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Tp53 and its potential therapeutic role as a target in bladder cancer

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Abstract

Introduction: Despite more than 30 years of research on p53 resulting in >50,000 publications, we are now beginning to figure out the complexity of the p53 pathway, gene ontology and conformational structure of the molecule. Recent years brought great advances in p53 related drugs and the potencial ways in which p53 is inactivated in cancer.

Areas covered: We searched for related publications on Pubmed and ClinicalTrial.gov using the following keywords "p53, Tp53, p53 and bladder cancer, p53 and therapeutic target". Relevant articles inproved the understanding on p53 pathways and their potential as candidate to targeted therapy in bladder cancer.

Expert opinion: Novel strategies developed to restore the function of mutants with chemical chaperones or by using compounds to improved pharmacokinetic properties are in development with potential to be applied in the oncology clinic. Other strategies targeting aberrantly overexpressed p53 regulators with wild-type p53 are also an active area of research. In particular, studies inhibiting the interaction of p53 with its negative regulators MDMX and MDM2 are an important field in drug discovery. Small molecules for inhibition of MDM2 are now in clinical trials process. However, personalized anticancer therapy might eventually advance through analyses of p53 status in cancer patients.

Article highlights

- Bladder Cancer is the most common malignancy of the urinary tract and comprises two long-recognized entities with distinct molecular features and clinical outcome.
- The identification of the genetic alterations may lead to the understanding of the nature of this disease and provide the possibility of more efective treatment.
- p53, a tumor suppress involved in a number of cellular processes, is regulated by the Mdm2, an oncoprotein that acts blocking its ability to regulate target genes.
- Mutations in the p53, frequently observed in human cancer, is an event with prognostic significance in patients with Bladder Cancer.
- MDM2-p53 negative feedback loop has been widely studied and presents an attractive target for cancer therapy. Therapeutic approach by restoring functional p53 protein using small peptides o molecules in cancer cells is now in process.

1. Introduction

Bladder cancer is the fourth most common tumor type diagnosed in developed countries, showing high morbidity and mortality rates (advanced muscle-invasive bladder cancer is the 9th most common cause of death worldwide) [1, 2]. Clinically, bladder cancer can be divided into three subtypes, completely different in terms of therapeutic management, pathological behavior and prognosis. First, non-muscle-invasive tumors (NMIBC), accounting for about 70% of bladder cancer, and classified into 3 diverse stages: pTis (flat carcinoma in situ – showing a high propensity to invade and metastasize), pTa (papillary non-invasive carcinoma – characterized by a tendency to recur locally, without invading and metastasizing), and pT1 (when cancer cells infiltrate the mucosa or submucosa of the bladder wall) [3]. Treatment (transurethral resection of bladder tumors, followed by intravescical chemotherapy or immunotherapy) aims at preventing recurrences (occurring in almost 50% of cases within 4 years from the diagnosis) and avoiding progression to a more advanced stage (approximately 10 - 15% of NMIBC progress to muscle invasive disease, after having acquired other genetic alterations)[4]. The second type includes muscle-invasive tumors (MIBC), which are at high risk of distant spread (80%–90% of cases) despite radical excision of the primary lesion (cystectomy). Finally, the third group concerns advanced recurrent or metastatic bladder tumors characterized by an extremely poor prognosis (5-year survival rate not exceeding 5%) [2, 4].

1.1. TP53 and Molecular Pathology of Bladder Cancer

Current emerging data supports two distinctive genetic pathways, probably mutually exclusive, responsible for urothelial bladder cancer pathogenesis and driven by FGFR3 or TP53 mutations respectively [5]. Low-grade noninvasive papillary tumors (pTa) frequently display a constitutive activation of the Ras pathway, characterized by activating mutations in the HRAS and

fibroblast growth factor receptor 3 (FGFR3) genes. The incidence of FGFR3 mutations varies acording to pathological grade and is seen in about 70% of low-grade noninvasive papillary tumors. Conversely, FGFR3 mutation is observed in 10-20% only of muscle invasive urothelial carcinoma. On the contrary, TP53 gene mutations, are seen in less than 5% of Ta urothelial tumors, but are more frecuently seen in association with high grade tumors, both flat carcinoma in situ and invasive carcinomas (AJCC category T1 or higher) [5]. As broadly known, p53 has a central role in different key cellular functions related to cancer development, progression, and response to therapy, including cell-cycle regulation, apoptosis, DNA repair, and angiogenesis. Therefore, the lack of p53 inactivation confers a genetic stability to pTa urothelial carcinomas, while genetic instability marks invasive bladder cancers carrying TP53 inactivating mutations [6] (Figure 1).

The poor prognosis of advanced BC patients relies on the intrinsic biologic aggressiveness of this tumor, showing a peculiar radio- and chemo-resistance. For this reason, efforts are directed at deeply understanding the pathogenesis of bladder carcinogenesis, so as to identify successful targets for therapy. The future clinical management for bladder carcinoma will likely use multimarker panels for prediction of prognosis and guiding targeting pathway-specific molecules therapies with a key role as drivers of tumor progression.

1.2. p53 pathway: activation, regulation and function

The tumor suppressor gene TP53, mapped on the short arm of chromosome 17 (17p13.1), is being considered as a 'genome guardian' acting as a cell-cycle regulator, arresting the cell cycle in response to DNA damage thus allowing the DNA repair and maintaining genomic integrity [7].

Complex molecular mechanisms contribute to regulate p53 activation, including interdependent control by protein-protein interactions, balance between protein synthesis and degradation, and post-translational modifications. A pivotal regulatory role is played by the interaction of p53 and the MDM2 protein [8]. Under normal conditions, the p53 tumor suppressor protein is a short-lived, latent transcription factor that is kept off by the inactivating binding with its negative regulator, MDM2. The oncogene MDM2 protein, in fact, inhibits p53 transactivation

function by binding to a region of the TP53 transactivation domain, thus concealing the activation domain of p53 from the cellular transcription machinery [7]. Moreover, the MDM2 enzyme causes rapid degradation of p53 through the MDM-promoted tie of the carboxy terminus of p53 with several small peptides (ubiquitin) resulting in the activation of the protein-degrading machinery (26S proteosome) responsible for p53 ubiquitin-mediated proteolysis [7]. A recent study suggested that there are distinct mechanisms regulating p53 function in accordance with the levels of Mdm2 activity. This study proposed that low levels of Mdm2 activity induce monoubiquitination and nuclear export of p53, whereas high levels promote p53's polyubiquitination and nuclear degradation [9]. Likewise, the stability, activity and functionality of wild type p53 may also be influenced by chaperone Hsp90 through binding DNA of the p53 promoter sequence, a fact that confers an additional level of control to p53 protein. [10, 11].

Interestingly, the MDM2 gene transcription is itself stimulated by p53-dependent transactivation, forming a feedback loop in which p53-stimulated increased levels of MDM2 lowers p53 concentrations therefore diminishing MDM2 transcription and allowing p53 to increase again [12]. On the contrary, the p53 network is activated in response to cellular DNA damage, mitotic spindle damage, or other numerous stressors (oxidative stress, osmotic shock, ribonucleotides deficiency), functioning as a delicate mechanism of damage "sentinel".

In particular, increased intracellular levels of the p53 protein can derived from:

1.2.1 DNA damage

The most investigated stimulus that triggers the p53 activation begins with DNA damage. Ionizing radiation (IR), DNA damage-inducing drugs and ultraviolet light (UV) - mediate breaks in double-stranded DNA; even a single break in double-stranded DNA can be sufficient, underlying the extreme sensitivity of the human cell to DNA harm. The DNA damage is recognized by 'sensing checkpoint proteins' that mediate the arrest of the cell cycle until the damage is repaired. The checkpoint proteins include different members of the PI-3-kinase family: the ATM kinase (ataxia telangiectasia mutated), the DNA-PK (DNA-dependent protein kinase), and the ATR kinase (ATM-

Rad3-related). Moreover, a key role is covered by Chk1 and Chk2 kinases, which are induced by ATM activation. These kinases induce multisite phosphorylation and acetylation of p53, key events in the activation of p53 in response to DNA injury. De novo p53 phosphorylation of the N-terminus of p53 at sites closed to the MDM2-binding region of the protein (mainly at serine 15, but also at serines 20, 33 and 37) impairs p53 interactions with MDM2 [13]. The disruption of the p53-MDM2 complex stabilizes p53 transactivation and prevents proteasome-mediated p53 degradation [14]. Besides the N-terminal phosphorylation, alterations also occur at the C-terminus of p53. In normal conditions, the carboxy terminus of p53 folds back blocking the DNA-binding domain of p53. In response to both IR and UV p53 is activated also through acetylation at C-terminal lysine residue 320 by PCAF and at 373 and/or 382 residues by p300/CBP. Acetylation of lysine residues or serine residues phosphorylation next to the p53 C-terminal induces a conformational change of the 53 protein by interfering with this folding, thus stimulating the p53-DNA binding [12] (Figure 2).

Of note, N-terminal phosphorylation favors acetylation of the C-terminus of p53, suggesting the importance of a harmonized and sequential series of p53 modifications in response to DNA-damage culminating in the dissociation of the p53-MDM2 complex, p53 stabilization, and enhanced p53 transcriptional activity [7, 12].

1.2.2 Ultraviolet light (UV), cytotoxic drugs (chemotherapy), and protein-kinase inhibitors.

UV light produces oxidative stress and causes pyrimidine dimers, which are repaired by excision repair. The induction of p53 is due to the phosphorylation of p53 at serine 15. UV cellular irradiation stimulates serine 15 kinase activation through the effector ATR (ataxia telangiectasia related) kinases - and not ATM -. ATR is also responsible for enhanced phosphorylation of serines 20, 33, and 37 in response to UV [15]. Moreover, differently from DNA strand break, UV irradiation determines the transactivation of p53 via the phosphorylation of serine 392 caused by casein kinase 2 (CK2) [14] (Figure 2).

1.2.3 Abnormal growth signals.

The induction of p53 can derive from the activity of dominant oncogene products that drive sustained cell proliferation in the absence of DNA damage. In particular, activating mutations of the oncogenes RAS, E1A or Myc trigger the p53 pathway via $p14^{ARF}$. These oncogenes stimulate the transcription of the INK4A tumor suppressor gene, which codes for two unrelated proteins, $p16^{INK4A}$ and $p14^{ARF}$ (also known as $p19^{ARF}$) which interact with the retinoblastoma (*RB*) and *TP53* tumor suppressors genes related proteins. These transcripts contains an alternate open reading frame (ARF) that responds to oncogenic signaling by neutralizing functions of Mdm2 through the formation of complexes binary such as the interactions ARF -Mdm2 and ARF- p53 and ternary as ARF–Mdm2–p53 [16, 17]. Specifically, $p14^{ARF}$ binds to MDM2 inhibiting its ability to negatively regulate the transactivation function of p53, and interferes with MDM2-mediated p53 degradation. The result is the p53 accumulation, leading to downstream effects of cellular growth restraint [18]. p53 can also be regulated by PTEN, a tumor suppressor protein, which inhibits PI3K/Akt signaling. This pathway is responsible to promote translocation of Mdm2 into the nucleus. Mdm2 is degraded when it is restricted to the cytoplasm. The ability of PTEN to inhibit the nuclear entry of Mdm2 increases the cellular content and transactivation of the p53 [19].

The activated p53 protein (marked by a prolonged half-life leading to a peculiar intracellular p53 accumulation, and by an increased transcriptional activity due to the phosphorylation of its N-terminal transcription activation domain) arrests the cell cycle to allow damage repair and cell survival, or induces apoptosis in case of irreparable harm. In particular, the peculiar ability of activated p53 to bind to specific DNA regions explains its role in controlling the transcription of several target genes, which are involved in:

1.2.3.1 Senescense

Reportedly, the p53 tumour suppressor is activated by numerous stressors to induce apoptosis, cell cycle arrest, or senescence. p53 regulates senescencia and in this sense some studies have shown that p53 mutant mice, that display early aging, induce downstream transcriptional

targets critical for senescence, such as p16 and PML [20-22]. To study the biological effects of altered p53 function, Tyner et al. generated a mice model with a deletion mutation in the first six exons of the p53 gene that express a truncated RNA capable of encoding a carboxy-terminal p53 fragment. The observed mutation confers phenotypes consistent with activated p53 rather than inactivated p53. As p53+/m mice age, they display an early onset of phenotypes associated with ageing. A fact data support that p53 has also a role in regulating ageing. An emerging issue is that defects in metabolism account for both cancer and aging [22].

1.2.3.2 Cell-cycle arrest.

The main functions of p53 concern the inhibition of the cell-division cycle. p53 directly regulates the transcription of the $p21^{WAF1/CIP1}$ gene, involved in the arrest of the G1-to-S transition. The $p21^{WAF1/CIP1}$ protein, a cyclin dependent kinase inhibitor (CDKI), binds to and inactivates cyclin/cyclin-dependent kinases (CDKs) complexes – mainly cyclin D-CDK4, and cyclin E-CDK2 complexes – crucial regulators of the transition from the cellular growth G1 phase to the DNA replication 'S phase' [23].

In addiction, p53 blocks the G2-to-Mitosis transition by enhancing the expression of protein 14-3-3σ (which sequesters cyclin B1/CDK1 complexes out of the nucleus) [24], B99 [25], and Gadd45 [26]. Moreover, the p53-dependent G2 arrest of the cell cycle can derive from an aberrant expression of the Reprimo gene, which is involved in the inhibition of both Cdc2 activity and cyclin B1 nuclear translocation [27] (Figure 2).

1.2.3.3 Induction of apoptosis.

Activated p53 induces programmed death via several mediators, including Bax, NOXA, p53AIP1, TNF, Fas/APO1, Pidd, Scotin, PERP, and KILLER/DR5 [12].

In particular, Bax is a p53 primary-response gene involved in a p53-regulated pathway for induction of apoptosis [28]. Moreover, the NOXA gene, directly activated by p53, codes for a Bcl-2 homology 3 (BH3)-only member of the Bcl-2 family of proteins. If ectopically expressed, the Noxa protein localizes within mitochondria thanks to the BH3-motif and interacts with anti-apoptotic Bcl-2

family members, resulting in the activation of caspase-9 and p53-dependent apoptosis [29]. The BH3-only proteins are divided into two complex dualists: 1) Models in which p53 functions as"enabler" type BH3 only proteins. In these models p53 is able to disrupt the interaction between anti-apoptotic proteins (such as BclxL, Bcl2 and Mcl1) and pro-apoptotic proteins. This could directly relieve the inhibition of Bax and Bak or free 'activator' type BH3 only proteins (Bid and Bim), which can then activate Bax and Bak; and 2) Models in which p53 functions as an "activator" type BH3 only proteins. Activation of Bax and Bak might involve binding and releasing them from interaction with the anti-apoptotic proteins. Alternatively, p53 itself may be held inactive by interaction with the anti-apoptotic proteins. In this case it is possible that other 'enabler' BH3-only proteins like PUMA might be able to displace p53 from the anti-apoptotic proteins, thus allowing it to activate Bax/Bak [30]. p53 also induces transcriptional activation of other pro-apoptotic factors such as IGF-BP3 (insulin-like growth factor binding protein 3) [31], PUMA (p53 upregulated modulainteractor of apoptosis with strong pro- apoptotic activity) [32] and FAS receptor [33].

In addition, it is also known that overexpression of p21 (CDK inhibitor) promote bile acid-induced apoptosis p53-dependent. A study carried out in primary hepatocytes have also demonstrate that CDK inhibitors suppress MDM2 levels and enhances p53 expression that facilitates bile acid-induced, ceramide-dependent CD95 activation to induce both apoptosis (a toxic response) and autophagy (a survival response) [34].

Analogously, phosphorylation of p53 at Ser-46 induces the aberrant transcription of the p53AIP1 (p53-regulated Apoptosis-Inducing Protein 1) gene, which migrates into the mitochondria leading to apoptotic cell death through dissipation of mitochondrial A(psi)m [29]. In addiction, p53 promotes cell death by regulating the expression of pro-apoptotic effector genes (including Fas, TNF receptor and Pidd [35]), and by stimulating the mitochondrial production of extremely toxic reactive oxygen species.

1.2.3.4 Angiogenesis, migration and cell motility inhibition.

p53 has a key role in the negative regulation of angiogenesis by stimulating the expression of genes that prevent blood-vessel formation. In particular, p53 can induce in fibroblasts the synthesis of thrombospondin-1 (TSP-1) - a potent inhibitor of angiogenesis - [36]. Furthermore, p53 inhibits growth of primary human endothelial cells through the transcriptionally activation of the alpha(II) collagen prolyl-4-hydroxylase [alpha(II)PH] gene, leading to increased synthesis and extracellular release of antiangiogenic collagen fragments [37]. Therefore, inactivating mutations of p53 result in increased neo-angiogenesis, which is necessary to support cancer cells uncontrolled proliferation and tumor growth. The transcription of the vascular endothelial growth factor (VEGF), an angiogenic factor that promotes the proliferation of endothelial cells, is activated by v-Src in presence of wt-p53 suggesting that wild-type p53 may play a role in suppressing angiogenesis [38]. In addition, to angiogenesis, cell migration and mobility are also essential functions in various physiological processes. Fibronectine (FN) is a glycoprotein present in a extracellular matrices and plasma that is involved in cell adhesion, motility. The downregulation of FN promoter seem to facilitate the migration of tumor cell and contribute to the wild-type p53-induced arrest of the cells in G1 [39]. Other studies have shown that p53 down-regulated may contribute to joint degeneration through the regulation of MMP-1 principally responsible of the irreversible destruction of collagen in articular tissue in rheumatoid arthritis [40].

Another study, reviewed the role of TNFalpha in regulating the cortical actin-containing structures. This study, concluded that TNFalpha prevents filopodia formation and cell migration through the activation of the mitogen-activated protein kinase (MAPK) p38, which in turn activates p53 [41]. Numerous studies to date have defined the Rho family of small GTPases (Rac, Cdc42 and Rho) as key regulators of actin cytoskeleton and control cell protusions during migration, reorganization and adhesion mechanisms. An increased activity of RhoA and Rac is related to a loss of p53 function (through the activation of the Akt/PI3K module) and causes overabundance of Cdc42-dependant filopodia formation. On the other hand, p53 has been

shown to interact with other proteins that are localized in the cytoplasm such as tubulin, vimentin and F-actin, sugesting that p53 also plays a part in cell cycle regulation [42].

1.2.3.5 Genetic stability

p53 plays a role in the maintenance of genetic stability, probably controlling the transcription of genes involved in DNA repair processes (chromosomal recombination, chromosome segregation, 'nucleotide-excision' repair of DNA) [12]. In addiction, the key function of p53 to induce cell cycle arrest, avoid cells to proceed through S phase under inappropriate growth conditions (i.e. paucity of ribonucleotide pools), or stimulate cell death programs in case of unrepairable DNA damage, prevent genomic instability [43]. As already known, p53 plays a important role in determinating the mutant frequency and the mechanism of mutation. An increase in mutation on both a molecular and chromosomal level can contribute to the progression to a malignant phenotype and compromise its ability to preserve genomic integrity [44]. The exonuclease activity of wild-type p53 protein seems to be reponsible of this genomic integrity [45]. Some studies have suggested that the association of BRCA1 and p53 is required for transcriptional regulation of genes involved in cell replication and DNA repair pathways. A recent study indicated that p53 mediated homologous recombination through inhibiting BRCA1 over-function via mechanism of transcription regulation in response to DNA repair [46]. On the other hand, loss of p53 results in impaired 53BP1 (p53 Binding Protein 1) focal recruitment to sites of DNA damage induced by ionizing radiation, and therefore p53 plays a role in the regulation of double-strand break (DSB) repair pathway choice [47]. Rb probably plays a role in the activation of the p53 and GADD45 act as link between the p53-dependent cell cycle checkpoint and DNA repair [48, 49].

TP53 genes function to restrain mobile elements and recent observations indicate that transposons become derepressed in human cancers. Together, these emerging lines of evidence

suggest that cancers driven by p53 mutations could represent "transpospoathies," i.e. disease states linked to eruptions of mobile elements. The transposopathy hypothesis predicts that p53 acts through conserved mechanisms to contain transposon movement, and in this way, prevents tumor formation. Currently, the process on how transposon eruptions provoke neoplasias is not well understood [50].

2. p53 and cancerogenesis: the role in urothelial bladder cancer

Mutation of the TP53 tumor suppressor gene is a crucial step of human carcinogenesis, representing the most recurrent defect in many human tumors, including bladder cancer [51]. In fact, TP53 inactivating mutations lead to the disruption of normal cell cycle checkpoints, favoring genomic instability with subsequent cancer cell transformation [52].

The p53 gene family members p53, p73, and p63 display several isoforms derived from the presence of internal promoters and alternative splicing events. p53, p73, and p63 are tumor suppressor genes that promote differentiation, senescence, and apoptosis. p53, unlike p73 and p63, is frequently mutated in cancer often displaying oncogenic "gain of function" activities correlated with the induction of proliferation, invasion, chemoresistance, and genomic instability in cancer cells. A mechanism based on promoting aberrant transcriptional cooperation of mutant p53 (mutp53) with transcription cofactors (e.g., NF-Y, E2F1, Vitamin D Receptor, Ets-1, NF-kB and YAP) or by the interaction with the other p53 family members, p73 and p63, determining their functional inactivation. The interference of mutantp53/p73 and/or mutantp53/p63 interactions, thereby restoring p53, p73, and p63 tumor suppression functions, could be among the potential therapeutic strategies for the treatment of mutant p53 human cancers [53]. Specifically, p53 family is abnormally expressed in bladder cancer and a recent study has hypothesized that high levels of p63 correlate with non-muscle invasive tumours with frequent relapses, whereas p73 overexpression is associated with a more aggressive tumour phenotype [54]. Importantly, DeltaNp63 expression has been shown to be associated with a poor prognosis in invasive tumors [55].

Different mechanisms have been described leading to p53 dysfunction in human cancers. Direct inactivating mutations of the TP53 gene account for about half of cases, including amino-acid-changing point mutation within the DNA-binding domain of the p53 protein (impairing the binding of p53 to specific DNA sequences) or deletion of the C-terminal domain [8]. In the remaining circumstances, impaired p53 function is an indirect consequence of alterations affecting different components of the p53-pathway: abnormal p53 degradation secondary to amplification of the MDM2 gene or caused by deletion of the $p14^{ARF}$ gene; confinement of p53 in the cytoplasm outside the nucleus; increased p53 inactivation and/or destruction by proteins of viral oncogenes [12].

Accordingly, in bladder cancer the TP53 gene alterations are usually missense point mutations, the majority of which are located in the hot-spot region of the TP53 gene. The majority of mutated p53 proteins found in human tumors have amino acid substitutions in the core domain of p53, involving either residues that are implicated in direct contacts with DNA (e.g. Arg 248 and Arg 273) - DNA contact mutations -, or residues that have a key role in maintaining the structural integrity of the core domain (e.g. Arg 175 and Arg 249) - structural mutations [56]. As a result, these mutant proteins are partially or completely unable to specifically bind to DNA.

Mutated p53 protein, differently from the wild-type p53 protein, has a prolonged half-life because of its tie with a heat shock protein (hsc70) conferring greater metabolic stability and resistance to ubiquitin degradation [57]. The prolonged mutant p53 protein half-life (many hours compared to 6 to 30 minutes of wild-type p53) is responsible for nuclear accumulation of p53, thus allowing detection of p53 mutations by immunohistochemistry.

Although a correlation between the immunohistochemical detection of p53 protein and TP53 gene mutations has been described, discordant data exist [58]. Indeed, on the one hand the lack of nuclear p53 protein accumulation does not rule out a mutated p53 gene [59]. Some unusual TP53 gene mutations (null mutations including nonsense, deletions, insertions, and splicing junction mutations that determine premature stop codons and shortened proteins) can be not detectable by

immunohistochemistry [60]. For example, mutations in exons 5 and 8 of the TP53 gene may manifest with a p53 wild-type protein status [61].

Also, wild-type p53 protein can also accumulate in the nucleus in the absence of TP53 gene mutations, as a result of various cellular stressors and/or activated oncogenes upstream regulators of the p53 pathway [62]. An interesting study has investigated the significance of the discordance between TP53 gene mutations (detected by the complete exon sequencing of the entire coding region of the TP53 gene) and p53 protein alterations (by immunohistochemical detection of nuclear p53 accumulation). The results of this study confirm the presence of a discrepancy (albeit in a minority of cases) between the p53 protein and the TP53 gene status. In addiction, since both p53 protein and TP53 gene status independently predict poor clinical outcome, determining the status of both the gene and the protein might provide additional information about bladder cancer patient's prognosis [61]. In fact, nuclear accumulation of p53 protein has been suggested to be a predictive marker of poor clinical outcome in invasive bladder cancer, especially in organ-confined, node-negative MIBC (T1-2bN0) [63, 64] . Moreover, the specific site of the TP53 gene mutation may be significant in predicting outcome of bladder cancer patients.

Indeed, some specific TP53 gene mutations (i.e. mutations in exons 5 and 8, which do not cause p53 inactivation) do not seem to confer the high recurrence risk associated with mutations at other sites, therefore resulting in a better clinical outcome [61]. Obviously, further investigations are required to confirm the role of the site of the mutation in predicting patient's prognosis and sensibility to treatments.

Besides TP53 mutations, other genomic alterations indirectly related to p53 can influence bladder cancer progression [65]. Loss of p21WAF/CIP1 (p21) expression contributes to bladder cancer progression. Bladder cancer patients (especially node-negative) carrying p21-negative and p53-mutated tumors have a greater recurrence and shorter survival rate than those with p21-positive tumors, regardless of tumor grade or pathologic stage. The maintenance of p21 expression seems to nullify the detrimental effects of p53 alterations on bladder cancer progression. Moreover,

alterations of the $p14^{ARF}$/MDM2 regulatory pathway are affected in bladder carcinogenesis. Inactivation of the $p14^{ARF}$ gene (responsible for the transcriptionally inhibition of the MDM2 gene) caused by homozygous deletion or by hyper-methylation of the promoter region correlate with poor prognosis of bladder cancer patients independently from directed alterations of p53 [66, 67]. Amplification of the MDM2 gene, the main negative regulator of p53, has been detected in superficial and invasive bladder tumors [68-70], suggesting the potential use of inhibitors of the MDM2-p53 interaction to restore p53 function [71]. Mutations or deletions of the CDKN2A gene (which encodes for p16) are frequently observed in bladder cancer, correlating with high tumor grade and increased disease recurrence and progression [72].

The current active process of implementation of next generation technologies in oncology practice has identified multiple regions of somatic copynumber alteration, including amplification of PPARG, E2F3, EGFR, CCND1 and MDM2, as well as loss of CDKN2A and RB1. Sequencing of candidate pathways have identified recurrent mutations in TP53, FGFR3, PIK3CA, TSC1, RB1 andHRAS [73]. Using whole-exome and targeted sequencing, some authors found that truncating somatic alterations in the CDH1 gene occur in 84% of plasmacytoid carcinomas and are specific to this histologic variant. Consistent with the aggressive clinical behavior of plasmacytoid carcinomas, which frequently recur locally, CRISPR/Cas9-mediated knockout of CDH1 in bladder cancer cells enhanced cell migration [74].

A recent report based on whole-genome, exome, and transcriptome sequencing of 38 bladder tumors, including four metachronous tumor pairs and 20 superficial tumors, identified an APOBEC mutational signature in one-third. The patient-specific APOBEC signature was negatively correlated to repair-gene expression and was not related to clinicopathological parameters. Mutations in gene families and single genes were related to tumor stage, and expression of chromatin modifiers correlated with survival. Evolutionary and subclonal analyses of early/late tumor pairs showed a unitary origin, and discrete tumor clones contained mutated cancer genes. Kdm6a showed a significantly higher mutation frequency in low-grade and low-stage tumor, and a

number of mutations were either not present or were seen at a significantly lower frequency (e.g., Tp53, Lphn3, and Wnk1). The ancestral clones contained Pik3ca/Kdm6a mutations and may reflect the field-disease mutations shared among later tumors [75]. Again, a discovery exome sequencing screen (n = 17), followed by a prevalence screen (n = 60), identified new genes mutated in this tumor coding for proteins involved in chromatin modification (*MLL2*, *ASXL2* and *BPTF*), cell division (*STAG2*, *SMC1A* and *SMC1B*) and DNA repair (*ATM*, *ERCC2* and *FANCA*). *STAG2*, a subunit of cohesin, was significantly and commonly mutated or lost in UBC, mainly in tumors of low stage or grade, and its loss was associated with improved outcome. Loss of expression was often observed in chromosomally stable tumors, and *STAG2* knockdown in bladder cancer cells did not increase aneuploidy. *STAG2* reintroduction in non-expressing cells led to reduced colony formation. These findings indicate that *STAG2* is a new UBC tumor suppressor acting through mechanisms that are different from its role in preventing aneuploidy [76]. An expanding genomic analysis of bladder cancer by both whole-genome and whole-exome sequencing of 99 patients with bladder cancer confirmed recurrent mutations in genes previously identified and discovered frequent alterations in *STAG2* and *ESPL1*, two genes involved in the sister chromatid cohesion and segregation (SCCS) process. Furthermore, recurrent fusion involving *FGFR3* and *TACC3*, another component of SCCS was also detected. Overall, 32 of the 99 tumors (32%) harbored genetic alterations in the SCCS process. [77].

3. Molecular Taxonomy of Bladder Cancer and oncogenic function of TP53

In recent years, several comprehensive genomic profile-based molecular studies have been carried out to identify clinically relevant genomic alterations – and potential targets for therapy – of advanced urothelial bladder carcinoma [73, 78-81]. In particular, a comprehensive genomic profiling of hundreds of bladder cancer-related genes could aim at overcoming limits related to the low accuracy of immunohistochemistry in detecting the expression of presumed mutated p53 protein, the absence of single gene assays available (widely used in the colorectal cancer treatment

algorithm) for molecularly selecting bladder cancer patients, and the lack of current systemic therapy impact derived from identifying a TP53 altered gene in bladder cancer patients [79].

TP53 gene mutations have been identified in more than half of the samples of metastatic bladder tumors investigated, for example, Ross et al.[79] detected 54% out of the 35 advanced urothelial tumors analyzed with next-generation sequencing; similarly, 55.6% (TP53 gene substitution, truncation, and rarely gene homozygous deletion) of the 295 recurrent/refractory bladder cancers examined in the more recent study of Ross et al [80], or 49% of the 131 urothelial carcinoma samples analyzed by the Cancer Genome Atlas project. This data is in accordance with the 50% p53 nuclear staining (nuclear accumulation) rate identified by IHC [60]. Furthermore, alterations of other components of the p53 network can frequently occur (i.e. MDM2 gene amplification in 9%, and overexpression in 29%), further increasing the rate of TP53 function inactivation (reaching 76% of case) [73].

As shown above, current molecular biology knowledge on bladder carcinogenesis have identify two main molecular genetic alteration related pathways involved in urothelial carcinoma development, one, related to FGFR3 activating mutations and the other one related to TP53 inactivating mutations [5] (Figure 1). This molecular pathway alterations have been reinforced by a recent analysis performed combining information on gene expression, genomic, and gene mutation levels [78]. Two major molecular subtypes of urothelial carcinoma have been identified based on this data, the MS1 and MS2 subtypes, characterized by distinct gene mutation patterns: MS1 tumors marked by FGFR3/PIK3CA activating mutations, while TP53/MDM2 alterations and RB1 losses represent the hallmark of MS2 tumors. Of note, MS2 tumors, and among those with high rates of TP53 mutations and showing also significant genomic instability (higher rate of focal amplifications resulting in tumors with highly rearranged genomes). However, it is important to underline that TP53/MDM2 mutations are not sufficient and might not be necessary to drive the genomic instability phase. Therefore, the high occurrence of TP53/MDM2 alterations observed in MS2 tumors would be a consequence of a subsequent selection for TP53-impaired cells, as cells

refractory to apoptotic signals are more likely to survive a phase of genomic instability than cells that are not [78].

The close link between TP53 mutations and genomically unstable bladder tumors has been confirmed in a subsequent much more comprehensive analysis of 308 tumors, which classify urothelial cell carcinoma in 5 major molecular subtypes (taxonomic classification): urobasal A (FGFR3 and PIK3CA mutated), genomically unstable, urobasal B, SCC-like, and a heterogeneous infiltrated class of tumors [81].

The genomically unstable subtype – having grossly rearranged genomes as key feature - is characterized by frequent TP53 mutations (48%), CCNE and ERBB2 expression, and low cytokeratin expression. Clinically, genomically unstable tumors represent a high-risk group, being muscle invasive lesions in nearly 40% of cases [81].

A growing number of studies suggest that the nature of a p53 mutation in a cell can impact upon cellular properties, clinical responses to therapy and prognosis of a tumor and a particular p53 mutation may confer upon tumor cells a selective survival advantage during chemotherapy. These findings define a new type of mutant p53 selective gain of function, which may compromise the efficacy of cancer chemotherapy [82]. As recently shown, mutant forms of p53 protein interact with NF-Y. The expression of cyclin A, cyclin B1, cdk1, and cdc25C, as well as the cdk1-associated kinase activities, is upregulated after DNA damage, provoking a mutant p53/NF-Y-dependent increase in DNA synthesis. Mutant p53 binds NF-Y target promoters and, upon DNA damage, recruits p300, leading to histone acetylation. The recruitment of mutant p53 to the CCAAT sites is severely impaired upon abrogation of NF-YA expression. Endogenous NF-Y, mutant p53, and p300 proteins form a triple complex upon DNA damage. Aberrant transcriptional regulation underlies the ability of mutant p53 proteins to act as oncogenic factors [83]. Further, novel therapeutic strategies that aim to rescue the function of p53 cancer mutants are now under development [84]. ID4 (inhibitor of DNA binding 4) is a member of a family of proteins that function as dominant-negative regulators of basic helix-loop-helix transcription factors. Growing evidence links ID proteins to cell

proliferation, differentiation and tumorigenesis. ID4 has been identified as a transcriptional target of gain-of-function p53 mutants R175H, R273H and R280K. Depletion of mutant p53 protein severely impairs ID4 expression in proliferating tumor cells. The protein complex mutant p53-E2F1 assembles on specific regions of the ID4 promoter and positively controls ID4 expression. The ID4 protein binds to and stabilizes mRNAs encoding pro-angiogenic factors IL8 and GRO-alpha. This results in the increase of the angiogenic potential of cancer cells expressing mutant p53. The role of transcriptional mutant axis p53, E2F1 and ID4 it is recognized still as undefined molecular mechanism contributing to tumor neo-angiogenesis [85]. On the other hand, different missense mutations in p53 may confer unique activities and thereby offer insight into the mutagenic events that drive tumor progression. [86].

Genomic instability is a common feature of many human cancers. High levels of mutp53 protein facilitate DNA damage accumulation and severely impair BRCA1 and RAD17 expression in proliferating cancer cells. The recruitment of mutp53/E2F4 complex onto specific regions of BRCA1 and RAD17 promoters leads to the inhibition of their expression. BRCA1 and RAD17 mRNA expression is reduced in HNSCC patients carrying TP53 mutations when compared to those bearing wt-p53 gene. Collectively, these findings highlight the direct involvement of transcriptionally active gain of function mutant p53 proteins in genomic instability through the impairment of DNA repair mechanisms [87].

Recent mechanistic developments underlying the importance of mutant p53 (mutp53) gain of function (GOF) and mutp53-induced chemoresistance. Interactions between mutp53 and transcriptional factors, proteins or DNA structures, as well as epigenetic regulation, contribute to mutp53 GOF. As mentioned earlier, major mechanisms of mutp53-induced chemoresistance include enhanced drug efflux and metabolism, promoting survival, inhibiting apoptosis, upregulating DNA repair, suppressing autophagy, elevating microenvironmental resistance and inducing a stem-like phenotype. In bladder cancer, mutp53 did not predict resistance, whereas in

some breast and ovarian cancers, it might be associated with sensitivity to certain chemotherapeutic agents [88].

Bringing small peptides into the clinic remains challenging, mainly owing to the need to deliver the peptides efficiently into the tumor cells. Nevertheless, their greater specificity, relative to small molecules of the types described above, bears the hope for minimal non-specific toxicity, rendering such approach potentially highly promising in the long run [89]. In bladder cancer, the tumour suppressor gene TP53 undergoes frequent missense mutations that endow mutant p53 proteins with oncogenic properties. Until now, a universal mutant p53 gain-of-function program has not been defined, but recent multi-omics approach, including proteome, DNA interactome (chromatin immunoprecipitation followed by sequencing) and transcriptome (RNA sequencing/microarray) analyses, potentially identified the proteasome machinery as a common target of p53 missense mutants. The mutant p53-proteasome axis globally affects protein homeostasis, inhibiting multiple tumour-suppressive pathways, including the anti-oncogenic KSRP-microRNA pathway. In cancer cells, p53 missense mutants cooperate with Nrf2 (NFE2L2) to activate proteasome gene transcription, resulting in resistance to the proteasome inhibitor carfilzomib. Combining the mutant p53-inactivating agent APR-246 (PRIMA-1MET) with the proteasome inhibitor carfilzomib is effective in overcoming chemoresistance in triple-negative breast cancer cells, creating a therapeutic opportunity for treatment of solid tumours and metastasis with mutant p53 [90].

4. The prognostic and predictive role of p53 in bladder cancer patients

Mutation of the TP53 gene (detected by either immunohistochemistry or molecular analysis) is an early event in bladder cancer pathogenesis, and has been associated with tumor progression and poor prognosis [91, 92].

The prevalence of altered p53 expression in early stages of bladder cancer (noninvasive papillary tumors – pTa) is relative low (22%). However, despite the low prevalence, nuclear

overexpression of the p53 protein has been proposed as an independent biomarker of tumor progression [93].

On the contrary, TP53 mutations are considered an early event in the development of carcinoma in situ lesions, where they occur much more frequently (highly significant difference) compared to superficial papillary tumors. The prevalence of TP53 gene alterations in CIS is indeed comparable to that observed in invasive tumors. Inactivation of p53 may therefore lead to increased propensity of CIS lesions to progress to invasive carcinomas, compared to other superficial tumors [94].

The value of pre-treatment p53 status in predicting the risk of tumor progression in pT1 NMIBC represents an interesting source of scientific debate. Several studies reported conflicting data. In particular, various analyses suggested a correlation between p53 overexpression and pT1 NMIBC disease progression and survival [95, 96], whereas other studies failed in demonstrating a prognostic relevance (prediction of disease recurrence and progression) of the p53 phenotype in "high-risk" superficial bladder tumors (T1G3, Tis, and Ta-T1, non-G3 tumors with submucosal lymphatic affection) [97-99].

Moreover, nuclear accumulation of mutated p53 has been postulated as a prognostic biomarker and a predictive factor of clinical response to intravescical bacillus Calmette-Guerin (BCG) therapy in T1 bladder tumors, with inconclusive results [100-102].

Recently a meta-analysis of 12 studies and 712 patients has been performed trying to definitely solve the enigma about the value of p53 over-expression in predicting progression of pT1 NMIBC tumors. This analysis suggests a correlation between p53 over-expression and increased risk of progression of pT1 NMIBC (RR 2.32, 95% CI 1.59 – 3.38), especially in T1G3 patients treated with intravescical BCG [103]. Prospective studies are required to validate this observation, so as to consider changing/intensifying the treatment program and follow-up in this subgroup of patients with superficially invasive bladder cancer at high risk of disease recurrence/progression.

Protein p53 overexpression seems to predict disease progression also in MIBC stage, even without conclusive evidence. Mutations of the TP53 gene are one the first genetic alterations demonstrated to occur in a high proportion of primary invasive bladder cancers [104]. Indeed, nuclear p53 protein accumulation seems to correlate with a more aggressive MIBC phenotype: high proliferative activity, high tumor grade and stage [105], a propensity for tumor progression independently from tumor grade and stage [91], and signs of genomic instability. Furthermore, altered expression of p53 has been proposed as a marker of poor clinical outcome in bladder cancer patients [106, 107]. However, data on the prognostic value of inactivating TP53 mutations in patients with MIBC are often contradictory [108, 109], derive from small sample size and often retrospective series and/or single-center studies thus limiting the possibility to draw definitive conclusions [63, 64].

There is a need for standardization of the assay procedure and the assessment criteria of the specimens, along with the beginning of a prospective multicenter trial to provide definite answers about the role of nuclear p53 protein overexpression as a prognostic marker in bladder cancer.

It is known that TP53 mutations is the most commun genetic altertion in MIBC. The Cancer Genome Atlas (TCGA) analysis revealed that nearly 49% of the samples have TP53 mutations and are mutually exclusive in their relationship with overexpression and amplification of MDM-2, thereby TP53 function is inactivated in 76% of samples. Therefore, alterations in p53 and its associated proteins may be a prognostic marker (especially in organ-confined disease) but this approache has not been confirmed in the prospective setting [73].

Interestingly, besides considering the prognostic significance of p53, several studies have suggested the potential predictive value of TP53 inactivating mutations in conferring a benefit from DNA-damaging therapy. In fact, the lack of normal functioning p53 protein (which normally mediates the G2-M cell-cycle arrest in response to drug-induced DNA damage) increases the susceptibility of cancer cells to DNA-damaging chemotherapies [110]. According to this hypothesis, nuclear p53 overexpression demonstrated a prognostic value for survival in MIBC patients receiving neoadjuvant chemotherapy [111]. However, mutated TP53 dropped its prognostic and

predictive significance in patients treated with MVAC (combination of methotrexate, vinblastine, doxorubicin, and cisplatin) adjuvant chemotherapy [112]. A recent study better delineate a p53-like molecular subtype of MIBC showing primary and acquired resistance to neoadjuvant MVAC chemotherapy characterized by wild-type p53 gene expression signatures [113]. Future studies should determine the molecular basis of these p53-like signatures so as to develop therapeutic approaches to overcome de novo and/or prevent acquired chemo-resistance.

5. Targeting p53 pathway: future therapeutic perspectives

Long-term outcome of bladder cancer patients currently remain far from being acceptable. The intrinsic biological aggressiveness, the high rates of tumor recurrence, along with the primary chemoresistance of this tumor explain the short median survival of patients affected by this incurable cancer. Given the pivotal role of TP53 in bladder cancer development, progression, prognosis, and resistance to immune and chemotherapy, increasing interest is focused on the development of novel anti-cancer therapeutic strategies that recognize p53 as a new potential target. In particular, restoring the physiological function of wild-type p53 tumor suppressor protein could lead to inhibition of tumor cells growth by inducing the expression of downstream genes, such as WAF1/p21/Cip1, Bax and Fas/APO-1, which have crucial roles in arresting the cell cycle and/or inducing apoptosis (Figure 2).

Synthetic peptides derived from the p53 C-terminal domain able to restore the specific DNA sequences binding and transcriptional function of mutant p53 have been identified, leading to p53-dependent apoptosis in tumor cells. In particular, the synthetic 22-mer peptide (peptide 46), corresponding to the carboxy-terminal amino acid residues 361-382 of p53, binds to both the core and C-terminal domains of mutant p53. The tie at the core domain displaces the negative regulatory C-terminal domain, activating p53 [114]. Peptide 46 can therefore activate specific DNA binding of wild-type p53 in vitro and can reestablish the transcriptional function of some mutant p53 proteins, resulting in the reinstatement of growth suppressor function of mutant p53 proteins, thus selectively

destroying cancer cells [115]. Also, a short 22-mer peptide derived from the p53 core domain (peptide 14) has been identified, responsible for the inhibition of p53 activity. Peptide 14 inhibits p53 specific DNA binding, impairs the ability of p53 to transactivate a reporter gene, and blocks p53-induced apoptosis in cell lines. Therefore, the anti-apoptotic effects of this peptide indirectly reinforce the key role of TP53 as a tumor suppressor gene [116]. However the lipophilic nature of these peptides prevents to cross the cell membrane, impairing their intracellular delivery. To overcome this major limit, cell permeable peptides (CPPs) - short cationic peptides able to cross the plasma membrane efficiently - have been extensively investigated as delivery carrier for driving a functional molecule into the cell. The CPP named R11 (poly(11)-arginine) has been proposed as a delivery vehicle for bladder cancer therapy, given its high affinity for the bladder tissue after intravenous or intravesical instillation [117]. Therefore, recently the antitumor activity of a small synthetic peptide derived from the p53 C-terminus conjugated with R11 (R11-p53C) has been investigated in bladder cancer cell lines [118]. R11-p53C has been shown to efficiently and preferentially accumulate into bladder cancer cells, resulting in tumor growth inhibition regardless of the status of p53. Moreover, the R11-mediated delivery of the p53C peptide into p53 mutated tumor cells not only inhibits primary bladder tumor growth in vivo but prolongs animal survival in orthotopic and lung metastatic cancer models without a significant toxicity [118]. The R11-p53C peptide could represent a promising therapeutic agent for the treatment of bladder cancer, especially for metastatic patients.

Among several developing TP53 modulating agents, CP-31398 is a novel molecule potentially able to restore p53 signaling. The mutant p53-conformation modifying drug CP-31398 can reestablish the DNA-binding function of mutant p53 protein through the restoration of a wild-type DNA-binding domain conformation, thus inhibiting tumor cells growth in-vivo [119]. Recently, CP-31398 combined with the inhibition of polyamines biosynthesis using DFMO (an ornithine decarboxylase – ODC – inhibitor) has demonstrated a synergistic inhibitory activity on urothelial bladder tumor growth and prevention of tumor recurrence in a mouse model [120].

Invasive bladder cancer has high morbidity and mortality when metastatic, with almost no therapeutic improvement in many years. Although chemotherapy combined with Chk1 inhibition has been investigated in several cancer types in which TP53 mutation is seen, this combination treatment approach has not been studied in bladder cancer yet. Recently, cancer genome sequencing efforts have identified CDKN1A (p21) mutations at 14% frequency in invasive bladder cancer, co-occurring half the time with TP53 mutations. Hypothetically, combined CDKN1A-TP53 loss would make bladder cancer sensitive to combined treatment with gemcitabine and Chk1 inhibitor, and this has been substantiated in TP53-CDKN1A double-mutant bladder cancer cell lines, 647V and RT-112 which have a remarkable increase in p-Chk1 levels and G2-M arrest in response to gemcitabine treatment, with a heightened sensitivity to combination treatment with gemcitabine and either Chk1 inhibitor PF477736 or AZD7762, in comparison with other bladder cancer cell lines (either TP53 or p21 deficient). In addition, CDKN1A restoration in p21-deficient bladder cancer cells significantly reduced their sensitivity to combined treatment by protecting them from DNA damage and apoptosis. This combination or others involving genotoxic agents and Chk kinase inhibitors is a promising therapeutic approach for bladder cancer harboring these mutations [121].

The anti-cancer activity of PRIMA-1 and PRIMA-1MET (APR-246), two compounds which have been previously reported to reactivate mutant p53 and convert it to a form with wild-type properties, has been studied as targeted therapy in patients with triple-negative breast cancer (TNBC) [122]. Other molecules in study which could restore the p53 family's tumor suppressor functions are mutp53 peptides (SIMPs), NSC59984 and Aptamers[53].

Current data suggest that the challenge ahead is to develop molecular targeted agents that recognize as target a molecule (such as p53) that plays a crucial role mediating tumor progression so as to significantly improve bladder cancer patient's prognosis.

6. Conclusion

Recent years brought important advances on how p53 is inactivated in bladder cancer, and novel mechanistic strategies of functional restoration of structural p53 mutants are now in the horizon to

be applied in clinical oncology. The potential of targeting overexpressed p53 regulators in patients with wild-type p53 is also an active area of clinical research nowadays and is likely to provide future drug targets. To develop molecular targeted agents that recognize p53 as target molecule is now recognized as one important challenge in modern molecular oncology research.

7. Expert commentary

It is known that bladder cancer cannot be treated exclusively on the basis of pathologic staging. Detection of molecular alterations in individual tumors is the base for personalized approach to neoplastic diseases. Currently, the availability of high throughput–omic technologies has enabled an increased understanding of the molecular events that lead to urothelial tumorigenesis. Determination of centrally placed molecules, such p53, as potential targets is key to better treat these patients. p53 protein contains 393 amino acids and a single amino acid substitution lead to functional loss of the gene. In addition, p53 is inactivated by MDM2 (an E3 ligase responsible for ubiquitin-dependent degradation of p53). Inhibiting the p53-MDM2 interaction is a promising approach for activating p53 since this association is well characterized at the structural and biological levels. The p53–MDM2 interaction contains several structural features that are favourable to the design of inhibitors. The efficacy of this inhibitors have been studied in a large panel of tumour cell lines that express wild-type p53. In recent years, small-molecule inhibitors that block the MDM2-p53 interaction have been sought as an attractive strategy to restore the function of p53 and therefore some examples are now in clinical trials.

The rate of altered p53 expression in tumors has been shown to increase progressively from normal urothelium to nonmuscle-invasive bladder cancer, to muscle-invasive and metastatic disease. It is known that the transformation from papillary tumor to invasive phenotype is usually due to accumulation of additional alterations in the p53 pathway. Despite such evidence, controversy still exists on the prognostic role of p53 in bladder tumorigenesis and progression because there are discrepancies related to the diversity of p53 antibodies used in

immunohistochemical assays, variability in interpretation and stratification criteria, and other technical and specimen handling inconsistencies.

The hallmark of chemical damage induced by mutagens in bladder cancer represents fingerprints defined by the frequencies, types of changes, strand orientation, and location of base substitutions. The prevalence of p53 mutation varies significantly by cancer type (this mutation are present in almost half of cases in bladder cancer) and also depends on the developmental stage of a tumor (molecular alterations in p53 have been documented in non-invasive bladder cancer but are much more common in muscle invasive disease).

The restoration of p53 function is a promising anticancer therapeutic approach in bladder cancer patients. Several strategies have been developed in an attempt to restore p53 function (introduction of wild type p53 into the cells with mutant p53). There is alternative strategy using trans-splicing ribozymes which can simultaneously reduce mutant p53 expression and restore wt p53 activity in various human cancers but this has not been tested yet in bladder cancer models. p53 binds as a tetramer to specific DNA recognition sequences adjacent to p53 responsive genes. The mutant p53s lose sequence-specific DNA-binding properties and cannot regulate expression of those genes . Moreover, by binding to and forming inactive tetramers with wt p53, certain mutant versions of the p53 protein can transform cells neoplastically, presumably by inhibiting endogenous wt p53 function in a dominant-negative fashion. New agents are in progress with the aim of restoring p53 function in tumor cells using molecules that target broad classes of mutants to reactivate suppressive functions in tumor cells and also compounds that specifically target particular missense mutants to restore wild-type-like structure, but reportedly, many obstacles remain to optimize these strategies for use in humans.

The extensive knowledge about the molecular mechanisms of oncogenic perturbations of p53 function now opens a host of novel avenues for therapeutic intervention in bladder cancer. At present, the mechanisms by which p53 differentially regulates its diverse target genes remain poorly understood. Some of the p53-mutated proteins acquire new oncogenic functions that strongly

contribute to increasing cell proliferation, invasion, angiogenesis, genomic instability and chemoresistance. This strategy provides a large pool of potential targets for therapy. The use of tumor suppressor genes as anticancer therapeutics has been investigated rigorously in both experimental and clinical research. Some anticancer agents are Apaziquone EO9 that has completed phase II clinical trials for treating superficial bladder cancer and ALT-803 (p53-IL-2 fusion protein designed to target cancer cell that over-express p53, found with high frequency in bladder cancer). Interestingly, several of these pharmacological compounds appear to share a common chemical activity.

There are other indirect pathways to target p53-deficient tumor cells. Checkpoint kinase inhibitors may preferentially sensitize p53-deficient tumor cells to chemotherapy and radiotherapy. Several inhibitors of the Chk1 and Chk2 kinases are now in development, including AZD7762 (AstraZeneca) in Preclinical Pharmacology status, XL844 (Exelixis) in Phase I Clinical Trial status and PF-00477736 (Pfizer) a Phase I Clinical Trial using in combination with gemcitabine. The combination of targeting p53 signalling with other prominent pathways like polyamine biosynthesis can serve as a potential chemo-preventive strategy for invasive urothelial cancers.

The translation of TP53 mutational analysis into the clinic will require large structured clinical trials, in which patients (with defined p53 status) are recruited on the specific inclusion criteria, randomized for treatment according to determined regimens and followed up for long-term therapeutic and clinical end points. Databases such as the current TP53 mutation databases will have a critical role in collecting, structuring, and annotating these data, allowing for the interpretation of TP53 mutation and their use in standard and molecular pathology practice. Various studies have shown that combination of tumor suppressor gene therapy with conventional anticancer therapy can yield synergistic therapeutic benefits. Clinical trials with p53 gene have demonstrated favourable clinical responses and that the treatment is well tolerated.

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Declaration of Interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5-29.

3. Cambier S, Sylvester RJ, Collette L, et al. EORTC Nomograms and Risk Groups for Predicting Recurrence, Progression, and Disease-specific and Overall Survival in Non-Muscle-invasive Stage Ta-T1 Urothelial Bladder Cancer Patients Treated with 1-3 Years of Maintenance Bacillus Calmette-Guerin. Eur Urol 2016;69:60-9.

4. van Rhijn BW, Catto JW, Goebell PJ, et al. Molecular markers for urothelial bladder cancer prognosis: toward implementation in clinical practice. Urol Oncol 2014;32:1078-87.

5. van Rhijn BW, van der Kwast TH, Vis AN, et al. FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 2004;64:1911-4.

6. van der Kwast TH, Bapat B. Predicting favourable prognosis of urothelial carcinoma: gene expression and genome profiling. Curr Opin Urol 2009;19:516-21.

7. Zilfou JT, Lowe SW. Tumor suppressive functions of p53. Cold Spring Harb Perspect Biol 2009;1:a001883.

8. Poyurovsky MV, Katz C, Laptenko O, et al. The C terminus of p53 binds the N-terminal domain of MDM2. Nat Struct Mol Biol 2010;17:982-9.

9. Li M, Brooks CL, Wu-Baer F, et al. Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. Science 2003;302:1972-5.

10. Muller L, Schaupp A, Walerych D, et al. Hsp90 regulates the activity of wild type p53 under physiological and elevated temperatures. J Biol Chem 2004;279:48846-54.

11. Walerych D, Kudla G, Gutkowska M, et al. Hsp90 chaperones wild-type p53 tumor suppressor protein. J Biol Chem 2004;279:48836-45.

12. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000;408:307-10.

13. Siliciano JD, Canman CE, Taya Y, et al. DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev 1997;11:3471-81.

14. Meek DW. Mechanisms of switching on p53: a role for covalent modification? Oncogene 1999;18:7666-75.

15. Wu X, Shell SM, Yang Z, et al. Phosphorylation of nucleotide excision repair factor xeroderma pigmentosum group A by ataxia telangiectasia mutated and Rad3-related-dependent checkpoint pathway promotes cell survival in response to UV irradiation. Cancer Res 2006;66:2997-3005.

16. Sherr CJ, Weber JD. The ARF/p53 pathway. Curr Opin Genet Dev 2000;10:94-9.

17. Weber JD, Jeffers JR, Rehg JE, et al. p53-independent functions of the p19(ARF) tumor suppressor. Genes Dev 2000;14:2358-65.

18. Pomerantz J, Schreiber-Agus N, Liegeois NJ, et al. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell 1998;92:713-23.

19. Mayo LD, Dixon JE, Durden DL, et al. PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. J Biol Chem 2002;277:5484-9.

20. Li T, Kon N, Jiang L, et al. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. Cell 2012;149:1269-83.

21. Flores ER, Lozano G. The p53 family grows old. Genes Dev 2012;26:1997-2000.

22. Tyner SD, Venkatachalam S, Choi J, et al. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002;415:45-53.

23. Bertoli C, Skotheim JM, de Bruin RA. Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol 2013;14:518-28.

24. Chan TA, Hermeking H, Lengauer C, et al. 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. Nature 1999;401:616-20.

25. Utrera R, Collavin L, Lazarevic D, et al. A novel p53-inducible gene coding for a microtubule-localized protein with G2-phase-specific expression. EMBO J 1998;17:5015-25.

26. Carrier F, Georgel PT, Pourquier P, et al. Gadd45, a p53-responsive stress protein, modifies DNA accessibility on damaged chromatin. Mol Cell Biol 1999;19:1673-85.

27. Ohki R, Nemoto J, Murasawa H, et al. Reprimo, a new candidate mediator of the p53-mediated cell cycle arrest at the G2 phase. J Biol Chem 2000;275:22627-30.

28. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995;80:293-9.

29. Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000;288:1053-8.

30. Yee KS, Vousden KH. Complicating the complexity of p53. Carcinogenesis 2005;26:1317-22.

31. Buckbinder L, Talbott R, Velasco-Miguel S, et al. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 1995;377:646-9.

32. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001;7:683-94.

33. Roos WP, Kaina B. DNA damage-induced cell death by apoptosis. Trends Mol Med 2006;12:440-50.

34. Zhang G, Park MA, Mitchell C, et al. Multiple cyclin kinase inhibitors promote bile acid-induced apoptosis and autophagy in primary hepatocytes via p53-CD95-dependent signaling. J Biol Chem 2016;291:20823.

35. Lin Y, Ma W, Benchimol S. Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis. Nat Genet 2000;26:122-7.

36. Dameron KM, Volpert OV, Tainsky MA, et al. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 1994;265:1582-4.

37. Teodoro JG, Parker AE, Zhu X, et al. p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase. Science 2006;313:968-71.

38. Mukhopadhyay D, Tsiokas L, Sukhatme VP. Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. Cancer Res 1995;55:6161-5.

39. Iotsova V, Stehelin D. Down-regulation of fibronectin gene expression by the p53 tumor suppressor protein. Cell Growth Differ 1996;7:629-34.

40. Sun Y, Wenger L, Rutter JL, et al. p53 down-regulates human matrix metalloproteinase-1 (Collagenase-1) gene expression. J Biol Chem 1999;274:11535-40.

41. Gadea G, Roger L, Anguille C, et al. TNFalpha induces sequential activation of Cdc42- and p38/p53-dependent pathways that antagonistically regulate filopodia formation. J Cell Sci 2004;117:6355-64.

42. Roger L, Gadea G, Roux P. Control of cell migration: a tumour suppressor function for p53? Biol Cell 2006;98:141-52.

43. Wahl GM, Linke SP, Paulson TG, et al. Maintaining genetic stability through TP53 mediated checkpoint control. Cancer Surv 1997;29:183-219.

44. Morris SM. A role for p53 in the frequency and mechanism of mutation. Mutat Res 2002;511:45-62.

45. Mummenbrauer T, Janus F, Muller B, et al. p53 Protein exhibits 3'-to-5' exonuclease activity. Cell 1996;85:1089-99.

46. Dong C, Zhang F, Luo Y, et al. p53 suppresses hyper-recombination by modulating BRCA1 function. DNA Repair (Amst) 2015;33:60-9.

47. Moureau S, Luessing J, Harte EC, et al. A role for the p53 tumour suppressor in regulating the balance between homologous recombination and non-homologous end joining. Open Biol 2016;6.

48. Smith ML, Chen IT, Zhan Q, et al. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. Science 1994;266:1376-80.

49. Smith ML, Zhan Q, Bae I, et al. Role of retinoblastoma gene product in p53-mediated DNA damage response. Exp Cell Res 1994;215:386-9.

50. Wylie A, Jones AE, Abrams JM. p53 in the game of transposons. Bioessays 2016;38:1111-6.

51. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer 2015;15:25-41.

52. Hollstein M, Sidransky D, Vogelstein B, et al. p53 mutations in human cancers. Science 1991;253:49-53.

•• The p53 mutational spectrum differs among in diverse types of human cancer and these differences may reflect the etiological contributions of both exogenous and endogenous factors to human carcinogenesis

53. Ferraiuolo M, Di Agostino S, Blandino G, et al. Oncogenic Intra-p53 Family Member Interactions in Human Cancers. Front Oncol 2016;6:77.

54. Papadogianni D, Soulitzis N, Delakas D, et al. Expression of p53 family genes in urinary bladder cancer: correlation with disease aggressiveness and recurrence. Tumour Biol 2014;35:2481-9.

55. Karni-Schmidt O, Castillo-Martin M, Shen TH, et al. Distinct expression profiles of p63 variants during urothelial development and bladder cancer progression. Am J Pathol 2011;178:1350-60.

56. Cho Y, Gorina S, Jeffrey PD, et al. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 1994;265:346-55.

57. Finlay CA, Hinds PW, Tan TH, et al. Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. Mol Cell Biol 1988;8:531-9.

58. Gao JP, Uchida T, Wang C, et al. Relationship between p53 gene mutation and protein expression: clinical significance in transitional cell carcinoma of the bladder. Int J Oncol 2000;16:469-75.

• p53 overexpression and mutation are not superior to TNM staging as prognostic markers

59. Erill N, Colomer A, Verdu M, et al. Genetic and immunophenotype analyses of TP53 in bladder cancer: TP53 alterations are associated with tumor progression. Diagn Mol Pathol 2004;13:217-23.

60. Shariat SF, Bolenz C, Karakiewicz PI, et al. p53 expression in patients with advanced urothelial cancer of the urinary bladder. BJU Int 2010;105:489-95.

• Integration of p53 into a panel of other biomarkers might be necessary to capture a more accurate picture of the biological potential of advanced bladder cancer

61. George B, Datar RH, Wu L, et al. p53 gene and protein status: the role of p53 alterations in predicting outcome in patients with bladder cancer. J Clin Oncol 2007;25:5352-8.

62. Abdel-Fattah R, Challen C, Griffiths TR, et al. Alterations of TP53 in microdissected transitional cell carcinoma of the human urinary bladder: high frequency of TP53 accumulation in the absence of detected mutations is associated with poor prognosis. Br J Cancer 1998;77:2230-8.

63. Malats N, Bustos A, Nascimento CM, et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. Lancet Oncol 2005;6:678-86.

64. Schmitz-Drager BJ, Goebell PJ, Ebert T, et al. p53 immunohistochemistry as a prognostic marker in bladder cancer. Playground for urology scientists? Eur Urol 2000;38:691-9;discussion 700.

65. Mitra AP, Datar RH, Cote RJ. Molecular pathways in invasive bladder cancer: new insights into mechanisms, progression, and target identification. J Clin Oncol 2006;24:5552-64.

66. Berggren P, Kumar R, Sakano S, et al. Detecting homozygous deletions in the CDKN2A(p16(INK4a))/ARF(p14(ARF)) gene in urinary bladder cancer using real-time quantitative PCR. Clin Cancer Res 2003;9:235-42.

67. Kawamoto K, Enokida H, Gotanda T, et al. p16INK4a and p14ARF methylation as a potential biomarker for human bladder cancer. Biochem Biophys Res Commun 2006;339:790-6.

68. Lianes P, Orlow I, Zhang ZF, et al. Altered patterns of MDM2 and TP53 expression in human bladder cancer. J Natl Cancer Inst 1994;86:1325-30.

69. Schmitz-Drager BJ, Kushima M, Goebell P, et al. p53 and MDM2 in the development and progression of bladder cancer. Eur Urol 1997;32:487-93.

70. Maluf FC, Cordon-Cardo C, Verbel DA, et al. Assessing interactions between mdm-2, p53, and bcl-2 as prognostic variables in muscle-invasive bladder cancer treated with neo-adjuvant chemotherapy followed by locoregional surgical treatment. Ann Oncol 2006;17:1677-86.

71. Nag S, Zhang X, Srivenugopal KS, et al. Targeting MDM2-p53 interaction for cancer therapy: are we there yet? Curr Med Chem 2014;21:553-74.

* Hghlight the various roles that the MDM2-p53 interaction plays in cancer

72. Abat D, Demirhan O, Inandiklioglu N, et al. Genetic alterations of chromosomes, p53 and p16 genes in low- and high-grade bladder cancer. Oncol Lett 2014;8:25-32.

73. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014;507:315-22.

•• A comprehensive landscape of molecular alterations of bladder cancer

74. Al-Ahmadie HA, Iyer G, Lee BH, et al. Frequent somatic CDH1 loss-of-function mutations in plasmacytoid variant bladder cancer. Nat Genet 2016;48:356-8.

75. Nordentoft I, Lamy P, Birkenkamp-Demtroder K, et al. Mutational context and diverse clonal development in early and late bladder cancer. Cell Rep 2014;7:1649-63.

76. Balbas-Martinez C, Sagrera A, Carrillo-de-Santa-Pau E, et al. Recurrent inactivation of STAG2 in bladder cancer is not associated with aneuploidy. Nat Genet 2013;45:1464-9.

77. Guo G, Sun X, Chen C, et al. Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. Nat Genet 2013;45:1459-63.

78. Lindgren D, Frigyesi A, Gudjonsson S, et al. Combined gene expression and genomic profiling define two intrinsic molecular subtypes of urothelial carcinoma and gene signatures for molecular grading and outcome. Cancer Res 2010;70:3463-72.

79. Ross JS, Wang K, Al-Rohil RN, et al. Advanced urothelial carcinoma: next-generation sequencing reveals diverse genomic alterations and targets of therapy. Mod Pathol 2014;27:271-80.

80. Ross JS, Wang K, Khaira D, et al. Comprehensive genomic profiling of 295 cases of clinically advanced urothelial carcinoma of the urinary bladder reveals a high frequency of clinically relevant genomic alterations. Cancer 2016;122:702-11.

81. Sjodahl G, Lauss M, Lovgren K, et al. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res 2012;18:3377-86.

82. Blandino G, Levine AJ, Oren M. Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy. Oncogene 1999;18:477-85.

83. Di Agostino S, Strano S, Emiliozzi V, et al. Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. Cancer Cell 2006;10:191-202.

84. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53 and cancer-associated mutants. Adv Cancer Res 2007;97:1-23.

85. Fontemaggi G, Dell'Orso S, Trisciuoglio D, et al. The execution of the transcriptional axis mutant p53, E2F1 and ID4 promotes tumor neo-angiogenesis. Nat Struct Mol Biol 2009;16:1086-93.

86. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012;26:1268-86.

87. Valenti F, Ganci F, Fontemaggi G, et al. Gain of function mutant p53 proteins cooperate with E2F4 to transcriptionally downregulate RAD17 and BRCA1 gene expression. Oncotarget 2015;6:5547-66.

88. He C, Li L, Guan X, et al. Mutant p53 Gain of Function and Chemoresistance: The Role of Mutant p53 in Response to Clinical Chemotherapy. Chemotherapy 2016;62:43-53.

89. Oren M, Tal P, Rotter V. Targeting mutant p53 for cancer therapy. Aging (Albany NY) 2016;8:1159-60.

90. Walerych D, Lisek K, Sommaggio R, et al. Proteasome machinery is instrumental in a common gain-of-function program of the p53 missense mutants in cancer. Nat Cell Biol 2016;18:897-909.

91. Esrig D, Elmajian D, Groshen S, et al. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 1994;331:1259-64.

92. Lipponen PK. Over-expression of p53 nuclear oncoprotein in transitional-cell bladder cancer and its prognostic value. Int J Cancer 1993;53:365-70.

93. Sarkis A, Zhang Z, Cordoncardo C, et al. P53 nuclear overexpression and disease progression in ta-bladder carcinoma. Int J Oncol 1993;3:355-60.

94. Spruck CH, 3rd, Ohneseit PF, Gonzalez-Zulueta M, et al. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 1994;54:784-8.

95. Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, et al. Prognostic factors in stage T1 grade 3 bladder cancer survival: the role of G1-S modulators (p53, p21Waf1, p27kip1, Cyclin D1, and Cyclin D3) and proliferation index (ki67-MIB1). Eur Urol 2004;45:606-12.

96. Serth J, Kuczyk MA, Bokemeyer C, et al. p53 immunohistochemistry as an independent prognostic factor for superficial transitional cell carcinoma of the bladder. Br J Cancer 1995;71:201-5.

97. Gil P, Allepuz C, Blas M, et al. Significance of protein p53 overexpression in the clinical course of high-risk superficial bladder cancer. Urol Int 2003;70:172-7.

98. Dalbagni G, Parekh DJ, Ben-Porat L, et al. Prospective evaluation of p53 as a prognostic marker in T1 transitional cell carcinoma of the bladder. BJU Int 2007;99:281-5.

99. Lopez-Knowles E, Hernandez S, Kogevinas M, et al. The p53 pathway and outcome among patients with T1G3 bladder tumors. Clin Cancer Res 2006;12:6029-36.

100. Saint F, Le Frere Belda MA, Quintela R, et al. Pretreatment p53 nuclear overexpression as a prognostic marker in superficial bladder cancer treated with Bacillus Calmette-Guerin (BCG). Eur Urol 2004;45:475-82.

101. Park J, Song C, Shin E, et al. Do molecular biomarkers have prognostic value in primary T1G3 bladder cancer treated with bacillus Calmette-Guerin intravesical therapy? Urol Oncol 2013;31:849-56.

102. Peyromaure M, Weibing S, Sebe P, et al. Prognostic value of p53 overexpression in T1G3 bladder tumors treated with bacillus Calmette-Guerin therapy. Urology 2002;59:409-13.

103. Du J, Wang SH, Yang Q, et al. p53 status correlates with the risk of progression in stage T1 bladder cancer: a meta-analysis. World J Surg Oncol 2016;14:137.

104. Sidransky D, Von Eschenbach A, Tsai YC, et al. Identification of p53 gene mutations in bladder cancers and urine samples. Science 1991;252:706-9.

105. Esrig D, Spruck CH, 3rd, Nichols PW, et al. p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 1993;143:1389-97.

106. Shariat SF, Tokunaga H, Zhou J, et al. p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol 2004;22:1014-24.

107. Tzai TS, Tsai YS, Chow NH. The prevalence and clinicopathologic correlate of p16INK4a, retinoblastoma and p53 immunoreactivity in locally advanced urinary bladder cancer. Urol Oncol 2004;22:112-8.

108. Frank I, Cheville JC, Blute ML, et al. Prognostic value of p53 and MIB-1 in transitional cell carcinoma of the urinary bladder with regional lymph node involvement. Cancer 2004;101:1803-8.

109. Fleshner N, Kapusta L, Ezer D, et al. p53 nuclear accumulation is not associated with decreased disease-free survival in patients with node positive transitional cell carcinoma of the bladder. J Urol 2000;164:1177-82.

110. Ferreira CG, Tolis C, Giaccone G. p53 and chemosensitivity. Ann Oncol 1999;10:1011-21.

111. Sarkis AS, Bajorin DF, Reuter VE, et al. Prognostic value of p53 nuclear overexpression in patients with invasive bladder cancer treated with neoadjuvant MVAC. J Clin Oncol 1995;13:1384-90.

112. Stadler WM, Lerner SP, Groshen S, et al. Phase III study of molecularly targeted adjuvant therapy in locally advanced urothelial cancer of the bladder based on p53 status. J Clin Oncol 2011;29:3443-9.

113. Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25:152-65.

114. Selivanova G, Ryabchenko L, Jansson E, et al. Reactivation of mutant p53 through interaction of a C-terminal peptide with the core domain. Mol Cell Biol 1999;19:3395-402.

115. Selivanova G, Iotsova V, Okan I, et al. Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nat Med 1997;3:632-8.

116. Protopopova M, Selivanova G. Inhibition of p53 activity in vitro and in living cells by a synthetic peptide derived from its core domain. Cell Cycle 2003;2:592-5.

117. Hsieh JT, Zhou J, Gore C, et al. R11, a novel cell-permeable peptide, as an intravesical delivery vehicle. BJU Int 2011;108:1666-71.

118. Zhang T, Wu K, Ding C, et al. Inhibiting bladder tumor growth with a cell penetrating R11 peptide derived from the p53 C-terminus. Oncotarget 2015;6:37782-91.

119. Takimoto R, Wang W, Dicker DT, et al. The mutant p53-conformation modifying drug, CP-31398, can induce apoptosis of human cancer cells and can stabilize wild-type p53 protein. Cancer Biol Ther 2002;1:47-55.

120. Madka V, Mohammed A, Li Q, et al. TP53 modulating agent, CP-31398 enhances antitumor effects of ODC inhibitor in mouse model of urinary bladder transitional cell carcinoma. Am J Cancer Res 2015;5:3030-41.

•• The restoration of p53 signaling using DFMO, an ODC-inhibiting agent, and CP-31398 (CP) appear to be a promising strategy for MIBC prevention

121. Liu Y, Kwiatkowski DJ. Combined CDKN1A/TP53 mutation in bladder cancer is a therapeutic target. Mol Cancer Ther 2015;14:174-82.

122. Synnott NC, Murray A, McGowan PM, et al. Mutant p53: a novel target for the treatment of patients with triple-negative breast cancer? Int J Cancer 2016.

Figure 1. Molecular pathology modeling of bladder cancer. Non-muscle invasive bladder cancer (low-grade papillary tumors) are typically characterized by activating mutations of FGFR3 and PIK3CA, and deletions of chromosome 9 (more common 9q than 9p) at the molecular level. It is believed that they arise via urothelial hyperplasia, a lesion recognized by pathologic evaluation of the bladder. These are considered as genetically stable tumors. However, muscle invasive carcinoma seems to arise via the flat carcinoma In Situ/dysplasia with TP53 mutation to occur early, together with rare FGFR3 mutations and 9p deletion more common than 9q deletions. These are considered genetically unstable tumors since are they accumulate genomic alterations, including RB1 inactivation, PTEN deletions, and many other genetic events.

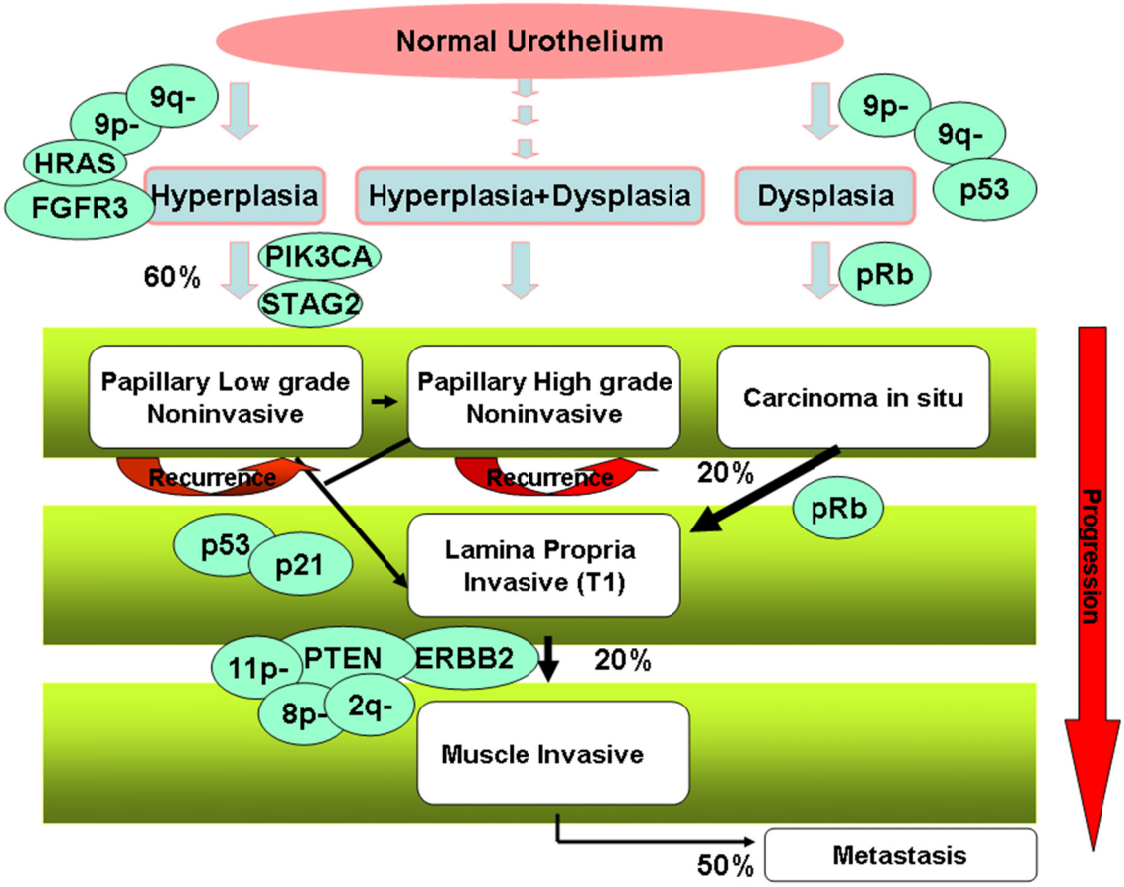

Figure 2. TP53 is a tumor suppressor gene mainly implicated in controlling cell cycle, angiogenesis and apoptosis. Using these capacities, TP53 controls cell cycle progression and therefore avoids replication of damaged and mutated cells.