Review

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Biomarkers in Medicine

The clinical and therapeutic uses of MDM2 and PSMA and their potential interaction in aggressive cancers

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Prostate-specific membrane antigen (PSMA) overexpression is observed in the neovasculature of solid tumors, but not in the vasculature of normal tissues. Increased PSMA expression is positively associated with tumor stage and grade, although its function in cancer remains unclear. Mouse double minute 2 (MDM2) is a negative regulator of the p53 tumor suppressor and is reported to regulate VEGF expression and angiogenesis. Both proteins have been considered as biomarkers and therapeutic targets for advanced solid tumors. Our work and a recent microarray-based gene profiling study suggest there could be signaling interplay between MDM2 and PSMA. We herein review the mechanisms underlining the outgrowth of tumors associated with PSMA and MDM2, their potential interaction and how this may be applied to anticancer therapeutics.

Keywords: interplay • MDM2 • PSMA • therapy • tumor

Tumorigenesis & clinical relevance of MDM2

The transformation potential of Mouse double minute 2 (MDM2) was discovered when it was revealed that MDM2 can bind to the p53 tumor suppressor and thus inhibit its transactivation. Since then, *in vivo* experiments have provided compelling evidence toward the importance of the MDM2/p53 interaction [1].

The p53 protein transcriptionally activates many genes, including the *mdm2* gene [2]. Therefore, p53 is regulated at protein level by MDM2, but once active, p53 triggers the transcription of the *mdm2* gene, locking the proteins into a tight negative feedback loop, vital for cell survival [3].

Apart from its involvement in p53-dependent activities, MDM2 also plays a role in p53-independent cellular functions which contribute to tumorigenesis [4]. It is now known that MDM2 binds and regulates many proteins independent of p53, including proteins involved in DNA repair, DNA replication, cell–cycle control and apoptosis. These pathways work in chorus to preserve the integrity of genetic information and it has been suggested that MDM2 may act as a central node in the regulation of genome stability and, hence, transformation [5].

MDM2 is overexpressed due to amplification in around 10% of all human cancers, and overexpression via other mechanisms also occurs in many human malignancies [6]. This means that development of a therapy involving the inhibition of MDM2 could be used to treat many different patients with various cancer types. Therefore, MDM2 is a major target for drug companies in the development of therapies for cancer patients.

MDM2 as a therapeutic target

The main focus of most therapeutics targeted at MDM2 is to decrease the level of MDM2 protein in cells and therefore allow the reactivation of p53. There are several approaches undertaken to accomplish this: reducing MDM2 levels in cancer cells, inhibiting the E3 ubiquitin ligase complex of MDM2 or the disrupting the interaction between p53 and MDM2 [7].

A basic strategy to decreasing MDM2 protein expression is to specifically target

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the gene using small interfering RNA (siRNA), short hairpin RNA (shRNA) or miRNA approaches [8]. The downregulation of MDM2 using antisense oligonucleotides has led to the stabilization and activation of the p53 pathway in cancer cells growing in culture and in tumor xenograft mice. Interestingly, mutant p53 cells have responded equally as well as those harboring wild-type p53. This result supports the notion that MDM2 has other p53-independent activities involved in its contribution to tumor growth and progression [9].

Another way to reactivate p53 activity is to inhibit the ubiquitin ligase activity of MDM2 [10]. Recently, small-molecule inhibitors have been discovered which specifically target the E3 ligase activity of MDM2. Numerous compounds from this group of inhibitors have been shown to inhibit *in vitro* p53 ubiquitination [11]. Studies using cancer cells reported that these molecules activate p53 signaling and thus induced apoptosis. However, these compounds have shown low potency and selectivity, with more optimization being vital before assessment of the therapy's potential [11,12].

Small molecule inhibitors of the MDM2-p53 interaction have been identified, with the logic that disruption of binding will lead to a degradation of p53. In the past decade, much effort has been invested in this approach, with a recent yield of the first potent and selective pharmacological activators of wild-type p53. A few of these small molecules do represent viable leads for the development of therapeutic agents. The first of these MDM2 antagonists, the nutlins, were identified from a class of compounds named cis-imidazoles [13,14]. The nutlins displace p53 from MDM2 *in vitro* and crystal structures have shown that they bind to the p53 pocket of MDM2 in a way which remarkably mimics the molecular interactions between the two proteins. Proliferating cancer cells have been shown to be effectively blocked in the G1 and G2 phases and undergo apoptosis following treatment with these inhibitors [13]. The nutlins were the first molecules to prove that activation of wild-type p53 using pharmacological inhibitors of the MDM2-p53 interaction was a feasible therapeutic concept. As predicted by the molecular mechanism, it seems that only cells with wild-type p53 are sensitive to these compounds, so p53 status of tumors would need to be determined before any therapeutic approach is undertaken. *In vitro* and *in vivo* studies conducted using the nutlins have verified their antitumor effect [15].

Recently, there has been an influx of small molecule MDM2 inhibitors undergoing clinical trials, with seven currently in Phase I, all of which target the interaction between MDM2 and p53 [16]. The first of these, AM 232 was discovered through studies into AM 8553, a compound produced using *de novo* design strategy based on the structure of MDM2. AM 232 targets a shallow cleft on the surface of MDM2; has been found to be potent and selective; and has shown notable antitumoral activity *in vivo* [17,18]. Roche currently have two compounds in trials, R05045337 (RG7112) and R05503781 (RG7388), with R05045337 being based on the original Nutlin family of inhibitors [19]. R05503781 is the second generation of R05045337, with superior potency and selectivity [20]. Novartis have developed a drug named CGM097 which has been optimized and moved to clinical trials, with analogs currently being developed and their efficacy assessed *in vivo* [21]. A fifth inhibitor, named DS-3032b was developed by Daiichi Sankyo following a miniaturized thermal denaturation assay used to screen chemical libraries, leading to a unique series of benzodiazepinedione antagonists of the MDM2-p53 interaction being discovered [22]. SAR4058383 was developed by the University of Michigan and Sanofi, with promising early studies showing that a single optimized oral dose of the compounds leading to complete tumor regression in the SJSA-1 cell line model [23]. Finally, MK-8242 was developed by Merck Sharp & Dohme Corp and a clinical trial of patients with solid tumors was recently completed [16].

It is well known that, following DNA damage, p53 is activated and this leads to arrest of the cell cycle and apoptosis in sensitive tissues [24]. Therefore, a main concern of using therapeutics to activate p53 is the effect of this act in normal tissues. Mice with MDM2 reduced to around 30% of its normal level show increased p53 in all tissues tested. Apart from slight disturbances in hematopoiesis and an increase in apoptosis in the small intestine, these mice developed normally [12,25]. Further, nude mice can tolerate nutlin-3 for 3 weeks at doses that cause inhibition and regression of tumors [15]. It seems that these studies suggest that perhaps activation of p53 through MDM2 inhibition may be a promising therapeutic option and can be well tolerated *in vivo* [12].

Although use of these inhibitors can be extremely useful for cancer therapeutic development, their effectiveness depends on multiple factors. First, as already mentioned, the therapeutic effect of p53 activation could be abolished through the potential cell cycle arrest or cell death caused by p53 activation. Second, MDM2 is not the only regulator of p53 in cells, so other interactors may hinder the cellular response to MDM2 antagonists. For example, MDMX, another p53-binding protein, cannot be displaced by nutlin-3, so the effectiveness of nutlins can be compromised in tumor cells which overexpress MDMX [12].

Therefore, although MDM2 represents a useful and potent target for inhibitors in the impedance of can-

cer progression, an ideal therapeutic has not yet been identified. However, with our new understanding of the functions of p53-dependent and -independent MDM2 and accelerating speed of drug development, it is possible that an MDM2-targeted therapy could be effectively applied to halt tumor outgrowth in patients (clinical trials of MDM2-targeted therapeutics summarized in Table 1 [26]).

Clinical relevance of PSMA

Prostate-specific membrane antigen (PSMA) has only a few sites of expression in normal tissues: the prostate epithelium, the kidney proximal tubules, the nervous system glial cells and the small bowel jejunal brush border [27,28]. At the jejunal brush border the protein is better known as FOLH1 and here it converts dietary folate (pteroylpolyglutamate) to monoglutamated folate [29,30]. In the nervous system, however, PSMA carries out its N-acetylated alpha-linked acidic dipeptidase (NAALADase) function and hydrolyses N-acetylaspartylglutamic acid (NAAG), the most abundant peptide neurotransmitter in the mammalian nervous system [30,31]. The presence of PSMA in the prostate and proximal tubules of the kidneys is not yet understood but it has been suggested that this could be due to the reuptake of folate in the kidneys and the release of monoglutamated folates into the seminal fluid [30].

The cell surface expression of PSMA has been shown to increase directly in cancers of higher grade, metastases, prostate cancer which is castration-resistant and cancers giving an adverse clinical outcome [32,33]. Furthermore, PSMA expression was observed to decrease in the prostate cancer cell line, LNCap, when incubated with androgen dihydrotestosterone and, conversely, cells grown in androgen-stripped media displayed increased PSMA expression [34]. It is clear that increased expression and enzymatic activity of PSMA in aggressive tumors are telling of a selective advantage bestowed by PSMA upon tumor cells and this contributes to prostate carcinogenesis [35].

PSMA has also been reported to be expressed in the neovasculature of a considerable majority of malignant solid tumors (bladder, breast, kidney pancreas, lung and melanoma), but not in the corresponding normal vasculature [36].

PSMA has been identified as an excellent target for imaging and therapy of cancer for several reasons. The specificity of its expression is a key factor, with only a limited number of normal tissue types expressing the protein, along with PSMA's large extracellular region allowing therapeutics to be exclusively targeted to the

Table 1. Clinical trials targeting mouse double minute 2 for cancer treatments.

Intervention	Target	Cancer type targeted	Affiliates	Trials stage	Current status
Biological: CD105/Yb-1/SOX2/CDH3/MDM2 multiplasmid vaccine	MDM2-expressing tumor cells	Advanced solid tumors, lymphomas	University of Washington	Phase I	Not yet recruiting
Drug: RO6839921	MDM2-p53 interaction	Neoplasms, leukemia, myelodysplastic syndrome	Hoffman-La Roche	Phase I	Recruiting
Drug: DS-3032	MDM2-p53 interaction	HER2-negative stage III–IV	Daiichi Sankyo Inc.	Phase I	Recruiting
Drug: RO5045337	MDM2-p53 interaction	Soft tissue sarcoma, neoplasms, leukemia	Hoffman-La Roche	Phase Ib	Active, not recruiting/ Completed
Drug: RO5503781	MDM2-p53 interaction	Neoplasms	Hoffman-La Roche	Phase I	Completed
Drug: thioureidobutyronitrile	p53 activator	Solid Tumors	Cellceutix Corporation	Phase I	Recruiting
Drug: HDM201	MDM2-p53 interaction	Advanced tumors (TP53wt) liposarcoma	Novartis Pharmaceuticals	Phase I	Recruiting
Drug: CGM097	MDM2-p53 interaction	Solid tumors with p53 wt states	Novartis Pharmaceuticals	Phase I	Recruiting
Drug: SAR405838	MDM2-p53 interaction	Neoplasm malignant	Sanofi	Phase I	Ongoing
Drug: MK-8242	MDM2-p53 interaction	Solid tumors	Merck Sharp & Dohme Corp.	Phase I	Completed
Drug: AM 232	MDM2-p53 interaction	Advanced solid tumors, multiple myeloma	Amgen	Phase I	Recruiting

tumor region and malignant cells. The fact that PSMA is a transmembrane protein is also important, as its extracellular region can be easily targeted by therapeutics. Also, the presence of an internalization sequence within the protein means that therapeutics targeted at PSMA could be internalized through binding. Finally, PSMA's peptidase activities, means that it could be involved in the processing of a pro-drug targeted at tumor cells [36–38]. Therefore, there is a very strong case for the use of PSMA as a biomarker and therapeutic target in the fight against cancer.

PSMA as a biomarker

Since prostate cancer tissues shows high PSMA expression and increased enzymatic activity of PSMA compared with normal and benign hyperplasia prostate tissues [39,40] the use of PSMA as a biomarker for prostate cancer is under investigation. A direct correlation has been identified in adenocarcinomas between the expression of PSMA and Gleason score, which is used to stage prostate cancer [40]. A study by Ross *et al.* suggests that PSMA could act as a biomarker for prognosis as it shows a significant correlation with adverse prognostic factors such as tumor grade, aneuploidy, biochemical recurrence and pathological stage [32].

The current standard for early detection of prostate cancer involves a digital rectal examination and a serum test for prostate-specific antigen (PSA). Despite its use, there is no definite level of PSA which can actively distinguish between men with prostate cancer and those with a benign hyperplasia, leading to false-positive results and overtreatment of men with limited disease [33].

PSMA immunohistochemistry was seen to have a higher (84%) sensitivity than PSA (58%) in staining of tissues from metastatic sites. Strong, diffuse staining was seen in 17 of 19 cases of metastatic prostate cancers, compared with 13 from PSA staining. Positivity for either of the molecules was seen in 89% of metastatic prostate cancer and this combination immunohistochemistry was slightly more sensitive than that of PSMA alone, indicating that a combination of PSMA and PSA immunohistochemistry could be a beneficial prognostic assessment for patients [41].

Quantification of PSMA and PSA levels in peripheral blood showed significant differences among BPH, locally confined prostate cancer and metastasized prostate cancer in expression of PSA and PSMA. It was found that one cancer cell could be detected in 2×10^7 mononuclear cells [42].

The first clinical agent targeting PSMA in prostate cancer was the monoclonal antibody 7E11/CYT-356, which was labeled with Indium-111 and known as ^{111}In-capromab or ProstaScint [30,43,44]. The sensitivity and specificity of the antibody has differed in studies, with an average sensitivity of 60%, a specificity of 70%, a positive predictive value of 60% and a negative predictive value of 70% [45,46]. These poor results could be a consequence of ^{111}In-capromab recognizing an intracellular epitope, and therefore only binding molecules in cells with a damaged cell membrane [30].

This led to the development of second-generation antibodies which can bind to the extracellular region of PSMA and thus could be superior to the capromab pendetide. One of these developed antibodies, J591, has shown potential in imaging primary prostate cancer, as well as bone metastases. Clinical trials with ^{99m}Tc-labeled J591 established detection of primary prostate cancer, as well as prostate bed recurrence and distant metastases, again, including metastasis to bone [30,47]. Several other developed monoclonal antibodies (3/A12, 3/E7 and 3/F11) bind to different epitopes of PSMA [35]. A study using ^{64}Cu-3/A12 for PET imaging of prostate cancer xenograft showed a good tumor-to-background ratio [44]. A fourth monoclonal antibody targeting PSMA, 3C6, has been labeled with ^{111}In for imaging in prostate cancer [48].

Radiolabeled PSMA inhibitor N-[N-[-(S)-1,3-dicarboxypropyl]carbamoyl]-S-[^{11}C]methyl-l-cysteine (DCFBC) has been successfully used in PET imaging of xenografts expressing PSMA [49]. The molecule was labeled with ^{18}F, with studies into its biodistribution and imaging showing a high uptake of ^{18}F-DCFBC in PSMA-positive tumors but slight or no uptake in tumors negative for PSMA [50]. Urea-based compounds have also been identified as possible targets for imaging of prostate cancer with PET and SPECT [51]. MIP-1095 and MIP-1072, which are small-molecule inhibitors targeting PSMA, have shown a high affinity for PSMA and their uptake when labeled with ^{123}I has been successfully imaged by SPECT [52,53].

PSMA as a therapeutic target

PSMA has been exposed as an attractive therapeutic target due to its expression being 100- to 1000-fold less in normal cells in comparison to prostate carcinoma cells [54]. So far, antibody-based radiotherapy, antibody-drug conjugates (ADC), PSMA-targeted pro-drug therapy and PSMA-based immunotherapy have been investigated [30].

The leading PSMA antibody-based radiotherapeutic is Lutetium-177 J591, which showed acceptable toxicity and excellent metastatic site targeting in a Phase I clinical trial [55]. A recent Phase II clinical trial utilized Lutetium-177 J591 in patients with metastatic castration-resistant prostate cancer [56]. Just less than 60% of patients showed a decrease in PSA levels with 1/10 showing a reduction of more than half and the

Table 2. Clinical trials utilizing prostate-specific membrane antigen for cancer imaging and treatments.

Intervention	Use	Cancer type targeted	Affiliates	Trials stage	Current status
Drug: 68Ga-PSMA	Imaging/diagnosis	Prostate cancer	Ebrahim Delpassand	Phase II	Recruiting
Drug: PSMA ADC 2301	Treatment	mCRPC	Progenics Pharmaceuticals, Inc.	Phase II	Completed
Drug: PSMA ADC BrUOG 263	Treatment	Glioblastoma multiforme, gliosarcoma	Heinrich Elinzano, MD	Phase II	Active, not recruiting
Drug: PSMA ADC 1301	Treatment	mCRPC	Progenics Pharmaceuticals, Inc.	Phase I	Completed
Biological: peptide vaccine/ drug: poly IC-LC	Vaccine treatment	Prostate cancer	H. Lee Moffitt Cancer Center and Research Institute	Phase I	Active, not recruiting (has results)
Drug: Anti-PSMA designer T cells	Treatment	Prostate cancer	Roger Williams Medical Centre	Phase II	Active, not recruiting
Biological: rsPSMA protein plus alhydrogel vaccine	Vaccine treatment	Prostate cancer	Memorial Sloan–Kettering Cancer Centre	Phase I	Completed
Biological: anti-PSMA monoclonal antibody MDX1201-A488	Imaging/diagnosis	Prostate cancer	City of Hope Medical Centre	Phase I	Recruiting
Biological: gene modified T cells	Treatment	Prostate cancer	Roger Williams Medical Centre	Phase I	Active, not recruiting
Biological: engineered autologous T cells/drug: cyclophosphamide	Treatment	Prostate cancer	Memorial Sloan–Kettering Cancer Centre	Phase I	Recruiting
Biological: human PSMA plasmid DNA vaccine	Treatment	Kidneycancer	Memorial Sloan–Kettering Cancer Centre	Phase I	Active, not recruiting
Drug: 18F-DCFBC	Imaging/diagnosis	Prostate cancer	Sidney Kimmel Comprehensive Cancer Center	Phase II	Active, not recruiting
Device: ProxiScan (scintigraphic rectal probe)	Imaging/diagnosis	Prostate cancer	Sidney Kimmel Comprehensive Cancer Center	Phase I	Completed
Biological: PSMA prostate cancer vaccine/IL-12	Treatment	Prostate cancer	University of Chicago	Phase II	Completed
Biological: PSMA/PRAME (MKC1106-PP)	Treatment	Advanced cancer	Mannkind Corporation	Phase I	Completed
Drug: 123I-MIP-1072	Imaging/diagnosis	Prostate cancer	Molecular Insight Pharmaceuticals, Inc.	Phase I	Terminated
Drug: 89Zr-J591	Imaging/diagnosis	Glioblastoma multiforme, gliosarcoma	Memorial Sloan–Kettering Cancer Centre	Phase I	Recruiting
Drug: 111-In capromab pendetide	Imaging/diagnosis	Prostate cancer	Molecular Insight Pharmaceuticals, Inc.	Phase I	Completed
Biological: androgen ablation/dendritic cell vaccine	Treatment	Prostate cancer	Pawel Kanlinkski	Phase I	Recruiting
Drug: 89Zr-DFO-huJ591	Imaging/diagnosis	Prostate cancer	Memorial Sloan–Kettering Cancer Centre	Phase II	Active, not recruiting

Data taken from [63].

Table 2. Clinical trials utilizing prostate-specific membrane antigen for cancer imaging and treatments (cont.).

Intervention	Use	Cancer type targeted	Affiliates	Trials stage	Current status
Drug: G-202	Imaging/diagnosis	Glioblastoma multiforme, advanced hepatocellular carcioma	GenSpera, Inc.	Phase II	Recruiting Phase II
Drug: 99mTc MIP 1404	Imaging/diagnosis	Prostate cancer	Molecular Insight Pharmaceuticals, Inc.	Phase I	Active, not recruiting
Radiation: [^{89}Zr]Df-IAB2M	Treatment	Prostate cancer	ImaginAb, Inc.	Phase II	Recruiting
Drug: EC1169	Treatment	Prostate cancer	Endocyte	Phase I	Recruiting
Drug: GVAX and ipilimumab	Treatment	mCRPC	VU University Medical Center	Phase I	Terminated

Data taken from [63].

therapeutic showed accurate targeting of metastatic sites [38]. The higher concentration used in the trials (70 mCi/m^2) led to longer survival of patients (almost 22 months, compared with 12 months), but resulted in increased grade 4 hematologic toxicity and platelet transfusions [30,56].

J591 antibody has also been utilized in the production of ADC, which involves the linking of a drug or toxin to an antibody [38]. MLN2704 is an antimicrotubule agent which has been conjugated to J591. Phase I studies in over 20 patients showed PSA levels dropped by more than half in two patients, although grade 3 toxicities occurred in three of the patients [30,57]. A multicentre Phase II/III clinical trial undertaken in 62 men with metastatic castration-resistant prostate cancer showed stabilization or decline in PSA in a majority of patients; however, limitation of treatment occurred due to toxic effects of the compound [30,38].

Work has been undertaken in xenograft LNCaP mice, using an immunotoxin consisting of the anti-PSA mAb E6 and deglycosylated ricin A, showing reduced tumor growth [38,58]. Another group coupled melitten-like peptide 101 to J591 and also saw a significant tumor growth inhibition in mice [38,59]. Monomethylauristatin E (MMAE) has also been conjugated to a mAb which recognized the PSMA external domain [60].

Recently, a group engineered a prodrug for tumor endothelial cells in prostate cancer therapy [61]. Their work involved the coupling of a PSMA-specific peptide to thapsigargin (inhibitor) of the sarcoplasmic/endoplasmic reticulum calcium adenosine triphosphate (SERCA) pump. SERCA is a vital cellular protein which is essential for the viability of all cell types. Before cleavage of the PSMA-specific molecule, the conjugate is inactive. However, post-cleavage, local SERCA inhibition ensues [30]. Preclinical xenograft models treated with thapsigargin showed significant prostate cancer tumor regression at doses which were modestly toxic to the host [61].

The use of immunotherapy in oncology has been long utilized, but only recently has work on PSMA as a target begun to be investigated [38]. This type of therapy is based on the concept that IL-2 stimulates natural killer cells, thus enhancing antibody-dependent cellular cytotoxicity. A Phase II trial of the anti-PSMA monoclonal antibody J591 was undertaken in patients with recurrent prostate cancer for 8 weeks, with patients receiving continuous low-dose subcutaneous IL-2 every day, with infusions of J591 weekly. Of 17 patients, nine had stable PSA, with declines of up to 34%. The therapy was well tolerated and the toxicity was low, with non-progressors showing a trend with significant natural killer (NK) cell expansion [30,38,62].

Thus, although PSMA-targeted therapy is yet to yield clinically important effects on the survival of patients without severe side effects ensuing, several fields are currently under study and as our molecular techniques and our understanding of tumor biology become more advanced, PSMA-therapeutics are likely to play an important role in the development of treatment for cancer patients [30] (clinical trials of PSMA as a biomarker and therapeutic target summarized in Table 2 [63]).

Tumor-associated angiogenesis mediated by MDM2 & PSMA

Vascular endothelial factor (VEGF) is a potent angiogenic factor that plays an important role in regulating normal physiological and pathological angiogenesis. Correctly timed expression of VEGF at appropriate levels is crucial for normal development of vascula-

ture and homeostasis, but also vital for solid tumor growth. VEGF is highly expressed in solid tumors and is required for the development and maintenance of blood vessels within the tumor, which is a prerequisite for successful tumor growth and metastasis.

A co-expression study was undertaken to evaluate the correlated expression of MDM2 and VEGF, finding that, over eight different cancer cell lines, higher MDM2 expression meant higher VEGF mRNA, with the cell lines with lost p53 function showing highest VEGF levels [64]. They verified their findings further by inhibiting MDM2 using a specific MDM2-specific antisense oligonucleotide (HDMAS5) and saw a significant decrease in VEGF mRNA and protein levels. Finally, they proved that transfecting the MDM2 gene in the prostate cancer cell line, LNCaP, produced a cell line overexpressing MDM2 and VEGF. The same group then identified MDM2 as a regulator of VEGF expression in cancer cells. HUVECs were treated with tumor-conditioned media from HMAS5-treated cancer cells. They found that VEGF release from cells and VEGF-dependent angiogenesis were significantly reduced *in vitro* [65].

Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor which generates a response to oxygen deprivation due to hypoxic conditions. Active HIF-1 is comprised of two subunits: HIF-1β is constitutively expressed in the cell, however, under normoxic conditions HIF-1α is covalently modified by prolyl hydroxylases, allowing VHL E3 ubiquitin ligase to polyubiquitinate and thus targets HIF-1α for degradation [66,67]. Factor inhibiting HIF-1 (FIH-1) can also hydroxylate HIF-1α, preventing coactivator binding and so inhibiting transcription of target genes [68]. Following a decrease in cellular oxygen levels, the rates of hydroxylation are decreased, VHL does not bind, HIF-1α is stabilized and the HIF-1 the heterodimer can form [69]. Overexpression of HIF-1α has been linked to angiogenesis, tumor invasion and a poor prognosis in many types of cancer [70–73]. The HIF-1 transcription factor binds to the 5′ flanking sequence of *vegf* and is essential for the transactivation of *vegf* during hypoxia (see Figure 2).

It has been known for some time that hypoxia is a physiological inducer of tumor suppressor p53, with p53 protein levels increasing under hypoxic conditions [74]. Since MDM2 is the most important negative regulator of p53, many groups began to look into the precise mechanism of the interaction between hypoxia and p53, and whether MDM2 was involved [68,75–84].

In 2005, a study showed that MDM2 positively activates HIF-1α in hypoxic tumor cells. Co-immunoprecipitation showed that MDM2 precipitates with HIF-1α, completely independently of p53 [75]. Evidence toward the involvement of MDM2 in the regulation HIF-1α expression under hypoxic conditions came from Lau *et al.* [76], who found that inhibitory effects on HIF-1α by the anti-cancer drug 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1), was MDM2-dependent and that overexpression of MDM2 reversed its inhibitory effects. A very recent study also suggested that, under hypoxic conditions, MDM2 is capable of ubiquitinating HIF-1α with its E3 ubiquitin ligase domain in a PTEN/PI3K-dependent manner. The group's results suggested that the PI3K-AKT signaling axis is a requirement for the preservation of HIF-1α stability during hypoxia [69] (see Figure 2).

Another study showed that nutlin-3 conferred anti-angiogenic activity. It was found that nutlin-3 dose-dependently suppressed the total tube length and the number of capillary connections developed from HUVECs. Also, the migration of endothelial cells was shown to be significantly inhibited by nutlin-3 in response to various chemoattractants [77]. In the same year, two more reports were published demonstrating the inhibition of HIF-1α by nutlin-3, leading to inhibited VEGF production and thus angiogenesis in tumors [78,79]. Lee *et al.* [80] then suggested a mechanism through which this occurred after finding that nutlin-3 downregulated HIF-1α in p53-positive cells but also functionally inactivated HIF-1α in p53-negative cells. Of these two occurrences, they found that the second mainly contributed to VEGF suppression by nutlin-3. It was reported that MDM2 competes with FIH which is a regulator of HIF-1α, by binding its C-terminal transactivation domain (CAD). FIH hydroxylates Asn803 in the CAD domain under normoxic conditions. However, when conditions are hypoxic, this hydroxylation is inhibited due to the limited oxygen and so HIF-1α becomes stable and active [68,81]. When MDM2 competes for binding of the CAD of HIF-1α, this hydroxylation is inhibited and so p300 is recruited. They found that nutlin-3 reinforced the FIH-mediated inactivation of HIF-1α through inhibiting any interaction between CAD and MDM2 [80]. This theory is in direct contrast to the report by LaRusch *et al.* [78], who reported that the N-terminal domain of HIF-1α was needed for binding of MDM2. This could imply that each domain of HIF-1α interacts individually in different ways with MDM2 or they cooperate to bind MDM2.

Therefore, it is widely accepted that hypoxia induces VEGF transcription through induction of HIF-1α. However, in 2011, a group set out to investigate the post-transcriptional regulation occurring, in which HIF-1α does not seem to be important [82]. Their work followed on from a study which showed that in rat cardiac myo-

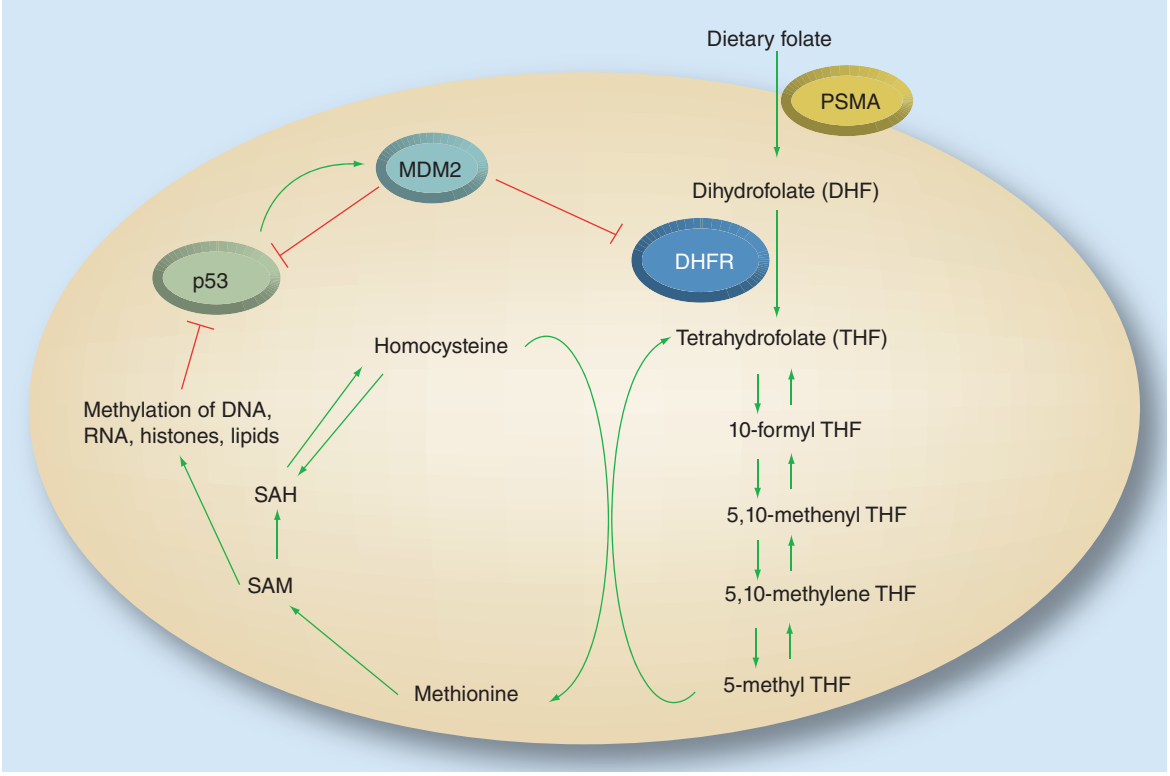

Figure 1. Hypothesized mouse double minute 2 and prostate-specific membrane antigen interaction through folate metabolism in aggressive tumors. PSMA metabolizes dietary folate to produce dihydrofolate, which is then converted to tetrahydrofolate by DHFR. DHFR is regulated by MDM2 through its RING-finger dependent E3 ligase activity, which is known to also regulate the p53 tumor suppressor. The p53 tumor suppressor is regulated by methylation of DNA, RNA, histones and lipids, which is governed by folate metabolism. Therefore, we hypothesize that in cancer cells, PSMA may be involved in the expression or activity of MDM2 through aberrant regulation of p53 methylation via folate metabolism.

cytes hypoxia can induce VEGF steady-state mRNA 25-fold; however, the hypoxia-mediated transcription rate of VEGF increases just 3.1-fold [85]. Their results showed that the RING domain of MDM2 can bind to AU-rich elements of the VEGF 3′ untranslated region (UTR) and regulate VEGF mRNA stability and thus its translation. Interestingly, they also demonstrated that during hypoxia, MDM2 was dephosphorylated and translocated to the cytoplasm from the nucleus, where it was able to induce high levels of VEGF in cancer cells [82]. The same group then undertook a study to elucidate whether p53 played a role in the interaction between MDM2 and VEGF. They did this through the use of two cell lines, MCF-7 which expresses wild-type p53 and MDA-MB-468, which expresses mutant p53. They studied the effect of nutlin-3 and anti-MDM2 antisense oligonucleotide (ASO), on these cell lines and saw that ASO significantly inhibited the VEGF transcript and protein levels in a dose- and time-dependent manner, whereas nutlin-3 had no effect. The effect of hypoxia was also studied, and it was observed that ASO treatment significantly inhibited HIF-1α expression at 3, 6 and 12 h of hypoxia in both cell lines. An inhibitory effect on HIF-1α was also seen in the nutlin-3 treated MCF-7 (wild-type p53) but not in MDA-MB-468 (mutant p53). The group used siRNA targeted at HIF-1α as well as ASO treatment, and found that HIF-1α only seems to have a role in VEGF production in early hypoxia (at 6 h, but not at 48 h). HIF-1α siRNA did not reverse the inhibitory effect of ASO on VEGF production. Therefore, the group surmised that ASO downregulates hypoxia-induced VEGF production via a HIF-1α-independent mechanism. When the same experiment was undertaken using nutlin-3, it was seen that nutlin-3 significantly inhibited the level of secreted VEGF from the MCF-7 cells at early hypoxia. When the cells were transfected with HIF-1α siRNA, nutlin-3 failed to inhibit VEGF production. This exhibits that the effect of nutlin-3 on VEGF regulation in early hypoxia is HIF-1α-dependent. ASO treatment of mice with tumors of each cell type showed a substantial decrease in serum VEGF levels, measured by ELISA. On the other hand, nutlin-3 treatment produced little effect on VEGF production [83].

A very recent study investigated the precise mechanism supporting the induction of VEGF transcrip-

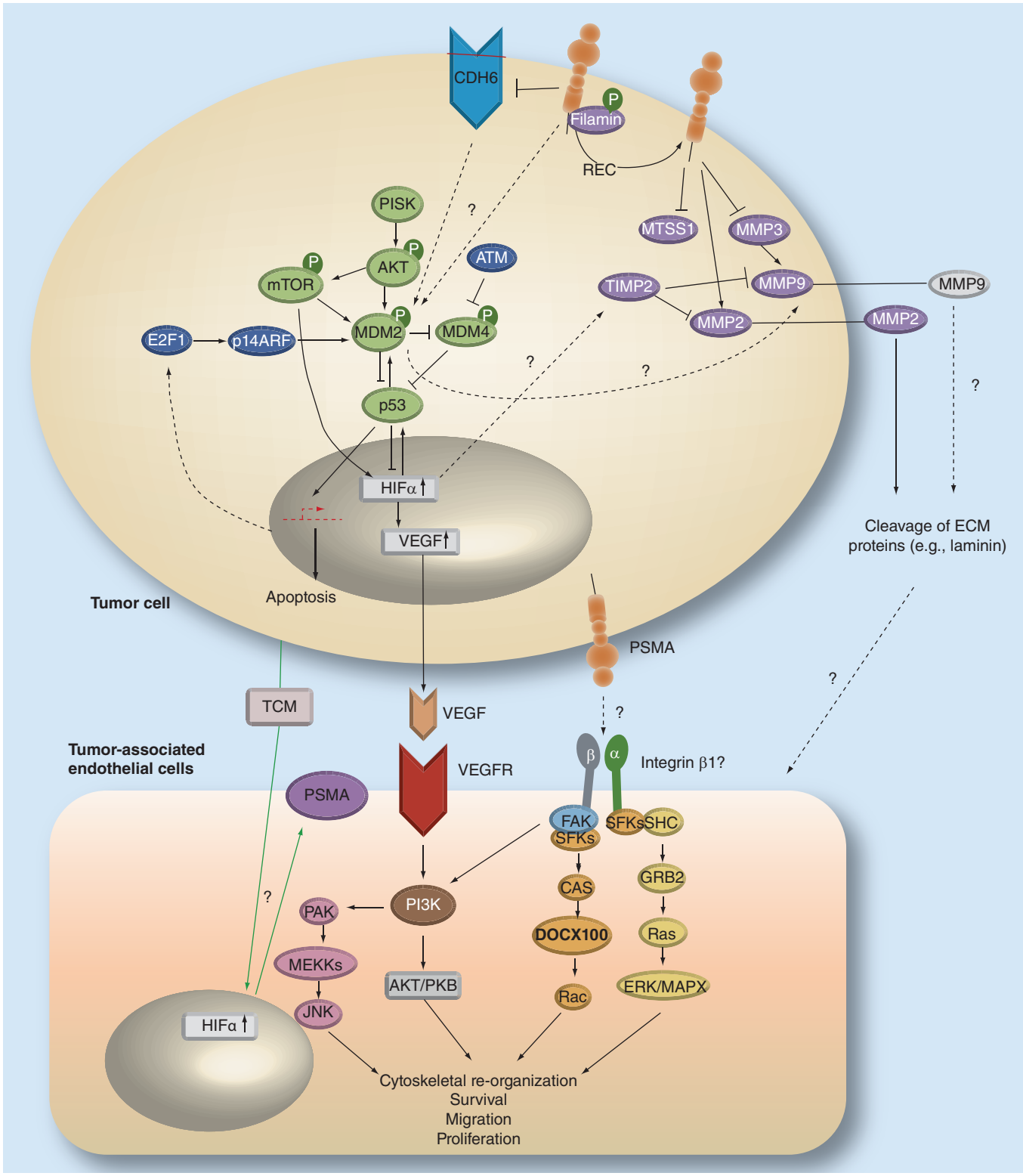

Figure 2. Proposed interplay roles of mouse double minute 2 and prostate-specific membrane antigen in tumor invasion through multiple signaling pathways. MDM2 and PSMA have both been linked to MMP-2 and MMP-9. HIF-1α is known to regulate the MMP inhibitor, TIMP-1. Since MDM2 activates HIF-1α, both PSMA and MDM2 could play a role in MMP regulation during hypoxia. MDM2 inactives p53 which is known suppress transcription of VEGF. The P13K and PAK pathways in endothelial cells can be activated directly by VEGF and also indirectly by PSMA through binding with integrin. Therefore MDM2 and PSMA may mediate angiogenesis which could permit the exertion of a synergetic pro-angiogenic effect between the proteins.

tion by MDM2. They used prostate cancer cell lines LNCaP and MDM2 transfected LNCaP (LNCaP-MST). As expected, they found that VEGF transcription was significantly higher in the LNCaP-MST cells compared with the nontransfected LNCaP [84]. Activation of the PI3K-mTOR pathway has previously been reported upon increase of VEGF expression in normoxic and hypoxic conditions [65]. Since HIF-1α is required as a primary member of this pathway, it is generally assumed that activation of the pathway is more effective under hypoxic conditions, in terms of induction of VEGF transcription. Yet, this study showed that in the LNCaP-MST cells, the PI3K-mTOR pathway seems to be activated and the basal HIF-1α appear high. They reported that MDM2 seemed to be triggering an elevated level of HIF-1α, in line with increasing expression of VEGF in normoxic cells, even when hypoxic conditions are lacking. The data presented also suggested that STAT3 and NF-κB may play important roles in MDM2-mediated activation of VEGF transcription, since their levels were increased in the LNCaP-MST cells compared with the nontransfected LNCaP cells [84].

It has also been suggested that p53 can negatively regulate VEGF expression. In 2000, Ravi *et al.* [86] claimed that homozygous deletion of p53 in human colon cancer cells promoted neovascularization and growth of xenograft tumors in nude mice. They showed that upon loss of p53, HIF-1 protein levels are enhanced and so VEGF expression is augmented. It was also demonstrated that forced HIF-1α expression in p53-expressing cancer cells promotes the expression of VEGF and this leads to neovascularization of tumor xenografts. Therefore, the group concluded that p53 acts as a molecular chaperone to HIF-1α, facilitating its recognition by MDM2 for ubiquitination. This work was disputed by a later study [75] which suggests that the group's results may be due to the use of hypoxia-mimicking agents such as cobalt and thus the proteins in complex could change.

In conclusion, despite the great amount of studies undertaken in order to elucidate the role of MDM2 in both angiogenesis and hypoxia, the precise mechanisms are yet to be exposed. It is widely accepted that MDM2 and VEGF levels are coordinated in cancer and that HIF-1α increase can upregulate VEGF transcription during hypoxia. It has been proved many times that MDM2 and HIF-1α interact during hypoxia, although whether this is a direct or indirect interaction, and whether it involves p53 tumor suppressor is under scrutiny. It has also been suggested that a second layer of regulation occurs between MDM2 and VEGF, at post-transcriptional level, independent of HIF-1α. Therefore, perhaps there are different points of regulation of VEGF levels by MDM2 during hypoxia and HIF-1α and p53 may play a role in some, but not others.

In terms of links of PSMA to VEGF, there are differing reports. A report by Tsui *et al.* [87] claimed that there was a correlation between PSMA and VEGF expression in the tumors of xenograft mice, when immunohistological analysis was undertaken. Forced PSMA expression in a prostate cancer cell line, RM-1 and quantification of secretion of VEGF by cells, led to the conclusion that stable transfection of PSMA promoted VEGF release. When these cells were injected into mice, immunohistochemistry was performed and VEGF levels were seen to be significantly higher in the mice injected with the cells expressing PSMA [88].

Since it is found in the neovasculature of many tumors, PSMA is thought to regulate angiogenesis. In 2006, a group demonstrated that PSMA is required for angiogenesis *in vivo* and invasion of endothelial cells *in vitro,* where it was exhibited to be involved in laminin-specific signaling and regulation of the dynamics of the cytoskeleton through the Rho GTPase effector molecule p21-activated kinase 1 (PAK-1). The group hypothesised that PSMA partakes in an autoregulatory feedback loop where, in its active state, it increases integrin signal transduction, PAK activation, followed by endothelial cell adhesion and invasion. This process leads to the dissociation the PSMA/filamin complex and a decrease in PSMA activity and therefore integrin-β1 activity is held in check [89] (see Figure 2).

In a subsequent study, the same group then went on to assess the role of PSMA in ocular neovascularization. To do this they used an oxygen induced retinopathy model (OIR) and it was observed that, after an initial decrease in retinal PSMA mRNA, transcript levels were progressively increased over the time of the relative hypoxia. Vessel formation was then assessed in the retina of PSMA null mice under these conditions of relative hypoxia. Again, it was seen that the loss of PSMA in these mice did not affect the development of normal retinal vasculature. However, mice undergoing OIR showed a remarkable difference between PSMA null and wild-type. The capillaries in the mid-periphery formed a dense, honeycomb of close vessels. In comparison, retinas from PSMA null animals after OIR showed a vascular pattern which closely resembled the normal structure, with less avascular area in the central region and more highly branched capillaries in the periphery. It was also seen that, in comparison to the wild-type, PSMA null mice vessels were better perfused and more functional. Finally, the study evaluated the use of 2-(phosphonomethyl)pentanedioic acid (2-PMPA) PSMA inhibitor in wild-type mice and obtained similar results. Therefore, the absence of

PSMA seems to lead to a less pathogenic phenotype in the retina. The involvement of PSMA in angiogenesis through this mechanism was seen to be independent of VEGF [90].

It is certain that PSMA is involved in angiogenesis; however, the precise mechanism by which PSMA exerts its effect is unknown. PSMA has been linked to VEGF levels in some reports, with increased and decreased PSMA levels being reflected in VEGF expression. However, a group who have released a number of related papers on the subject of PSMA in angiogenesis claim that the involvement of this protein is VEGF-independent. This suggests that PSMA may also play a number of roles in angiogenesis, some involving VEGF, others not.

Participation of MDM2 & PSMA in tumor invasion & metastasis

Due to the high expression of PSMA and MDM2 a number of cancer types, their roles in the invasion and subsequent metastasis of tumors have been studied. Migration and invasion through the extracellular matrix are reliant on the matrix metalloproteinases (MMPs) which are zinc-dependent remodeling endopeptidases implicated in many pivotal roles in tumor growth and the multistep processes of invasion and metastasis. Different members of the MMP family exert contradicting roles at various stages of cancer progression [91].

The most obvious feature of MDM2 involvement in the progressive properties of cancer is its interaction with p53. The ability of MDM2 to block p53 activity is exploited by tumor cells. However, there are other ways in which MDM2 contributes to the progression of cancer. It was shown that in breast cancer cells MDM2 can decrease E-cadherin protein level through ubiquitination and ectopic expression of MDM2 increases cell–cell dissociation, invasion and cell motility [92]. A study into patients with malignant melanoma showed that MDM2 expression level was directly associated with the thickness of a tumor and weakly with invasion level [93].

Immunohistochemical staining of invasive ductal breast carcinoma (IDC) showed a significant correlation between MDM2 and MMP-9 expression. *In vitro* studies in MDA-MB-231 and MCF-7 breast cancer cell lines have shown that siRNA targeted at MDM2-targeted siRNA significantly decreased cell invasion, migration and proteolysis, with the opposite seen in cells overexpressing MDM2. MDM2 overexpression in these cells was seen to induce MMP-9 expression in a dose-dependent manner [94]. A slightly later study also linked the expression of MDM2 and MMP-9 in the oncogenesis of lung cancer in rats [95] (see Figure 2).

A paper by Ghosh *et al.* [96] showed that, surprisingly, in prostate cancer cells, ectopic expression of PSMA in the PSMA-negative cell line PC-3 cells reduced their invasiveness. On the other hand, they found that knockdown of PSMA in the PSMA-positive cell line, LNCaP, increased their invasiveness fivefold. PSMA mutants lacking the carboxypeptidase activity of the protein were produced and showed that this reduced the impact of PSMA expression on invasiveness. Another study involving the injection of the mouse prostate cancer cell line RM-1 with stable expression of PSMA into mice showed the formation of lytic bone lesions and distinct MMP-9 expression compared with the control [88].

Recently, it was found that the sequential digestion of laminin, a predominant component of the extracellular matrix (ECM), occurs through PSMA working downstream of MMP-2, generating small peptides which enhance the invasive and adhesive abilities of HUVECs *in vitro*, providing evidence that these peptides activate adhesion through integrin$\alpha_6\beta_1$ and FAK. It was suggested that since PSMA is a glutamate-specific peptidase, cleavage of a laminin-derived peptide substrate could modify the overall charge of the peptide and so facilitate integrin binding [97].

Another study has linked the expression levels of PSMA and MDM2 in no uncertain terms. LNCaP (PSMA positive) and PC3 (PSMA negative) cell lines were used to assess metastasis-related genes which were downregulated in cells with silenced PSMA. It was found that MDM2 transcript levels were decreased over 80-fold following PSMA silencing. This paper also indicated that the treatment of the LNCaP cell line with PSMA-targeted siRNA led to an upregulation of MMP-3 and -13, and a downregulation of MMP-2 [98] (see Figure 2). This decrease has also been shown in breast cancer cell lines in our own laboratory (unpublished data). Since the degradation of the extracellular matrix and basement membrane by MMPs is pivotal to whether a tumor infiltrates and metastasises, it may be deduced that PSMA could interplay with MDM2 to regulate MMP secretion. This theory is supported by the many reports linking both proteins to a number of MMPs.

In conclusion, if Xu *et al.* [98] and our own findings concerning the correlated expression of MDM2 and PSMA are correct, it seems that conflicting data exists regarding invasion capacity as a result of MDM2 and PSMA knockdown and overexpression. Ghosh *et al.* [96], claimed that PSMA increase reduced *in vitro* invasion of prostate cancer cell lines, and vice versa; however, Chen *et al.* [94] saw that MDM2 decrease caused a decrease in invasion, and vice versa, in breast cancer cell lines. Since both proteins have been reported

to have correlations with MMP-9 [88,94,95] and PSMA has been linked to MMP-2 [97,98], it would be expected that a decrease in transwell cellular invasion would be seen. This apparent contradiction of results could be explained by the fact that different cell lines were used for each study, and these cancer cells were derived from very different areas of the human body (prostate and breast). However, it could also be the result of both proteins being decreased in expression when PSMA levels are decreased, and only MDM2 protein being decreased when the *mdm2* gene is targeted, as so far no studies have been published which show the expression levels of PSMA when the *mdm2* gene is knocked down. Therefore, the difference in results seen could highlight the pathways in which MDM2 and PSMA interact, and those in which MDM2 works alone. This may mean that when PSMA levels are decreased, factors that increase invasion are produced in the cells and this is a stronger force than those factors that are decreased when MDM2 levels are reduced, which would otherwise decrease invasion. Further work in our own and other laboratories will hopefully lead to an explanation regarding whether this is the case.

Signaling pathways of MDM2 & PSMA are intertwined by DHFR & HIF1α

As aforementioned, PSMA was originally identified as FOLH1, named for its important role in folate metabolism. Previously cited studies show that PSMA plays a critical role in the progressive properties of cancer. It has been found that PSMA expression gives LNCaP prostate cancer cells a growth advantage in media containing low (<1 nM) and physiological (25 nM) folate [99]. The same group then showed that PSMA-expressing PC-3 cells showed a growth advantage compared with wild-type PC-3 cells.

MDM2 has also been identified to have an involvement in folate metabolism, with its ability to directly bind to DHFR and catalyze the monoubiquitination of DHFR via its E3 ubiquitin ligase activity and reduce DHFR activity within cells, without changing levels of steady-state DHFR, in a RING finger-dependent and p53-independent manner [100]. DHFR is an important folate metabolizing enzyme which reduces dihydrofolic acid (DHF) to tetrahydrofolic acid, using NAPDH as an electron donor. Tetrahydrofolic acid can then be converted to many types of tetrahydrofolate cofactors using in one-carbon chemistry. This one-carbon transfer reaction catalyzed by DHFR is essential for DNA synthesis and homocysteine remethylation [101]. Changes in the level of DHFR expression and activity due to genetic polymorphisms can affect a patient's susceptibility to a variety of diseases including cancer. Likewise, variability in DHFR expression can affect the sensitivity of a patient to anticancer drugs such as folate antagonist methotrexate (MTX) [102].

There are two possible mechanisms which could explain the role of folate in tumor development and metastasis. First, folate deficiency causes a reduction of intracellular S-adenosylmethionine (SAM) and therefore can alter cytosine methylation in DNA, leading to an inappropriate activation of proto-oncogenes and induction of malignant [103]. Second, folate deficiency could cause an imbalance of DNA precursors, uracil misincorporation into DNA and chromosome breakage. A further link of MDM2 to folate metabolism is given in the demonstration that folate deficiencies induce DNA strand breaks and hypomethylation within the p53 tumor suppressor gene in animal models, suggesting that the relevance of folate activity stress to carcinogenesis could be p53-dependent [104]. Therefore, the involvement of both MDM2 and PSMA in folate metabolism could provide reasoning for their important role in cancer progression and their possible interplay, since the roles of the two are so closely connected within the pathway.

We hypothesize that in cancer cells, PSMA may be involved in the expression or activity of MDM2 through aberrant regulation of p53 methylation via its activity in folate metabolism Though this hypothesis needs to be fully investigated, it is partially supported by a recent microarray analysis previously mentioned, which suggests that silencing of PSMA using siRNA leads to downregulation of MDM2 expression [98]. Work by Yao *et al.* [99] also supports this theory, with their studies showing that increased PSMA expression in conditions of low or physiological folate leads to a growth advantage of prostate cancer cell lines. This work could imply that increased PSMA, means upregulated p53 methylation, greater MDM2 expression and thus elevated cell growth. Further, DHFR has been considered as a target of second-line cancer chemotherapy, due to its role in tetrahydrofolate synthesis, which is essential for cellular synthesis of DNA, RNA, thymidylates and proteins. As mentioned, this leads DHFR to be highly sensitive to tetrahydrofolate analogs such as methotrexate (MTX) [105]. Therefore, it may be possible that MDM2 inhibition sensitises the effects of certain DHFR-targeted therapy, or promotes the function of PSMA in cancer cells through the response of DHFR (hypothesis of interplay summarized in Figure 1).

MDM2 and PSMA have been proved to promote the activity of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which are secreted by cancer cells and degrade the extracellular matrix, allowing cells to migrate, invade and move from the primary cancer site [88,94,95,97,106]. The endogenous inhibitors

of MMP activity are the tissue inhibitors of metalloproteinases (TIMPs). Recently, it has been suggested in a number of papers that HIF-1α is a regulator of TIMPs [107], specifically TIMP-1 [108], and the levels of MMP-2 and MMP-9 have been seen to correlate with higher levels of HIF-1α in polytetrafluoroethylene grafts of patients [109]. Therefore, given the evidence that MDM2 activates HIF-1α, we hypothesize that both PSMA and MDM2 play a role in the regulation of MMPs during hypoxia. Additionally, VEGF induces

Executive summary

Tumorigenesis & clinical relevance of MDM2

- Involved in both p53-dependent and -independent roles in the cell.
- Important in transformation of cells and its overexpression frequently observed in many human cancers, meaning it is a good therapeutic target.

MDM2 as a therapeutic target

- Therapeutics aim to decrease protein levels, thus reactivating p53. Undertaken through: reducing protein levels in cancer cells, inhibiting E3 ubiquitin ligase activity or disruption of interaction with p53.
- No ideal therapy involving MDM2 has been identified, but studies are ongoing and still viewed as a promising target.

Clinical relevance of prostate-specific membrane antigen (PSMA)

- Known to play a role in folate metabolism and hydrolyse neurotransmitter N-acetylaspartylglutamic acid.
- Expression increases in metastasised and later stage/grade cancers.
- Excellent target for therapy: specific expression (prostate, kidneys, nervous system and small intestine), large extracellular region, transmembrane protein, internalization sequence and peptidase activity (activation of prodrug).

PSMA as a biomarker

- Since there is a positive correlation between PSMA and adverse clinical outcome, it has been suggested and studied as a biomarker for prostate cancer progression.
- More sensitive and specific than PSA detection.
- Currently, second-generation antibodies which bind extracellular epitopes are under trial. One of these, J591 has shown potential in imaging of prostate cancer.

PSMA as a therapeutic target

- PSMA has been targeted using many different strategies: antibody-based radiotherapy, antibody-drug conjugates, prodrug therapy and immunotherapy.
- There is yet to be a clinically important effect on survival in patients due to PSMA-based therapeutics.

Tumor-associated angiogenesis mediated by MDM2 and PSMA

- MDM2 and VEGF levels are coordinated in cancer.
- HIF1α is regulated by MDM2 and through this can upregulate VEGF expression during hypoxia. It is not known if p53 is involved in this interaction.
- There may be different points of regulation of VEGF by MDM2 – at gene and protein level – some may involve p53, some may not.
- PSMA also linked to VEGF in some reports but its involvement in angiogenesis has been said to be VEGF-independent in others.
- PSMA involved in autoregulatory feedback loop in endothelial cells where it increases integrin signal transduction, PAK activation, followed by endothelial cell adhesion and invasion.

Participation of MDM2 and PSMA in tumor invasion & metastasis

- Both proteins have been linked to MMPs: MMP-2 and MMP-9.
- Overexpression of MDM2 has been linked to increased migration and invasion in breast cancer cell lines.
- Overexpression of PSMA has been linked to decreased migration and invasion in prostate cancer cell lines.
- PSMA and MDM2 expression is linked in prostate cancer cells (published data) and breast cancer cells (our own unpublished data), with decreased PSMA protein expression leading to a significant decrease in MDM2 gene expression.

The signaling pathways of MDM2 and PSMA are intertwined by DHFR and HIF1

- MDM2 linked to DHFR, a key enzyme in folate metabolism.
- PSMA could be involved in the expression/activity of MDM2 through aberrant regulation of p53.
- MDM2 inhibition may sensitise the effects of certain DHFR-targeted therapy or promote function of PSMA in cancer cells through response of DHFR.
- PSMA and MDM2 may coordinate to regulate MMPs during hypoxia.
- Both MDM2 and PSMA could mediate angiogenesis through PI3K pathways and this could permit exertion of a synergetic pro-angiogenic effect between the proteins.

angiogenesis partly through activation of the P13K signaling pathway in endothelial cells [110] and integrin, which is known to be regulated by PSMA in endothelial cells, can also activate P13 through p21-activated kinase (PAK) [89]; as well as MDM2 being shown to regulate HIF-1α in a PTEN/PI3K-dependent manner [69]. Thus, both MDM2 and PSMA could mediate angiogenesis or, in particular, hypoxia-mediated angiogenesis, through PI3K pathways and this could permit the exertion of a synergetic pro-angiogenic effect between the proteins [40].

Future perspective

Although, to date, no ideal therapeutics targeted to MDM2 or PSMA have been identified, an understanding of their interaction could make the future for these fields bright. First, since none of the routes of MDM2-targeting have shown satisfactory results in clinical trials, the observation that PSMA siRNA can decrease MDM2 transcript levels [98] could provide another way of lessening MDM2 protein levels in tumor cells. This is an even more enticing idea when the expression of PSMA solely on tumor cells in most areas of the body is considered. Another therapeutic potential of this interaction could be the dual targeting of the proteins, to further lower MDM2 and PSMA levels in cells. Thus, further investigation of the interaction of these proteins is likely key to the improvement of MDM2- and PSMA-targeted therapeutics.

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