insulin activates a phosphorylation cascade leading to the translocation of GLUT4 from cytoplasmic vesicles to the cell membrane enabling GLUT4 to act as a rate-limiting step in the insulin-induced glucose uptake in skeletal muscles (Bjornholm and Zierath, 2005). ER α -/- mice are glucose-intolerant and insulin-resistant (Heine et al., 2000). In Aromatase-deficient mice (ArKO) data suggest a suppressive role of ER β on GLUT4 expression while tamoxifen, the ER α antagonist, had no effect on glucose tolerance or insulin sensitivity in muscle in wt or ER β -/- mice, but in ER α -/- mice, tamoxifen increased GLUT4 expression and improved insulin sensitivity (Barros et al., 2006). These results indicated that ER α modulates GLUT4 translocation to the cell membrane and glucose uptake whereas ER β is a repressor of GLUT4 expression and both receptors have distinct actions in glucose uptake in skeletal muscle.

Stirone et al. were the first to demonstrate that treatment of cerebral arteries with physiologically relevant levels of estrogen *in vivo* greatly affects mitochondrial function, increasing the capacity for oxidative phosphorylation while consecutively decreasing production of reactive oxygen species (ROS) (Stirone et al., 2005). The authors demonstrated localization of ER α and subunit I of complex IV (i.e., a mitochondrial marker) in the cerebral vascular smooth muscle layer. This study provided another possible mechanism underlying the vasoprotective effects of estrogen and furthermore suggests that estrogen may serve as a coordinator of gene expression between mitochondrial and nuclear genomes. Taken together, the effect of estrogen in protecting cerebrovascular mitochondrial function has the potential to significantly affect the incidence and sequence of a number of diseases, including Alzheimer's, vascular dementia, and stroke. Similarly, in MCF-7 breast cancer cells, exposure to estrogen increases binding of ER α and ER β to mitochondrial DNA and also causes a significant increase in transcript levels of the mitochondrial genes that encode cytochrome c oxidase subunits I and II. (Chen et al., 2004b). A schematic representation of ER signaling in glucose metabolism and mitochondrial respiration is shown in Figure 2.

It is clear from studies detailed above that estrogen affects mitochondrial function by modifying the expression of both nuclear- and mitochondrial-encoded proteins. Furthermore, it has been reported that tamoxifen and E2 act on the flavin mononucleotide site of complex I of isolated liver mitochondria leading to mitochondrial failure independent of estrogen receptors (Moreira et al., 2006). In this way, estrogen has the potential to coordinate interactions between nuclear and mitochondrial genetic compartments. However it is still not clear if estrogen is associated with coordination of mitochondrial and nuclear gene expression to regulate cellular oxidative capacity.

9. p53 and ER Cross Talk in Cancer Cell Nucleus and Mitochondria

Most of the information about ER-p53 crosstalk in cancers comes from studies in breast cancer. In comparison to other cancers, overall frequency of p53 mutation in breast cancer is about 20%; however, wild type p53 is functionally incapacitated. A novel mechanism by which ER α , generally up-regulated in luminal breast cancer, suppresses p53 function was discovered in our laboratory: interaction between nuclear ER α and p53 results in the inhibition of p53 function in breast cancer cells and xenograft tumors and consistent with this finding, clinical studies by us and others showed that ER α -positive patients expressing wildtype p53 were more responsive to tamoxifen therapy (Bergh et al., 1995; Berns et al., 2000; Konduri et al., 2010; Liu et al., 2009; Liu et al., 2006; Miller et al., 2005; Sayeed et al., 2007; Yamashita et al., 2006). Vast majority of investigations done on tumor development have focused on events in understanding the molecular and genetic hallmarks of the disease (Hanahan and Weinberg, 2011). There is increasing evidence for the important role of mitochondria, bioenergetics, and metabolism in pathophysiology of various diseases including cancer (Wallace, 2013). Several studies have shown that tumor cells use glycolysis even when oxygen is accessible ("Warburg effect") (Moreno-Sanchez et al., 2007; Warburg, 1956). Metabolic underpinnings of such molecular alterations have been largely ignored until recently. Several lines of evidence have shown that the preservation of wild type p53 is linked with the maintenance of oxidative phosphorylation. Loss of p53 function in cells is associated with significant deficiency in mitochondrial biogenesis and decrease in oxygen consumption. Furthermore, p53 can avert metabolic transformation by restraining the glycolytic pathway (Bensaad and Vousden, 2007; Cheung and Vousden, 2010; Galluzzi et al., 2008; Matoba et al., 2006; Vousden, 2009; Wang et al., 2012). Similar to p53, ER is reported to play a functional role in the mitochondria in the regulation of mitochondrial genes that encodes respiratory chain proteins (Chen et al., 2009; Leigh-Brown et al., 2010). As discussed earlier, ERs influence the activity of metabolic gene network by regulating the expression of transcription factors implicated in metabolic control. ERs are required in cells to sustain mitochondrial oxidative respiration through the regulation of genes that control the TCA cycle. In contrast, breast cancer cells that are positive for ER expression display defects in mitochondrial oxidative phosphorylation (Owens et al., 2011) and show increase in glucose uptake (Ko et al., 2010). While accumulating results clearly indicate ER participation in the regulation of mitochondrial oxidative phosphorylation, the correlation between ER and expression of enzymes that regulate the glycolysis pathway in breast cancer cells is lacking. Also, whether ER α and ER β have contradictory roles in the establishment of a Warburg-like phenotype in breast cancer cells remains unknown.

Numerous clinical studies and transgenic mouse investigations have now shown a correlation between E2 and several aspects of the metabolic syndrome (MS) pointing to a role of E2 in glucose homeostasis and obesity (Mauvais-Jarvis et al., 2013). It is clear that both ER α and ER β participate in numerous mechanisms and intricate regulation of organs such as brain, skeletal muscle, adipose tissue, pancreas, liver, and heart and has fundamental roles in metabolism (Barros and Gustafsson, 2011). It remains to be determined whether the ER-p53 crosstalk observed in breast cancer is also relevant in other cancers such as ovarian, endometrial, cervical, prostate, colon, and lung cancers. As witnessed in tissues with high metabolic rate, ERs could sustain the oxidative metabolic pathway in normal cells while on the other hand promoting a glycolytic profile required for the proliferation of rapidly dividing cancer cells. Figure 3 depicts our hypothetical model of ER's ability to negatively affect mitochondrial p53 function that leads to metabolic reprogramming in a tumor cell. On the basis of knowledge gained about the roles of both ERs, the intricacy of metabolic reprogramming by ERs may depend on the specificity of action of each ER subtype resulting from their relative level of expression in different cells and tissues.

10. p53 and ER in Aging

p53 plays an important role in cellular senescence and aging. Senescence, an irreversible cell cycle arrest, is important in aging and counteracts oncogenic insults, and therefore contributes to tumor suppressor function of p53. The emerging model on the role of p53 in aging is that normal physiological p53 activity prevents cancer and aging, whereas uncontrolled excessive activation of p53 is still tumor suppressive but is detrimental to healthy aging (Rufini et al., 2013). DePinho group supports a model for ageing by linking telomeres, p53 and mitochondria, highlighting the roles of mitochondria on lifespan. They propose that telomere dysfunction-induced p53 activation leads to mitochondrial deregulation through the suppression of the master regulators of mitochondrial biogenesis and function, PGC1 α and PGC1 β . The repression of both these proteins impair overall mitochondrial biogenesis and function (Sahin and DePinho, 2012). Age-related changes in estrogen production and estrogen receptor expression based on cell or tissue types form the basis for the role of estrogen receptor signaling in aging and age-related diseases such as osteoporosis, cardiovascular disease, Alzheimer's disease, and Parkinson disease (Cui et al., 2013). It is intriguing to note that p53 has also been implicated in these diseases in a way that is opposite to that of ER. Although abnormal mitochondrial function has been observed in many of these diseases, direct crosstalk between nuclear and/or mitochondrial ER and p53 in these diseases has not been reported. Future research should provide insights into this important issue that would have tremendous therapeutic implications.

11. Conclusion and Perspectives

ERs and p53 are important players in normal and pathological physiology. Delicate balance between their pro-proliferative and anti-proliferative functions is necessary for normal development and functioning of mammals including humans. Interaction between ER α and p53 and its consequences have been demonstrated (D'Assoro et al., 2008; Diaz-Cruz and Furth; Duong et al., 2007; Katayama and Sen, 2011; Konduri et al., 2010; Liu et al., 2000; Liu et al., 2009; Liu et al., 2006; Sayeed et al., 2007). Moreover, there is a feedback loop

between transcription of ER α and p53 genes (Angeloni et al., 2004; Berger et al., 2012; Duong et al., 2007; Fuchs-Young et al., 2011; Shirley et al., 2009). These observations suggest necessity for coordinating expression levels and activities of these proteins. Such mechanisms to balance the functions of ER α and p53 appear to have gone awry in diseases such as cancer. Although non-nuclear, non-transcriptional roles of p53 in the mitochondrial compartment have been well documented, all the studies on ER α -p53 crosstalk have been confined to their nuclear roles. Therefore, the importance of analyzing whether such crosstalk occurs in the mitochondria, and if so, understanding its cellular consequences cannot be overstated. While several studies have demonstrated crucial roles of p53 in maintaining oxidative phosphorylation and the inhibition of aerobic glycolysis, whether ERs may promote tumor growth by shifting the metabolism from oxidative phosphorylation to aerobic glycolysis by antagonizing p53 activity remains unknown. Does ER α -p53 interaction in the nucleus impact directly or indirectly interaction and/or function of mitochondrial ER α and p53? Does ER α -p53 interaction play any role in subcellular localization of these proteins? Do they antagonize or cooperate in a context-dependent manner? Does nuclear and mitochondrial ER β bind to p53, and if so, how does it impact each other's function? Understanding the role of ERs in regulating mitochondrial p53 is essential in deciphering how these proteins influence each other's function and coordinate their nuclear and mitochondrial roles both in normal and disease states. That the knowledge gained from such studies in this fertile research area could be exploited for preventing and treating ailments including metabolic syndrome, cancer, and neurodegenerative diseases is an exciting future possibility.

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Highlights

- Estrogen receptors and tumor suppressor protein p53 have important roles in the nucleus and mitochondrion
- p53 regulates cell cycle, apoptosis, and cellular metabolism
- Nuclear estrogen receptor-alpha (ER α) binds and antagonize p53; ER α functions as suppressor of a tumor suppressor
- ER-p53 interaction is functionally important
- Whether ER-p53 interaction occurs in mitochondria is being intensively investigated
- Deciphering ER-p53 crosstalk in coordinating nuclear and mitochondrial functions could have important implications in understanding, preventing and treating various ailments such as cancer, obesity, aging, osteoporosis, Alzheimer's disease, and Parkinson's disease.

Fig: 1. Schematic representation of p53-mediated functions in glucose metabolism, mitochondrial respiration, mitochondrial homeostasis and cell death

p53 is able to transcriptionally repress glucose transporters GLUT1 and GLUT4 along with the insulin receptor (IR) to inhibit cellular glucose uptake into the cell. Through transcriptional activation of TP53-induced glycolysis and apoptosis regulator (TIGAR), p53 decreases the rate of glycolysis and redirect glycolytic intermediates into the pentose phosphate pathway (PPP). Glycolysis is also dampened by negative regulation of phosphoglycerate mutase (PGM) by p53. In contrast, transcriptional activation of hexokinase II (HK II) by mutant p53 stimulates glycolysis. The mitochondrion is the site of ATP generation via the tricarboxylic acid (TCA) cycle and the electron-transport chain (ETC). p53 promotes oxidative phosphorylation (OXPHOS) through transcriptional activation of synthesis of cytochrome c oxidase 2 (SCO2), a regulator of complex IV and apoptosis-inducing factor (AIF) that acts directly on complex I. By regulating transcription and stability of ribonucleotide reductase subunit (p53R2), p53 maintains mitochondrial homeostasis and mitochondrial genome integrity. p53 is able to transcriptionally regulate and interact with the nuclear encoded mitochondrial transcription factor A (TFAM) and play a role in mitochondrial DNA (mtDNA) transcription and in regulating mtDNA content.

Genotoxic stress signals trigger cytoplasmic p53 to undergo MDM2-dependent mono-ubiquitination that induces translocation of p53 to the mitochondria. P53 is then deubiquitinated by herpesvirus-associated ubiquitin-specific protease (HAUSP), which generates a mitochondrially-restricted, apoptotically capable pool of p53 protein. Mitochondrial p53 has been shown to interact and inhibit anti-apoptotic members of the Bcl-2 protein family and can directly trigger apoptosis by interacting with Bax and Bcl-xL. Combined role of transcriptional and mitochondrial functions of p53 in regulating apoptosis is evident in involvement of Bcl-x_L, PUMA, and NOXA. Mitochondrial p53 has been demonstrated to block the antioxidant function of manganese superoxide dismutase (MnSOD). Role of p53 in regulating metabolic pathways is an emerging rapidly moving area of research, and the influence of p53 on metabolism is likely to be much more complex than illustrated here.

Fig 2. Schematic representation of ER signaling in glucose metabolism and mitochondrial respiration

ER α activated by E2 controls gene expression in the nucleus by binding to EREs in target gene promoters followed by recruitment of coactivator complexes (COAC1, COAC2, COAC3) leading to transcriptional activation. Genes activated by ER α include those that encode proteins involved in mitochondrial biogenesis such as nuclear respiratory factors (NRF1) and still unknown metabolic proteins and enzymes that may participate in various pathways (as indicated by black broken arrows) regulating glycolysis and oxidative phosphorylation (OXPHOS). The mitochondrial localized transcription machinery (mt-TM) and TFAM are encoded in the nucleus and their expression is controlled by ER α and NRF-1. E2 dependent increased transcription and protein expression of NRF-1 induces mitochondrial biogenesis. Both ER α and ER β are reported to modulate insulin signaling and glucose uptake. When Insulin signaling is activated, numerous proteins are phosphorylated leading to the translocation of GLUT4-containing vesicles to the cell membrane, where GLUT4 facilitates the glucose uptake into the cell. ER α modulates GLUT4 transcription and translocation to the cell membrane and glucose uptake, while ER β is a repressor of GLUT4 expression. The presence of both ERs in mitochondria in many mammalian tissues and cell

lines has been reported, but remains controversial. The question mark indicates an experimentally unresolved observation.

Fig 3. Hypothetical model for ER-mediated antagonism of p53 function in a tumor cell

In tumor cells ERs participate in activating insulin signaling that leads to the translocation of GLUT4-containing vesicles to the cell membrane where GLUT4 enables the entry of glucose to increase the rate of aerobic glycolysis (“Warburg effect”). Tumor cells largely convert most of the glucose to lactate via less efficient aerobic glycolysis that results in minimal ATP production. In breast cancer cells, ER has been reported to bind and inhibit wild type p53 function in the nucleus. In a tumor cell, ERs may promote establishing a prominent glycolytic profile by repressing p53-mediated enhancement of mitochondrial oxidative phosphorylation and transcription of antiproliferative gene networks. Likewise, ERs may repress genes regulated by p53 that are vital to mitochondrial hemostasis and genome integrity. Accumulating mtDNA damage will ultimately lead to the impairment of oxidative phosphorylation (OXPHOS). Impaired OXPHOS is reported to cause further genomic and mtDNA instability, decreased apoptosis, and a cellular environment indicative of the Warburg effect. Overall this model suggests that in a cancer cell, ER is able to negatively affect mitochondrial p53 function, leading to altered metabolic profile and tumorigenesis.