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The Role of JMY in p53 Regulation.

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Abstract

Following an event damaging the DNA, p53 levels increases inducing cell cycle arrest or apoptosis. JMY protein is a transcription co-factor involved in p53 regulation. After a DNA damage, also JMY levels increase and, as this protein accumulates in the nucleus, it forms a complex with P300 and Strap1 which increases the ability of p53 to induce transcription of proteins triggering apoptosis but not cell cycle. Therefore, Increase levels of JMY "direct" p53 activity toward triggering apoptosis. JMY expression is also linked to increased motility as it downregulates the expression of adhesion molecules of the Cadherin family and induces actin nucleation, making the cell less adhesive and more mobile. According to the scenario this gene can therefore have both a suppressive or a tumour promoting activity.

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Introduction

P53 was identified in SV40 transformed cells where it was associated with Large T Antigen. With time, it was discovered that TP53 was frequently mutated in many human tumours [1,2]. P53 turned out to be a prominent transcription factor whose function is essential in preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress (**Figure 1**) [2] [3]. In response to cellular stress such as DNA damage, hypoxia oncogene overexpression, viral infection, the p53 protein level is augmented. Following post translational modifications which include phosphorylation, acetylation, ubiquitination and methylation, p53 is able to activate multiple promoter elements of target genes which would affect cellular processes such cell cycle arrest, senescence and apoptosis [2,4].

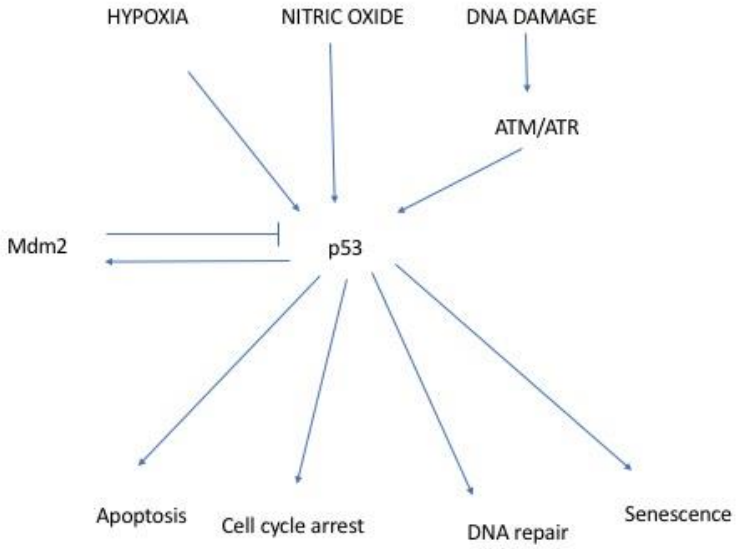

Figure 1 Basic functions of p53

P53 regulation of apoptosis (programed cell death) it is at the heart of neoplastic proliferative control. In mammalian cells, apoptosis can be either P53 transcription dependent or P53 transcription independent. The P53 transcriptional dependent mechanism is set in motion by cellular stress like DNA damage which triggers p53 post translational modification of phosphorylation and acetylation that activates and stabilizes/ enables it to bind and activate pro-apoptotic genes. It is highly speculated that p53 selectively activates transcription of pro-

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apoptotic target genes upon interaction with transcriptional co-activators such as p300/CREB-binding protein (p300/CBP), Junctional and regulatory protein(Jmy), Stress responsive activator of p53 (Strap) and apoptosis-stimulating protein of p53 (ASPP) [2,5].

Studies that have shown that inhibition of mRNA and protein synthesis that could inhibit transcription of p53 target genes did not deter p53 dependent apoptosis. A plausible explanation of this phenomena is the existence of an alternative p53 dependent apoptosis that is transcriptionally independent. According to Bossi et al, there are corroborating studies that have shown that upon DNA damage, p53 can localize to the mitochondria where it triggers a rapid apoptotic response that occurs even before p53 target genes are activated. This is attained by the p53 DNA binding domain directly binding pro-apoptotic proteins BCL-XL and BCL2 within the mitochondria which facilitates the release of BH3 protein that induces mitochondrial permeabilization and apoptosis [6].
P53 activity is tightly controlled and regulated at a multiplicity of levels and the importance of co-factors that influence p53 activity is becoming increasingly evident (**Figure 1**) [5]. Mdm2 for instance is a major negative regulator of p53 and is capable of doing this by direct p53 ubiquitination targeting it for degradation or indirect p53 regulation via p53 cofactor ubiquitination for degradation [5,7]. Additionally, Mdm2 through its E3 ubiquitin ligase activity promotes the proteasome-dependent p53 degradation and modulates nuclear export of p53[2] .

CBP and p300 are a family of acetyltransferase which act as transcriptional activators for several transcription factors including p53[8]. Following DNA damage, p53 is phosphorylated at Serin15 and, as a result, its stability and ability to link p300 increases. Upon ligation with p300, two sites on the C terminal region of p53 are acetylated. It has been proposed that following acetylation, p53 stability and recruitment of its targets are further increased leading to a preponderant transcription of p53 target proteins [8].

The Junctional Mediating and regulator Y protein (Jmy) was originally identified as a CBP/p300 co-factor regulating p53 response [9]. Upon DNA damage, it was observed that JMY interacts and forms a complex with p300 and Strap recruiting PRMT5 into a co-activator complex that drives the p53 response [10]. According to Coutts et.al Jmy functional role in p53 response is evident in Jmy associated increase of p53-dependent transcription and apoptosis. A transcriptional co-factor role of Jmy is observed as in its presence the transcription of factors downstream to p53 increases although the amount of p53 protein is constant [8]. Studies have

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also shown Jmy to be an MdM2 target for ubiquitination and degradation, the same mechanism trough which MDM2 regulates p53 activity. This is consistent with the fact that upon DNA damage, Jmy is released from MDM2, allowing it to contribute to the p53 response [8].

Jmy localizes both in nucleus and cytoplasm [11], but following stress or DNA damage Jmy start to migrate from the cytoplasm and to accumulate in the nucleus [10]. Metastasis are facilitated by increased cell motility, allowing tumors to invade and colonize surrounding as well as distant tissues: in addition to its effect on p53, Jmy has also another very interesting effect: enhancing of cell motility [10]. Jmy contains a series of WH2 domains that promote actin nucleation or elongation enabling it to promote cell motility [10]. Other studies have shown that Jmy nucleates actin in vitro and induces actin filament formation in vivo as a consequence of its inherent WH2 domain series [10,12]. Via its WH2 domain, Jmy is also able to down-regulate E-cadherin, an adherent junction protein required for cell-cell adhesion that is known to be lost during the course of tumor progression [13,14]. This loss of E-cadherin also favors cell motility, metastasis and invasion.

This in effect supports the hypothesis, generated by micro array study showing an inverse relationship between Jmy and Trap1[15]: hypoxia leads to slower proliferation with decreased Trap1 and increased Jmy, which in turn promotes cell motility facilitating escape from the hypoxic environment.

Interestingly Coutts et al have demonstrated that when Jmy shuttles into the nucleus, where enhances P53 transcription activity, Jmy contribution to cell motility diminishes [10].This hence implies that Jmy is playing a dual role. It is then important to reconcile the role of Jmy in the two different cellular processes of transcriptional/actin dynamic regulation and tumorigenesis.

Identification of Jmy

The Junctional Mediating and regulator Y protein (Jmy) was originally identified by Shikama et al [9] while using the two-hybrid method to screen for proteins which are participating in the p300/CPB proteins complex and are involved in the regulation of p53 transcription [9]. Jmy is a protein of 110kDa whose gene is located on chromosome 5 at the 5q 13.2 band. Upon DNA damage, it is observed that Jmy forms a complex with Strap and p300 that can also recruit PRMT5 into a co-activator complex driving the p53 response [10]. Zuchero et al have

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demonstrated in HL60 cells that Jmy primarily localizes in the nucleus but can move between the nucleus and cytoplasm [12].

JMY regulation by E2F1

The E2F protein family of transcription factors can lead to either cell proliferation or apoptosis hence suggesting a dichotomy in their function [16]. Jmy protein is ubiquitously expressed in both nuclei and cytoplasm of most human tissues [11]. Levels of Jmy protein increases in cells treated "in vitro" with DNA damaging compounds like ultraviolet light`, etoposide and actinomycin D [5], as Jmy transcription and JMY protein accumulation are induced by the transcription of E2F1 which follows the DNA damage[17]. E2F1 is also the most potent apoptotic inducer of the E2F family [18]. Following DNA damage (**Figure 2**), the E2F1 is phosphorylated but its function will change according to the phosphorylated residue: while pE2F1 Serin364 is linked to the interaction with the Retinoblastoma protein, pE2F1 Serine 31 remain free. Both the Rb-E2F1 complex and the free E2F1 leads to apoptosis [16]. Active E2F1 introduced into the human cell line U2OS induces transcription of Jmy and of three others pro-apoptotic p53 co-factors: Aspp1, Aspp2 and TP53Inp1, as a consequence the levels of the corresponding proteins are also increased[17]. Inhibition of protein synthesis does not prevent the increased transcriptions of these factor in presence of E2F1 suggesting that these genes are directly targeted by E2F1. While Aspp1, Aspp2 and TP53Inp1 have putative E2F1 binding sites, no such as consensus region has been found on JMY, although this does not exclude the possibility of interaction between E2F1 and Jmy [17].

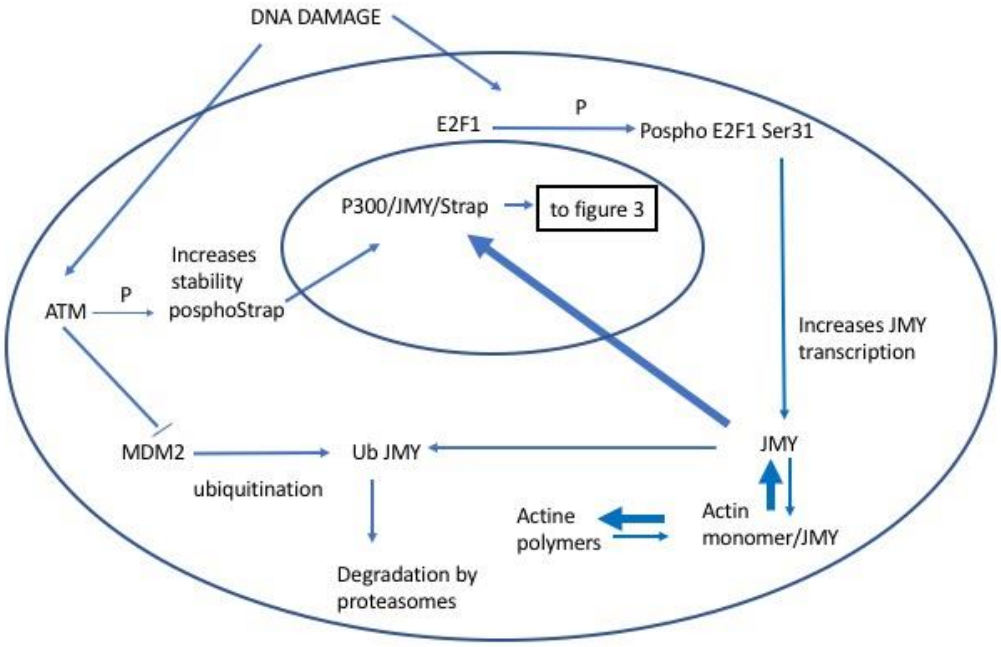

Figure 2 Jmy regulation

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Jmy regulation by Strap

Strap is another co-factor involved in the p53 regulation through its interaction with both p300/ Jmy complex and Mdm2. Strap contains six TPR (tetratricopeptide repeat) motifs, having several of these protein binding regions, one Strap protein can link to different substrates, forming complexes [8,19,20]. Following DNA damage, Atm phosphorylates Strap on Serine 203 (**Figure 2**). Phosphorylated Strap has an increased stability and accumulate in the nucleus. Here it links to the p300/ Jmy complex, as shown by co-immunoprecipitation of Strap with Jmy and with p300, further increasing its activity and increasing the p53 dependent transcriptional activity by improving the assembly of Jmy and p300, while its second function is to inhibits Mdm2 activity [19,20]. Jmy -binding and p300-binding regions have than identified on Strap and Strap- binding regions have been identified on both p300 and Jmy [19]. Using a two-hybrid assay and challenging free p300 and JMY with added Strap, the authors demonstrated that, eventually, the presence of Strap increases the number of p300/ Jmy complexes. Ultimately, through the stabilization of the p300/ Jmy complex lead to increased p53 activity by acetylating it (**Figure 3**)[8,19,20].

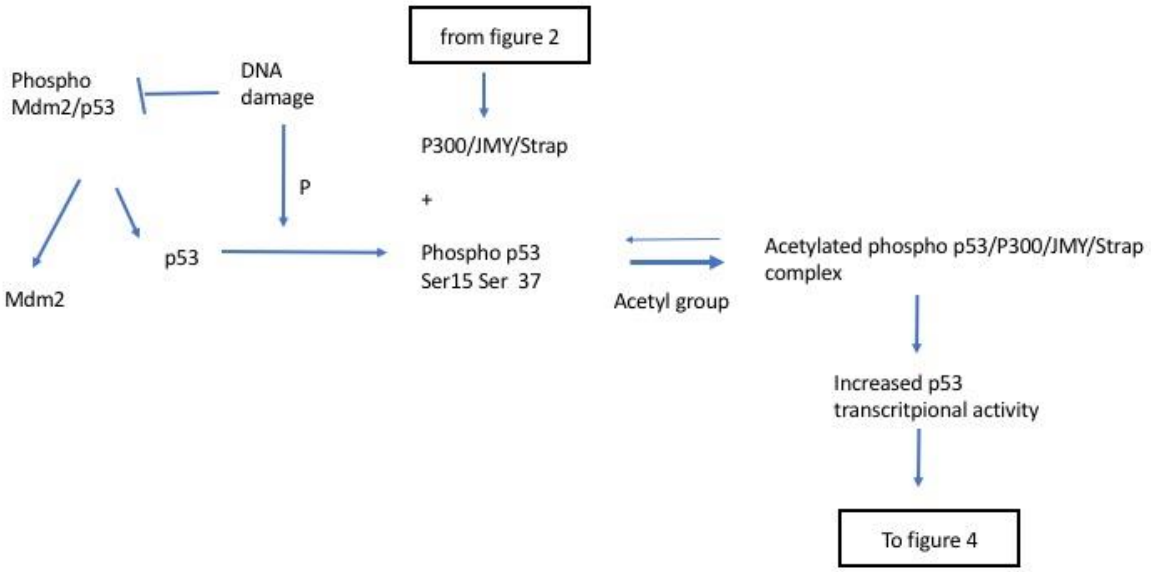

Figure 3. Jmy **effect on p53**

JMY regulation by MDM2

MDM2 ubiquitinates p53 causing its degradation, however when it is phosphorylated p53 interact less with MDM2, escaping degradation (**Figure 3**) [5] [8]. Levels of Jmy protein are also subject to regulation by MDM2 in stress-free cells: when treated with inhibitors of Mdm2 ligase activity, levels of Jmy protein increases in stress-free mouse embryonic fibroblasts. This finding

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suggests that, in absence of stress, production of Jmy protein is balanced by protein degradation mediated by Mdm2 and therefore the levels of Jmy protein are steady. Mdm2 links to, and co-precipitates with, Jmy protein: Mdm2 E3 ligase causes ubiquitination of its targets and it was found that, accordingly, co-expression of Mdm2 and Jmy induces polyubiquitination of the latter (**Figure 2**) while the proteasome inhibitor MG132 reduces Jmy degradation by Mdm2 [5]. Following induction of DNA double strand break by ultraviolet or by actinomycin-D, Jmy levels increases, not only because of increased transcription, but also because of diminished degradation by Mdm2 as the number of Mdm2/ Jmy complexes diminishes. The authors propose that Mdm2 ubiquitinates Jmy causing its degradation [5] [7]. Following reduced interaction with Mdm2, Jmy, Strap and p300 induces acetylation of p53, protecting it from degradation by Mdm2 [7]

JMY increases p53 dependent transcription leading to selective increase of Apoptosis.

Higher levels of p300/JMY increases p53 transcriptional activity leading to increased apoptosis but not cell cycle arrest [9] and, as discussed above, their activity is further increased in presence of Strap. Two p53 downstream genes were investigated: Bax which leads to apoptosis and p21 which induces cell cycle arrest. Levels of Bax were increased in presence of p300/ Jmy while the effects on the levels of p21 and Mdm2 were very modest (**Figure 4**)[9]. Immunoprecipitation experiments showed that both proteins co-precipitate with p53 and immunoprecipitated together, suggesting that a ternary complex is formed. When either truncated Jmy or p300 proteins are used, the effect of the complex is absent, further supporting the hypothesis of a large complex. The ability to increase p53 transcriptional activity is conditional to the integrity of p53 N-terminus activation domain [9]. The levels of Jmy and p300 are not affected by each other nor by p53. Furthermore, p300 and Jmy do not affect p53 levels, supporting the hypothesis that these protein increases the activity but not the number of p53 proteins present. P53 induces apoptosis and presence of Jmy increases the amount of apoptosis further. P300 by its own fails to increase p53 apoptotic activity but enhances it if also Jmy is present.

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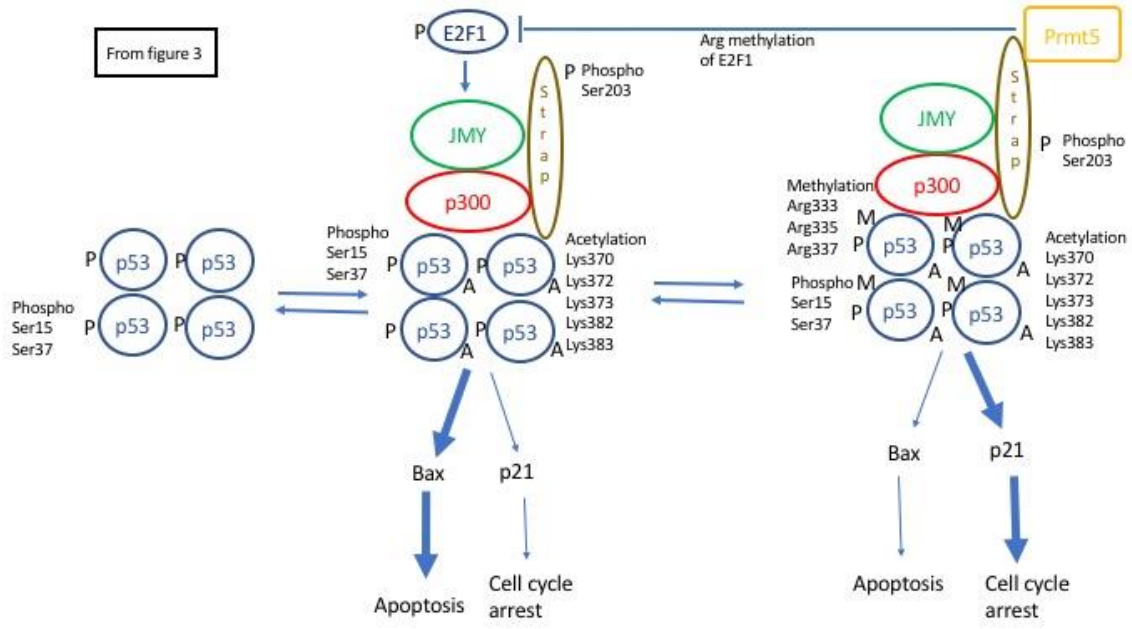

Figure 4. Formation of molecular complexes and their effects on apoptosis and cell cycle

Isoforms of Jmy lacking the C-terminus proline reach domain are less efficient in inducing p53 transcription of pro apoptotic proteins but display instead ability to increase the p53 efficiency to arrest cell-cycle [9] [8].

P53 activation can leads to both cell cycle arrest and apoptosis, or just one of the two: however how in some cases p53 activate just one of the two option has started to be unveiled recently has it has been discovered that p53 activation by Jmy, and the others "proapototic factors" increases the transcriptional activity of p53 targeting pro apoptotic proteins but not proteins blocking the cell cycle. Therefore, the first important action of Jmy is to increase p53 pro apoptotic activity only [17]. When Prmt5 is present it links to the Jmy /p300/Strap complex inducing instead increased transcription of p21 and cell cycle arrest rather than apoptosis (**Figure 4**) [21,22].

JMY and cell motility

The second main role of Jmy is to regulate motility by affecting actin nucleation and cell adhesion [10]. Actin filaments provide the structural basis for cell motility and are critical to numerous physiological processes such as morphogenesis, wound healing, migration, membrane

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transport and metastasis [23]. Actin filament formation occurs either via branching of already existing actin filament or alternatively via de-novo nucleation of actin monomers [23].

Spontaneous assembly and nucleation of actin trimers and dimers are kinetically unfavorable so to counteract this obstacle cells use actin nucleators and nucleation-promoting factors (NPF) to jump start actin nucleation and filament formation. Actin formation via branching is usually facilitated by the actin nucleator "Actin related protein 2/3 complex" (Arp2/3). Arp2/3 is activated by NPFs via their 'Wiskot-Aldrich Syndrome protein (WASp) homology-2'domain (also known as WH2 domain), which are actin binding motifs that enable assembly of actin monomers [24]. This de-novo nucleation is produced by nucleators such as 'Spire' that themselves contain WH2 domains and do not require activation by NPFs (Nucleation-Promoting Factors) [24] [12].

With the discovery of Jmy protein sequence homology to actin regulators and nucleators, came the knowledge of its involvement with actin [12]. Jmy capacity to regulate actin dynamics lies with it possession of WH2 domains that can either independently initiate actin filament formation in SPIRE-like fashion or activate actin nucleators such as Arp2/3 [24]. Experiments with myeloid lineage HL60 have shown that Jmy is mostly nuclear in these cells but when differentiation into neutrophil is induced some Jmy protein move to the cell edge where it co-localizes with actin. Jmy over expression was found to be associated with increased speed of migrating cells [12]. Additionally, Jmy expression and cytoplasmic co-localization with actin is increased under HIF1 stimulation[25]. Of note is that actin filament facilitates cell motility and migration. Hence Jmy association implies Jmy significant role in cell motility.

Zuchero et al. [12] and Firat-Karalar et al. [26] demonstrated that purified JMY biochemically activates Arp2/3 (**Figure 5**) induced actin polymerization in a dose dependent fashion. Jmy does not induce elongation of preformed filaments but is able to nucleate new filaments, make them to elongate faster and cut filaments to make barbed ends. The authors also demonstrated that Jmy possess WH2 domains and was able to catalyze new filament formation in a Spire-like fashion, always in a dose – dependent fashion, even in the absence of Arp2/3 activity [12] [23]. Jmy nucleation activity in presence of Arp2/3 complex occurs in the cytoplasm and lead to production of branched filaments. Once inside the nucleus, instead JMY acts with a mechanism similar to that used by the actin nucleation factor Spire and produces unbranched filaments in an Arp2/3 independent fashion.

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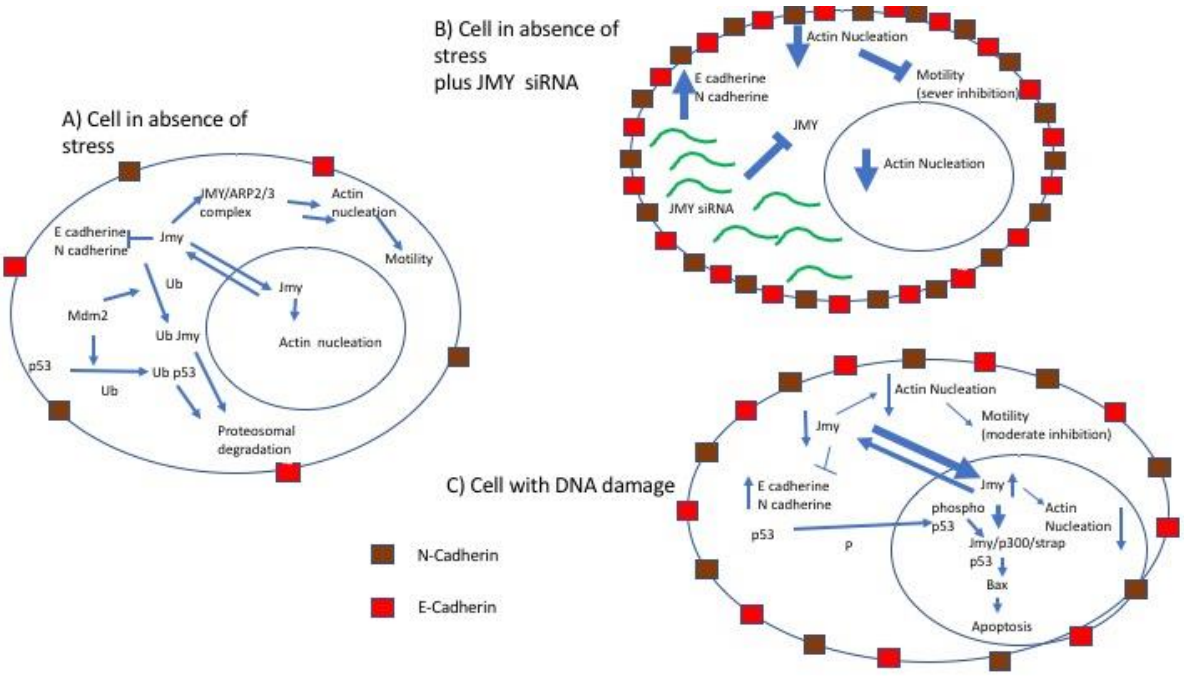

Figure 5. Model of Jmy **-mediated co-ordination between cell motility and DNA damage response in cell lines**

This implies that Jmy is capable of producing both forms of biochemical actin filament formations**.** Having both forms of biochemical actin polymerization would also imply that Jmy contribution to cell motility is via its nucleation filament and that JMY can promote rapid assembly of a new actin network by harnessing its ability to first nucleate new mother filaments and then activate Arp2/3 to branch off these filaments. This duality of JMY localization and functions between the cytoplasm and nucleus might be a gained evolutionary advantage of Jmy [12] [23].

We previously discussed how Jmy accumulate in the nucleus in response to DNA damage. However, another mechanism regulating the subcellular localization has been unveiled, this time in response to actin monomers which are able to link with theWH2 Jmy domains blocking its transfer to the nucleus [27]. This is supported by the effect observed on JMY intracellular localization after treatment with Jasplakinolide, a compound which induces polymerization of actin [28]. As all the monomers are recruited to form polymers, no free monomers as available to ling to Jmy are left and therefore Jmy is no longer trapped into the cytoplasm and can move to the nucleus [28]. The same results are obtained when a mutation into the WH2 domain is introduced that blocks binding to actin monomers [27]. An actin monomer prevents Jmy transfer to the nucleus, because it competes with Importin, as the Actin binding region overlaps with the

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Nuclear Localization Signal targeted by importin [27]. This mechanism has been eventually demonstrated as responsible for the transfer of Jmy to the nucleus after DNA damage [27]. As UV-induced DNA damage has been demonstrated to induce actin polymerization in the cytoplasm, just like treatment with Jasplakinolide [29] Zuchero et al demonstrated that nuclear accumulation of JMY is observed after exposure to UV or treatment with other DNA-damaging agents like Etoposide and Neocarzinostatin and that it is conditional to the availability of Importin. The authors conclude that the nuclear accumulation of Jmy following DNA damage may therefore be regulated by the assembly into polymers of actin induced by DNA damage [27].

The role played by Jmy in actin regulation does not affect only the motility of the cell but, in the case of the neurons, is also involved in the development of these cells as Jmy inhibits the formation of neurites [26]. This inhibiting activity appears to be due to its function as an actin-assembly protein [26].

The second mechanism by which Jmy affects motility is the regulation of the levels of E-Cadherin and N-Cadherin[10]as expression of Jmy is inversely correlated to the levels of these two adhesion molecules. Coutts et al have seen an upregulation of E-cadherin in Jmy depleted MCF7 cells and the opposite in Jmy rich cells [10]. Correlation studies done in our lab between Jmy and E Cadherin expression in 235 invasive breast carcinoma shown an inverse correlation between cytoplasmic Jmy and membranous E cadherin [11].

Linking p53 pathway and cell motility

Increasingly Jmy appears to have a special role in co-ordinating DNA damage response and cell motility. This follow the accumulation of evidences demonstrating that p53 has a role as inhibitor of cell motility (**Figure 5**) [30]. P53 has been found to be associated with tubulin, vimentin, F-actin and tubulin indicate a possible role in cytoskeleton regulation, Interaction with Cdc42 inhibits both filo podia formation, cellular polarization and “in vitro” cellular spreading. Furthermore, Inhibition or absence of p53 leads to increased activity of the Rho pathway with consequent increase in cell migration [30].

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The role played by Jmy both in regulating cell motility and cell damage response suggest that JMY could be a between the two pathways. In the model currently being explored, illustrated in **Figure 5**, after DNA damage, the neat Jmy concentration diminish in the cytoplasm and increases in the nucleus where most of the Jmy is sequestrated by the Cp300/Strap/p53 complex, making overall less JMY available for actin nucleation and cadherin inhibition [31] [10,27,32].

Conclusion

By being able to drive p53 towards induction of apoptosis on one side, and by promote invasiveness of the cancer cells on the other, Jmy is an example of genes that can act both as suppressor gene or a gene promoting tumour progression [33]. Despite this peculiar role played by JMY in cancer biology, still very little is known about its role in different types of cancers and in tumours at different stages.

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Figure legends

Figure 1 Basic functions of p53. P53 levels are tightly controlled and, in unperturbed cells, Mdm2 is its main regulator by inducing its degradation. Between the two proteins a negative feedback exists: P53 itself induces Mdm2 transcription therefore, as p53 levels increases, more Mdm2 is produced and p53 levels are brought down again. In presence of DNA damage and other stresses, p53 degradation stops and its levels increases. Increased levels of stable p53 induce transcription of proteins involve in different types of responses, the main one being cell cycle arrest and apoptosis.

Modified from: KEGG P53 pathway hsa04112 (http://www.kegg.jp/kegg-bin/show_pathway?map=hsa04115&show_description=show)

Figure 2 Jmy regulation. In unperturbed cells, JMY levels are maintained constant by a balance between transcription and degradation. The latter is controlled by Mdm2 which ubiquitinates Jmy leading to its degradation by proteasomes. Following DNA damage, the newly phosphorylated E2F1 induces increased transcription of Jmy, while ATM neutralises Mdm2 activity. Furthermore, actin monomers forms polymers and therefore are no longer available to link to Jmy, sequestering it in the cytoplasm. As it is no longer linked to actin monomers, JMY can link to Importin and moves to the nucleus. As a consequences levels of Jmy increases and form a complex with P300. Stability of this complex is further increases by linkage with phosphorylated Strap.

Based on: [19], [20],[17],[27].

Figure 3. Jmy **effect on p53.** Following DNA damage, p53 is phosphorylated and escapes degradation, therefore p53 levels increase. Therefore, this phosphorylated p53 links to the Jmy /p300/Strap complex and its transcriptional activity increases.

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Based on: [8].

Figure 4. Formation of molecular complexes and their effects on apoptosis and cell cycle. Following DNA damage, p53 is phosphorylated and is no longer linked to Mdm2 therefore remaining stable. It joins the p300/Jmy/Strap complex which causes acetylation, of variable intensity, of five Lysine residues located on the C terminus region of p53: this leads to an increased ability of p53 to transcribe Bax, but not p21, resulting in an increment of Apoptosis rather than cell cycle arrest. However, trough Strap, the available Prmt5 links to the Jmy /p300/Strap complex causing methylation of p53 and reducing the transcription of Bax but increasing transcription of p21, therefore decreasing apoptosis but inducing cell cycle arrest, Prmt5 further induces this switch by inhibiting, trough methylation, E2F1 and consequently reducing the Jmy transcription. Eventually the ability of p53 to induce more apoptosis than cell cycle arrest, or vice versa, is regulated by the balance between Jmy, p300, strap and Prmt5.

Based on and modified from: [8],[21,22,34] [35,36]

Figure 5. Model of Jmy **-mediated co-ordination between cell motility and DNA damage response in cell lines.** A model of how Jmy links p53 response to DNA damage to cell motility in cell lines. **A)** In a motile cell, standing in absence of stress, the amount of Jmy in the nucleus and in the cytoplasm, are maintained in equilibrium. The available Jmy protein in the cytoplasm inhibits, in a dose dependent fashion, E and N Cadherin adhesion molecules and induces nucleation, both in an Arp2/3 dependent and independent way. **B)** If such a cell is treated with siRNA targeting Jmy, the inhibition of Jmy transcription leads to a strong upregulation of the two cadherin, plus a drop of actin nucleation and therefore a severe inhibition of motility. **C)** When instead DNA damage occurs, a more modest drop in motility is observed. The hypothesis is that, on one side, the increased transfer of Jmy from cytoplasm to nucleus causes a diminution of the cytoplasm level which is, however, in part compensated by the overall increases of Jmy levels following the DNA damage. Therefore, the resulting inhibition is not as pronounce as after Jmy siRNA transfection.