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P53 Network in Ovarian Cancer

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1. Introduction

P53 (Tp53, tumor protein p53) is one of the most relevant human oncosuppressor genes. Accordingly, inactivation of p53 by direct mutation of the gene is one of the most frequent genetic lesions in human tumors.

In Ovarian Carcinoma (OC), p53 is altered in 30–80% of cases. Molecular and genetic studies have further confirmed the relevance of p53 in the development and progression of OC. Several studied have attempted to establish the p53 status as a marker of clinicopathological features. However, the predictive value of p53 alterations is still ambiguous, suggesting that multiple factors contribute to define p53 function. One of these factors may be related to the recent discovery of p53 variants in OC that may modulate, or even antagonize wild-type p53 function (Hofstetter et al., 2010).

Additional studies in molecular oncology have revealed alternative routes of p53 inactivation through deregulation of its negative regulators, MDM2 and MDM4. MDM family members are key regulators of p53 activity and levels, by acting as repressors of p53 transcriptional function and as a complex for the degradation of p53 protein. Their overexpression has been observed in many human tumors characterized by wild-type p53 status, supporting the model of multiple ways of p53 inactivation in tumor cells. In fact, p53 dysfunction measured as pathogenic mutations or altered copy number of MDM2 and MDM4, approaches 100% of confirmed high-grade serous carcinoma (Ahmed et al., 2010).

Recent data, also from our group, have contributed to define an even higher level of complexity in the p53 network. Indeed, it has been shown that the canonical inhibitors MDM2 and MDM4 may actually exhibit a dual mode of action (Shmueli & Oren, 2007; Mancini et al., 2009a). Particularly, following DNA damage, MDM4 functions as a cooperative factor in p53 apoptosis and promotes cell death in cisplatinum-treated ovarian cancer cells. Accordingly, MDM4 levels/p53 status correlates significantly with chemosensitivity of OC (Mancini et al., 2009b).

Interestingly, various studies have evidenced that the estrogen signalling pathway has a profound impact on the activity of MDM2/MDM4/p53 network (Bond & Levine, 2007) suggesting the relevance of hormonal status too in the prediction of p53 function.

Overall, these data suggest that a combined signature of p53 network may be a better prognostic factor for clinicopathological properties of ovarian cancer in agreement to what it has been recently published (Kalloger et al., 2011).

In this chapter, we will summarize all these data and try to compose potential scenario for novel predicting properties of p53 network in ovarian cancer.

2. P53

P53 is a central hub in the cellular response to a variety of stress signals, including DNA damage, hypoxia and aberrant proliferative signals, such as oncogene activation. Its activation results in the fulfillment of key cellular processes as cell-cycle arrest, senescence and, most importantly for tumor clearance, apoptosis.
P53 is a transcriptional factor able to bind specific DNA sequences and to modulate transcription of several targets by its transactivation domain. P53 transcriptional activities are mediated by its oligomerization (Figure 1).

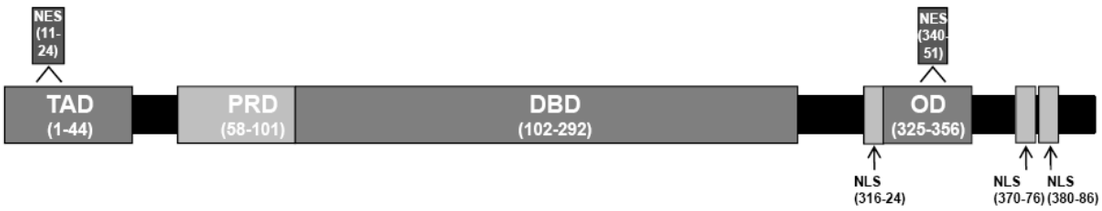

Fig. 1. P53 protein domains. TAD=Trans-Activation domain; PRD=Proline-Rich domain; DBD=DNA-binding domain; OD=Oligomerization domain; NES=Nuclear export signal; NLS=Nuclear localization signal.

The traditional view describing p53 activation in response to cellular stress comprises three basic steps: stabilization of p53, sequence-specific DNA binding, and transcriptional activation of target genes (Yee & Vousden, 2005). Promoter selection is dictated by numerous factors, including posttranslational modifications of p53 that can influence the recruitment of p53 binding proteins to specific promoters.
In addition to these nuclear activities, p53 possesses also cytosolic activities that can induce apoptosis in a transcription-independent manner (Green & Kroemer, 2009). Specifically, in response to various cell death signals, such as ionizing radiation, p53 rapidly localizes to the mitochondria where induces mitochondrial outer membrane permeabilization (MOMP) leading to the release of pro-apoptotic factors.
The relevance of this function in the tumor suppression has been demonstrated by mouse models expressing mitochondrial-targeted p53 variants (Galluzzi et al., 2008).
Because of its potent tumor suppressive activity, it is widely assumed that the complete molecular understanding of p53 action will produce fundamental insights into the natural processes that limit tumorigenesis and will contribute to identify key molecular targets for therapeutic intervention.

2.1 P53 role in ovarian cancer

Most of the epithelial ovarian cancers (EOC) are thought to arise from a single cell of the ovarian surface epithelium (OSE) which accumulates different genetic and epigenetic alterations which in turn lead to the malignant phenotype. The molecular events underlying this transformation are poorly understood. The inactivation of p53 oncosuppressor function seems to be an early event in the induction of hereditary ovarian cancer characterized by germ-line mutations of the BRCA1 tumor suppressor (Werness et al., 2000), suggesting that the loss of p53 function is required for a transformed cell to tolerate the loss of the BRCA1 function. Consistent with this, familial ovarian cancers have high frequency of p53 mutations (Ramus et al., 1999).

The cooperative action of p53 in BRCA1-driven tumorigenesis and in the induction of hereditary ovarian cancer is further strengthened by the phenotype of knock-out mice. *Brca1*$^{-/-}$ mouse embryos are embryonic lethal at embryonic day 6.5; if embryos are deleted simultaneously for both *Brca1* and *p53*, the embryonic lethality is delayed (Scully & Livingston, 2000). This suggests that p53 function antagonizes genome instability induced by BRCA1 loss, causing embryo lethality. Therefore, in order to promote tumor development, p53 activity must be lost so that the cell transformation process can go on easily.

According to this model, in ovaries removed prophylactically from women heterozygote for BRCA1, alteration of p53 was observed in all early stage I serous carcinomas as well as in the adjacent dysplastic surface epithelium (Pothuir et al., 2001). Although sporadic ovarian carcinomas were not analyzed in this study, the clinical and pathological features of BRCA-associated ovarian carcinomas and their sporadic counterparts are indistinguishable, suggesting that their histogenesis may be similar.

Overall, these observations support a general model in which *p53* inactivation is required not only for tumor progression but also for the early development of OC.

2.2 P53 mutation and ovarian cancer

Alterations of p53 pathway are one of the most frequent events in sporadic epithelial ovarian cancer (EOC). The majority of p53 mutations at its locus 17p13.1 are missense mutations that cause single residue changes, largely occurring in the DNA binding domain (Figure 1) (Sigal & Rotter, 2000). The p53 Web Site (http://p53.free.fr/index.html) reports that the most representative mutations found in ovarian cancers occur in the canonical hot spots of p53 gene, namely residues 273, 248, and 175 (ranging from 8% to 5%).

Although p53 mutations have been detected in all histological types of EOC, they are more strongly associated with high grade serous carcinomas then with low grade or borderline serous carcinomas (Kupryjanczyk et al., 1993; Kupryjanczyk et al., 1995; Skomedal et al., 1997; Zheng et al., 1995). The percentage of p53 gene mutations was reported to be lower also in others tumor types as endometrioid, mucinous, and clear-cell ovarian tumors (28%, 16%, and 10%, respectively) (Skilling et al., 1996).

The pathogenesis of ovarian carcinoma lacks of a defined tumor progression model. According to Kurman and Shih, the surface epithelial tumors are divided into two categories designated type I and type II tumors that correspond to two main pathways of tumorigenesis. Type I tumors tend to be low-grade neoplasms that arise in a stepwise manner from borderline tumors whereas type II tumors are high-grade neoplasms for which morphologically recognizable precursor lesions have not been identified (*de novo* development). According to this classification, high-grade serous carcinoma is the prototypic type II tumor whereas low-grade serous carcinoma and all other histological types are the prototypic type I tumor. Importantly, p53 gene mutation is the most common single genetic alteration observed in high-grade serous carcinomas, clinically the most important histological subtype of ovarian cancer (Kurman & Shih, 2011).

Recently, The Cancer Genome Atlas project has evidenced the presence of p53 mutations in almost all analyzed high-grade serous ovarian adenocarcinomas (96%) (Cancer Genome Atlas Research Network, 2011). Similarly, Ahmed et al., reported the presence of p53 mutation in 96.7% of high-grade serous carcinoma. Interestingly, molecular and pathological review of mutation-negative cases showed in these cases copy number gain of

MDM2 or MDM4, confirming the potential role of p53 network in contributing to p53 dysfunction (Ahmed et al., 2010). In this tumor context, therefore p53 mutation appears to be a driver pathogenetic event.

2.3 P53 predictive value in ovarian cancer

According to previous observations, several studies have tried to define the association of p53 with clinicopathological features of the OC. Because p53 mutation is almost invariably present in high-grade serous carcinoma, it is not of substantial prognostic or predictive significance in this tumor type.

On the contrary, considering the tumor stage, the prevalence of p53 genetic alterations appears to rise with increasing stage. Indeed, p53 gene mutations occur more often in stage III and IV ovarian cancers when compared to stage I and II, i.e., 58% versus 37% respectively (reviewed by Shelling et al., 1995), suggesting a positive selection of p53 mutation along tumor progression.

In the same direction, Bernardini et al. evidenced a correlation of the type of p53 mutation with the stage of the tumor. Early stage cancers have a significantly higher rate of null mutations (frameshift or chain terminating mutations that cause the lack of p53 protein) in comparison to late stage disease (38% vs. 8%) (Bernardini et al., 2010). These data suggest that along tumor progression p53 missense mutations are positively selected compared to null mutation, probably due to the "gain of function" of some p53 mutants that promote cell proliferation, tumor formation and invasion.

Accordingly, p53 overexpression (a specific feature of mutant p53 protein in cancer cells), has been associated with poor prognosis, poor overall survival and altered sensitivity to chemotherapy in patients with ovarian cancer (Fujita et al., 1994; Ferrandina et al., 1999; Sengupta et al., 2000; Reles et al., 2001; Hashiguchi et al., 2001; Tachibana et al., 2003; Bali et al., 2004; Bartel et al., 2008; Bernardini et al., 2010; Lee et al., 2011). However, others studies showed that overexpression of p53 is not associated with patient outcome (Havrilesky et al., 2003), has no prognostic value (Laframboise et al., 2000; Fallows et al., 2001) and is not predictive for responsiveness to platinum-based chemotherapy (Bauerschlag et al., 2010). Recently, a meta-analysis of studies on the prognostic value of p53 expression, showed that aberrant p53 status is associated only with poor overall survival (de Graeffet al., 2009), although there was ample heterogeneity among studies.

A number of factors can affect the predictive value of p53 alterations in OC. At first, it is increasingly evident that the overexpression of p53 protein, as usually detected by immunohistochemistry, is not strictly linked to its mutation. Indeed, Bartel et al. described a group of patients with p53 overexpression in which 49% of samples retain wild-type p53 (Bartel et al., 2008).

Moreover, the mere expression of p53 protein or its mutational status could not be sufficient to explain its behavior in the tumor context. P53 regulators, such as MDM2 and MDM4, can be altered (by overexpression or mutation) and differently modulate p53 wild-type functions (see next paragraphs).

Moreover, besides p53 regulators, other p53 alterations, as p53 alternative splicing (see next paragraph) can affect p53 function and, of note, create false results depending on the methodology of p53 detection. Particularly, immunohistochemical analysis cannot easily distinguish the protein form and the genetic status of a positive p53 staining.

All these data underline the necessity to assess the global functionality of p53 pathway and to distinguish the p53 genetic status in order to improve its prediction sensitivity in OC.

2.4 P53 splicing variants and p53 targets

Many cancer-associated genes, including the tumor suppressor p53, exhibit alternative pre-mRNA splicing. These variants may derive from canonical splice sites or by mutations that introduce new aberrant splicing sites.

In ovarian cancers cell lines and in primary ovarian cancers, different p53 splice variants were recently identified, some of them previously reported and others as novel cancer-specific forms (Hofstetter et al., 2010) (Figure 2).

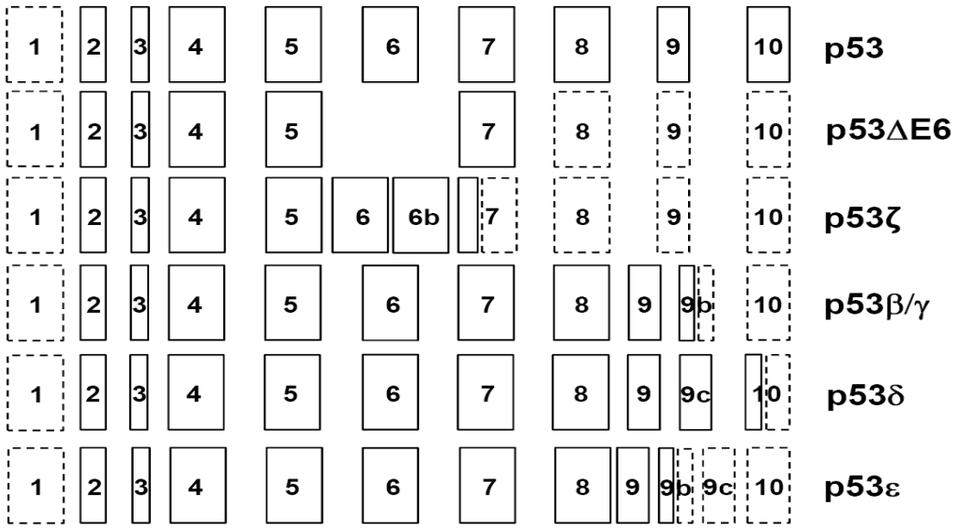

Fig. 2. Exon structure of the human p53 gene and p53 splice variants encoding C-terminally truncated proteins. Coding sequence (continuous lines); non-coding sequence (dashed lines).

Alternative splicing at the C terminus gives rise to p53β, containing 133 additional base pairs (bp) from intron 9 (exon 9b), and p53γ, retaining the distal 58 bp of exon 9b. These insertions result in a frameshift that introduces a premature termination codon. The p53β and p53γ proteins lack the basic regulatory domain and most of the oligomerization domain (OD) and possess unique short C-terminal tails of 10 and 15 new residues, respectively. Another splice variant, p53ΔE6, is missing exon 6 and encodes a C-terminally truncated p53 protein that lacks part of the DNA binding domain and the entire OD (Jolly et al., 1994). The three novel p53 splice variants identified in OC are p53ζ, p53δ and p53ε, arising from alternative splicing of exon 6 or intron 9. P53 splice variants were present in 18 of 34 ovarian cancer cell lines analysed (52.9%) and 134 of 245 primary ovarian cancers (54.7%). In this study, p53δ expression was associated with impaired response to primary platinum-based chemotherapy and its expression constituted an independent prognostic marker for recurrence-free and overall survival. P53β expression was associated with adverse clinicopathologic markers, that is, serous and poorly differentiated cancers and correlated with worse recurrence-free survival in patients exhibiting functionally active p53 (Hofstetter

et al., 2010). The other p53 splice variants differ in their clinical relevance, implicating that they possess different functions in vivo. The exact molecular function of these variants has not been completely ascertained; however, it has been hypothesized that they can modulate wild-type p53 function as well as be endowed of autonomous activity.

Overall, the discovery of the high frequency of p53 splice variants in ovarian cancer increases the complexity of the deregulation of p53 pathway in OC and therefore the understanding of p53 contribution to the pathogenesis of ovarian carcinoma.

Another layer of complexity in the prediction of p53 function, is represented by the analysis of some p53 targets. One of the most relevant and more studied is p21waf1/cip1. It is a cyclin-dependent kinase inhibitor that is usually induced through a p53-related pathway. P21waf1/cip1 has been shown to be integral to the control of the cell cycle after DNA damage. Indeed, up-regulation of p21waf1/cip1 by p53 is essential to sustain cell cycle arrest after DNA damage.

Although p21waf1/cip1 has been studied in EOC, the role of this protein as a prognostic indicator is still controversial (Sengupta et al., 2000; Geisler et al., 2001). Some studies confirm the importance of the combination of p21 and p53 staining in determining EOC prognosis. Indeed, expression of p53 protein in the absence of p21waf1/cip1 was a better marker of poor prognosis than either p53 or p21waf1/cip1 expression alone (Bali et al., 2004; Geisler et al., 2001; Werness et al., 1999).

Cai et al. suggested that since p21 expression may be an indicator of wild-type p53 function, lack of p21 in the presence of p53 expression may be predictive of an inactivated status of p53. Given that p53 inactivation precedes morphological transformation of the ovarian surface epithelium in most cases, the double analysis of these proteins might constitute an early marker of pre-neoplastic lesions (Cai et al., 2009).

Another important group of p53 targets involved in ovarian cancer was recently identified in the MicroRNAs molecules. MicroRNAs (miRNA) are a recently discovered class of noncoding RNAs that negatively regulate gene expression. Evidence indicates that miRNAs play an important role in cancer development. MiR-34b and miR-34c are the miRNAs most significantly affected by p53 and have been shown to cooperate in suppressing proliferation and transformation of neoplastic epithelial ovarian cells (Corney et al., 2007). Analyzing a group of EOC, Corney et al., showed that miR-34b/c expression is decreased in 72% of tumors with p53 mutation. Furthermore, expression of miR-34b/c is significantly reduced in stage IV tumors compared to stage III. These data suggest that miR-34 family plays an important role in EOC pathogenesis and that reduced expression of miR-34b/c may be particularly important for tumor progression to the most advanced stages (Corney et al., 2010).

Overall, these data highlight the importance of the analysis of p53 and of its targets as a tool with improved prediction properties in OC.

3. MDM2

MDM2 (for transformed mouse 3T3 cell double minute 2) is the first and best known negative regulator of p53. It has been isolated from a spontaneous transformed BALB/c 3T3 mouse cell line in 1992.

MDM2 interacts physically with p53 and brings this oncosuppressor to degradation besides to inhibit its transcriptional function masking the p53 activation domain. Molecular and genetic studies have confirmed the crucial role of MDM2 in the inhibition of p53 function,

leading to the concept that MDM2 overexpression may be an alternative way of p53 inactivation in human tumors (Marine & Lozano, 2010). Accordingly, MDM2 overexpression has been observed in many human cancers (Momand et al., 1998). Recent data have led to reconsider MDM2 not only as a p53 inhibitor but also as a modifier of p53 response. Indeed, after stress, MDM2 contributes to lower the protein levels of some proapoptotic factors (i.e. HIPK2, TIP60) that assist p53 in activating its apoptotic function. Therefore, increase or decline of MDM2 levels would affect p53 choice between growth arrest and apoptosis respectively (Shmueli & Oren., 2007). The relevance of this model in the oncosuppressive activity of p53 as well as in its role in chemosensitivity remains to be elucidated.

3.1 MDM2 alterations and ovarian cancer

MDM2 aberrant expression has been reported in human tumors, including ovarian cancer. Several ways of MDM2 aberrant expression have been recognized. The first way is the amplification of the gene. The human MDM2 gene (also HDM2) resides on chromosome 12q13-14 and is amplified in a large cohort of human tumors (about 7% in a survey of 28 tumor types). MDM2 overall amplification frequency in all ovarian cancer was reported to be 3.1% (Momand, 1998). However, analysing specific tumor subtype, MDM2 amplification has been recognized in 80% of serous borderline tumors (Mayr and al., 2006) often associated to co-expression of p21WAF1/CIP1 suggesting that in this histotype these cell cycle control proteins might be important for cancer phenotype (Palazzo et al., 2000).

In addition, MDM2 levels can be upregulated independently of gene amplification. Both enhanced MDM2 protein levels as well as high levels of MDM2 transcripts have been reported in different tumor histotypes although the molecular mechanisms that underlie such alterations have not been completely characterized. In OC, MDM2 overexpression has been reported by various reports (varying among 17%, 33%, and 47, 5%) (Baekelandt et al., 1999; Dogan et al., 2005; Cho et al., 2006). In one study, it has been demonstrated the independency from amplification events (Foulkes et al., 1995) confirming the existence of mechanism of MDM2 stabilization in the ovarian cancer too.

More recently, two single nucleotide polymorphisms (SNP) in the P2 promoter of the MDM2 gene able to modify MDM2 levels have been identified. The first one, at the 309th nucleotide in the first intron, alters the affinity of the transcriptional activator Sp1 resulting in different levels of MDM2 mRNA and protein. Particularly, the T to G nucleotide change extends the length of one Sp1 DNA binding site, increasing the transcription of the MDM2 gene. This in turn results in attenuation of the p53 activity and accelerated tumor formation (Bond et al., 2004). The presence of this polymorphism has been considered an oncogenic predisposing factor. Indeed the authors found out a significant correlation of SNP309G with earlier age of onset in a group of sporadic soft tissue sarcoma. Subsequently, the SNP309G effects appeared to be mediated by the hormonal status, being effective in the presence of an active estrogen signalling pathway (Bond & Levine, 2007). Therefore, the role of this polymorphism has been especially studied in breast and ovarian cancer. However, relative studies have reported controversial results indicating both association between SNP309G and OC risk (Yarden et al., 2008) or earlier age of onset in estrogen receptor-overexpressing FIGO stage III patients (Bartel et al., 2008) as well as the lack of its association with OC (Campbell et al., 2006) or cancer risk (Krekac et a., 2008). Recently, an important study has solved these controversies. It has been identified an additional SNP (at nucleotide 285)

whose activity profoundly impacts on the activity of SNP309G. In vitro, SNP285C strongly reduces the Sp1 binding to MDM2 promoter therefore counteracting the inhibitory activity of SNP309 towards p53 pathway. Indeed, the authors demonstrated that the presence of SNP285C antagonizes the activity of the SNP309G lowering the risk and the age of appearance of ovarian cancer. Interestingly, SNP285C has been evidenced in Caucasian individuals only, while being absent in Chinese population (Knappskog et al., 2011).

An additional way of MDM2 deregulation is the expression of MDM2 splicing variants. Indeed, besides full-length (fl) mRNA, more than 40 different splice variants of MDM2 transcripts have been identified in normal tissues and tumors including OC (Sigalas et al., 1996; Bartel et al., 2002), and tumorigenicity of some of these variants has been in vivo and in vitro assessed. Although the specific role of MDM2 variants in OC has not been studied, their presence may lead to a misinterpretation of MDM2 expression in tumor samples. Indeed, MDM2 detection by immunohistochemistry often lacks the sufficient specificity to distinguish wild type protein from splicing forms. This is of relevance taking into consideration the fact that many of these variants show a p53-independent function as they have lost, at least in part, the p53-binding domain. Therefore, their presence should be clearly ascertained when considering the p53 network.

3.2 MDM2 predictive value in ovarian cancer

Given the frequent alteration of MDM2 in OC, several studies have investigated the association of its expression with ovarian carcinoma properties. Conflicting results have been reported, suggesting that the analysis of sole MDM2 as well as of sole p53 are not good predictors. A recent study has supported this hypothesis demonstrating that a 9 marker set (including MDM2, CDKN2A, DKK1, HNF1B, PGR, TFF3, TP53, VIM and WT1) is the most predictive factor of ovarian cancer subtype (high-grade serous, clear cell, endometrioid, mucinous and low-grade serous) in a 322 archival ovarian carcinoma by tissue microarrays (Kalloger et al., 2011). Validation of this panel in two independent series of 81 cases demonstrated good to excellent ability to predict subtype (k=0.85 and 0.78). These data point to multiple immunohistochemical analysis as the gold standard for diagnostic accuracy in the future.

4. MDM4

Murine Mdm4 (for transformed mouse 3T3 cell double minute 4, also Mdmx) and human ortholog MDM4 (also HDMX) have been identified as the closest analogues of Mdm2 in 1996 (Marine et al., 2007).

Similarly to MDM2, MDM4 inhibits p53 transcriptional function although less efficiently than MDM2. However, at variance with MDM2, MDM4 is unable to degrade p53 protein or other targets. In the human cell, MDM4 heterodimerizes with MDM2 and their complex is considered the effective controller of p53 activity. In agreement with its inhibitory function, MDM4 overexpression has been observed in some human cancer, in some cases associated to simultaneous MDM2 overexpression (Macchiarulo et al., 2011).

However, recent evidence indicates that, in analogy to MDM2, MDM4 is not only a p53 inhibitor. It has been demonstrated that upon stress, MDM4 contributes to p53 activation by stabilizing its levels and promoting the mitochondrial apoptotic response (Mancini et al., 2009a). Noteworthy, this MDM4 activity is able to modify chemosensitivity of cancer cell

lines by altering the p53 apoptotic response. This scenario is even more complicated by the observation that under stress, MDM2 may degrade MDM4 and therefore inhibit MDM4-mediated proapoptotic function. These molecular data lead to a reconsideration of the role of MDM4 and/or MDM2 in p53 suppression. Their relative balance rather than the single molecules could be relevant for their function in the regulation of p53 (Mancini et al., 2010). Interestingly, a recent mathematical model provided support for this hypothesis: it shows that MDM4 may stabilize or even amplify DNA damage-induced p53 response, depending on the balance with MDM2, the main regulator of MDM4 levels (Kim et al., 2010) (Figure 3).

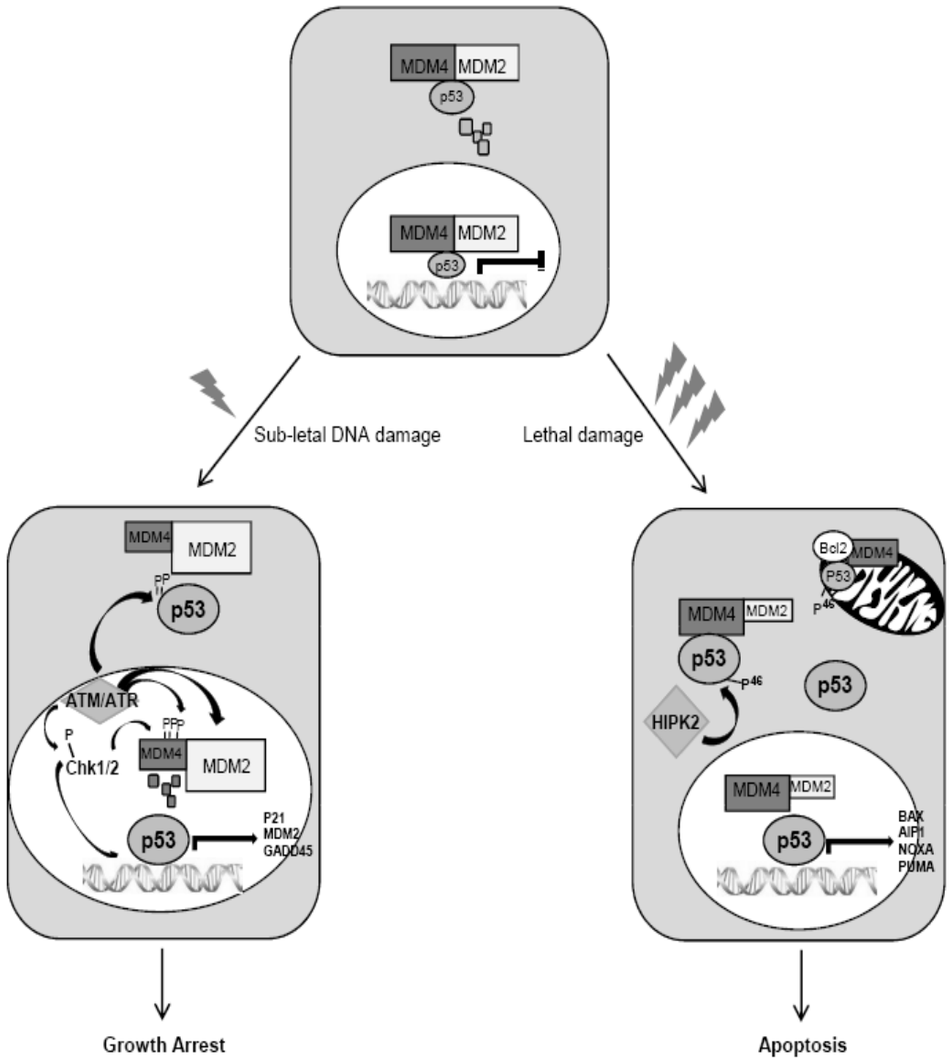

Fig. 3. P53 network following different DNA damages.

4.1 MDM4 and ovarian cancer

To date, MDM4 overexpression or amplification have not been reported in OC. On the contrary, a significant downregulation of MDM4 mRNA and protein levels has been observed in a group of wt-p53 carrying OC characterized by resistance to platinum-derived

therapy in comparison to the responsive ones (Mancini et al., 2009b). These data have been correlated to the ability of MDM4 to promote p53-dependent mitochondrial apoptosis. Although few data have been reported on the role of mitochondrial apoptosis in the sensitivity to particular chemotherapeutic drugs, it has been shown that the expression of mitochondrial proteins BCL2 (antiapoptotic) and BAX (proapoptotic) have predictive value in ovarian cancer patients treated with platinum-based chemotherapy (Kupryjańczyk et al., 2003), confirming that the mitochondrial pathway may have a relevant function in the response to this chemotherapy in these tumors. Of note, these data may contribute to explain the difficulties to correlate p53 status with chemotherapy response. Indeed, not only p53 status but also the status of its regulators may profoundly affect the chemosensitivity of ovarian cancer.

These findings are supported by evidence that in other human tumours MDM4 levels are significantly downregulated in association with more aggressive features (Prodosmo et al., 2008) and that in breast cancer MDM4 presence is considered a positive prognostic factor (Abdel-Fatah et al., 2010).

Further studies about the role of MDM4 in OC have highlighted that the estrogen pathway is an important modifier, in analogy to what observed for MDM2. Indeed, it has been recognized a SNP at position 34091 in the 3' untranslated region (UTR) of MDM4, just 32 nucleotides downstream of the stop codon. SNP34091C introduces an illegitimate binding site for a miRNA, miR-191, that is ubiquitously expressed in human normal and cancer tissues.

The presence of SNP34091C is correlated to a decrease in the MDM4 levels. Interestingly, SNP34091A correlates with increased MDM4 expression in a group of 66 primary ovarian carcinomas and with significant decreased overall survival and increased risk of tumor-related death (Wynendaele et al., 2010). Noteworthy, this occurs only in patients negative for estrogen receptor (ER) expression suggesting the MDM4 oncogenic function is modified by ER signalling pathway although no ER binding sites are present in the MDM4 gene. Intriguingly, ER status affects MDM4 and MDM2 in an opposite way. It counteracts oncogenic activity of MDM4 while potentiates that of MDM2. The understanding of the molecular mechanism underlying these effects will further clarify the role of these proteins in the development and progression of ovarian cancer. It has to be emphasized that SNP34091A does not correlate with p53 status suggesting that MDM4 may exert oncogenic function independently of p53 too (Wynendaele et al., 2010).

In a second genetic study, the authors identified additional SNP that confer an earlier age of onset of familial and sporadic OC in 3 different populations of Caucasian of different ethnic background and in 1 population of African Americans. However, the effects of these SNP on MDM4 levels and/or activity were not identified as well as any relationship with estrogen signalling (Atwal et al., 2009).

Finally, in analogy to MDM2, alternative splicing of MDM4 has been described as well (Mancini et al., 2009c). Particularly, a tumor-specific form, MDM4-211, derived from an aberrant splicing between exon 2 and exon 11, is frequently present in OC (unpublished data). This form lacks the p53-binding domain and therefore cannot directly modulate p53 function. However, it can stabilize MDM2 and in turn inhibit p53 function (Giglio et al., 2006). The relevance of this variant in OC features remains to be elucidated. However, as previously described, the presence of these variants suggests measures of caution in the interpretation of MDM4 IHC positive results.

5. Conclusion

P53 is a central hub in the stress response and its function plays a major role in human oncogenesis.
In ovarian cancer, it seems to be a key determinant in the appearance as well as in the progression of the tumor. In addition, its status affects the response to the chemotherapy.
Despite this, the numerous studies aimed to use p53 detection as a marker for prediction of clinicopathological features of OC have provided some conflicting results. Accordingly, clinical trials based on the status of p53 are not currently in progress. Increasing evidence from literature suggests that the assessment of p53 function and therefore its predictive/diagnostic value might be foreseen more effectively by the analysis of its network, particularly of its regulators MDM4 and MDM2 and of some of its targets, as p21 and Bcl2. The recent work by Kalloger (Kalloger et al., 2011) using tissue microarray gave strong support to this hypothesis. Moreover, it is assuming increased relevance integrated genomic analyses for simultaneous analysis of mRNA, miRNA and promoter methylation to delineate transcriptional subtype associated to clinicopathological properties of tumor (Cancer Genome Atlas Research Network, 2011).
In the future, the integration of disease-specific transcriptional profile analysis and protein detection could represent the optimum for patient diagnosis and cure.

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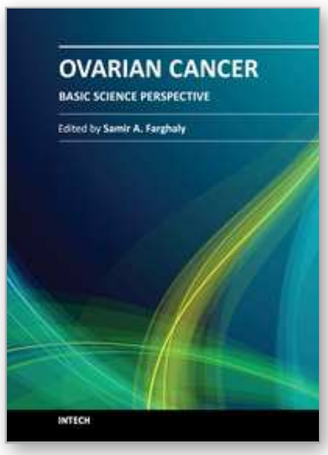

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Worldwide, Ovarian carcinoma continues to be responsible for more deaths than all other gynecologic malignancies combined. International leaders in the field address the critical biologic and basic science issues relevant to the disease. The book details the molecular biological aspects of ovarian cancer. It provides molecular biology techniques of understanding this cancer. The techniques are designed to determine tumor genetics, expression, and protein function, and to elucidate the genetic mechanisms by which gene and immunotherapies may be perfected. It provides an analysis of current research into aspects of malignant transformation, growth control, and metastasis. A comprehensive spectrum of topics is covered providing up to date information on scientific discoveries and management considerations.

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