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MDM2 promotes cell motility and invasiveness through a RING-finger independent mechanism

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ABSTRACT

Recent studies connect MDM2 with increased cell motility, invasion and/or metastasis proposing an MDM2-mediated ubiquitylation-dependent mechanism. Interestingly, in renal cell carcinoma (RCC) p53/MDM2 co-expression is associated with reduced survival which is independently linked with metastasis. We therefore investigated whether expression of p53 and/or MDM2 promotes aggressive cell phenotypes. Our data demonstrate that MDM2 promotes increased motility and invasiveness in RCC cells (N.B. similar results are obtained in non-RCC cells). This study shows for the first time both that endogenous MDM2 significantly contributes to cell motility and that this does not depend upon the MDM2 RING-finger, i.e. is independent of ubiquitylation (and NEDDylation). Our data suggest that protein–protein interactions provide a likely mechanistic basis for MDM2-promoted motility which may constitute future therapeutic targets.

1. Introduction

In addition to its well-documented and proven role as critical negative regulator of p53 (reviewed in [1]), MDM2 has been shown to display p53-independent oncogenic activities (reviewed in [2]). For example, studies in NIH3T3 cells have shown that naturally occurring splice variants of MDM2 that lack the ability to bind to p53 retain transforming activity [3]. Additional evidence from in vivo studies of mice expressing an *MDM2* transgene from a β-lactoglobulin promoter have demonstrated abnormal mammary development and cell cycle deregulation in both *p53* wild-type and null backgrounds [4]. Further evidence has been provided by studies of mice that over-expressed MDM2 from the *MDM2* promoter which developed a different spectrum of tumours c.f. *p53* null mice [5], regardless of their *p53* status. *MDM2* expression has also been shown to abrogate the growth inhibitory activities of Transforming Growth Factor-Beta1 (TGFβ1) in a p53-independent manner in cells in culture [6]. More recently, we have shown that MDM2 mono-ubiquitylates DHFR, altering cellular sensitivity to methotrexate and inhibiting DHFR activity in tumour cells in a p53-independent manner [7], thus implicating MDM2 in the regulation/de-regulation of one carbon metabolism with potentially wide-ranging consequences.

Recent work has suggested links between MDM2 and cell motility, invasion and metastatic potential although the mechanisms leading to this remain unclear (discussed below) [8,9]. Our study was initiated to investigate renal cancer cells because unlike the situation pertaining in most other cancers, we have recently found that in renal cell carcinoma, the most common form of renal cancer, up-regulated p53 is usually wild-type and is linked with up-regulation of MDM2 (see [10] for review of the literature and also Noon et al., submitted). Since co-expression of p53 and MDM2 is linked with poor outcome, we speculated that this might promote more aggressive behaviour/s in renal cells such as increased motility and invasiveness leading to increased metastasis. We therefore set out to investigate whether p53 and MDM2 expression in renal cancer cells altered their motility and invasiveness and in the course of these studies have also found that these properties are not limited to renal cells, but extend to other cell types.

In a recent study, it was proposed that MDM2 promotes motility through the ability of its E3 ubiquitin ligase activity to target SLUG (SNAI2) for degradation [8]. This study showed that in cells expressing high levels of MDM2, SLUG levels were reduced and motility increased. In another study it was found that MDM2 levels in breast cancer cells inversely correlated with E-cadherin levels

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and that high MDM2/low E-cadherin was associated with axillary lymph node metastasis [9]. Whilst these authors did show that ectopic expression of MDM2 increased motility in MCF-7 cells, neither they, nor the authors of the other study directly assessed the contribution of endogenous MDM2 to motility or invasion, e.g. through RNAi studies (it was addressed only through correlation studies). Also, whilst several correlative experiments were described, no data were presented in either study that tested whether an intact MDM2 RING-finger, a prerequisite for its function as an ubiquitin ligase, is required for MDM2-promoted motility or invasion. We therefore included analysis of these properties in the present study and show for the first time that endogenous MDM2 contributes to cell motility and that mutant MDM2 which lacks a functional RING finger, retains the ability to promote increased cell motility. It may be noteworthy that in parallel studies, we found no evidence for any detectable change in SLUG levels in these more motile cells, nor did we find changes in E-cadherin levels in highly motile cells expressing wt or RING finger mutant MDM2 in our analyses.

Thus a key conclusion from our studies is that MDM2 possesses motility promoting activity that does not require its function as an ubiquitin ligase. This not only suggests that previously unidentified mechanism/s determine the capacity of MDM2 to promote motility and invasion, but also is most compatible with a protein–protein interaction-based mechanism. This may be of particular importance in renal cancers where this mechanism likely contributes to the observed link between p53/MDM2 co-expression and reduced patient survival and thus we predict that a novel protein–protein interactive site is present that may be targeted for therapeutic intervention.

2. Materials and methods

2.1. Antibodies

The mouse monoclonal antibody for β-actin (C-2) used as a protein loading control in SDS–PAGE, was purchased from Santa Cruz Biotechnology. The mouse monoclonal antibodies used in western analysis for MDM2 (Ab-1/clone IF2) and p53 (Ab-6/clone DO-1) were purchased from EMD Biosciences. Secondary antibody anti-mouse was from GE Healthcare.

2.2. Plasmids and siRNAs

The expression vectors for human p53 (pCEP4-p53), MDM2 (pCMVneobam-MDM2) and RING-finger mutant of MDM2 (pCMV-

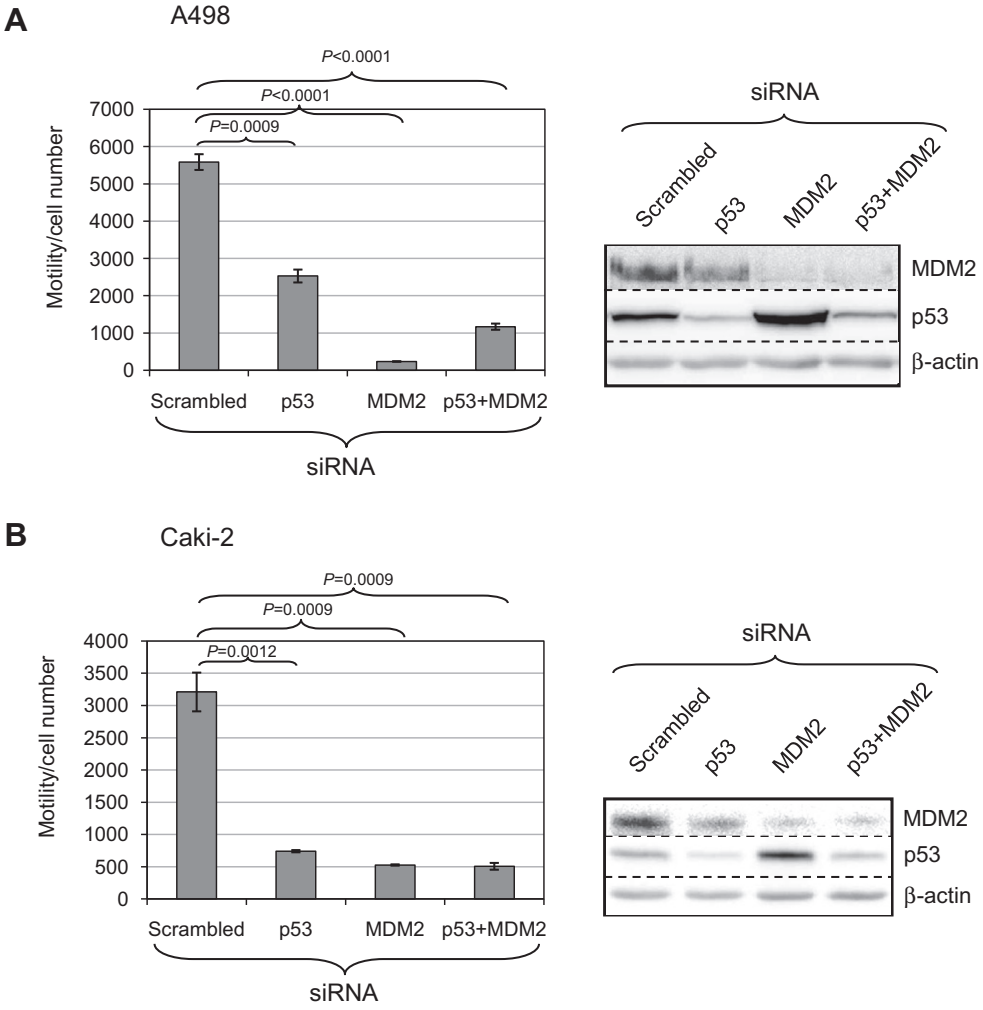

Fig. 1. p53 and/or MDM2 promote cell motility in RCC cells. Histograms display results of motility experiments with the indicated cell lines (A = A498 and B = Caki-2), transfected with the indicated siRNAs. Cells were transfected with siRNA and 24 h later were seeded into Boyden chambers. Eighteen hours later the membranes were stained prior to counting. Results are mean ± S.E.M. Experiments shown are typical from a single experiment and for each condition three independent transfections were performed and analysed separately ($n = 3$). Statistical analysis performed using Student's t-test. Panels to the right of histograms display western blot analyses of samples from A and B probed with the indicated antibodies.

neobam-MDM2-C464A) and the empty vector controls CMVneobam and pCEP4 (obtained from Invitrogen) have been described previously [11]. A dominant negative mutant of p53 (R175H) pCB6+p53 175His, kindly provided by Prof. Karen Vousden, was sub-cloned to create pCEP4-p53(R175H). The GFP expression vector pSUPER-EGFP was obtained from Oligoengine. siRNAs for p53 (5′ GGACAUACCAGCUUAGAUU 3′), MDM2 (5′ GCCACAAAUCUGAUAGUAU 3′), and a scrambled control (5′ GGACGCAUCCUUCUUAAUU 3′) have been described previously [12–14] [12]and were synthesized by Dharmacon.

2.3. Cell lines and transfection

The cell lines A498 (primary RCC, p53 wild-type), Caki-2 (primary clear cell RCC, p53 wild-type), 117 (p53 wild-type-kindly provided by Prof. W.M. Linehan), H1299 (non-small cell lung carcinoma, p53 null) and Clone 9 (a H1299 derivative which stably over-expresses MDM2) have been described previously [7,14]. A498, and 117 cells (and clonal derivatives) were maintained in Eagle's MEM supplemented with 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate and 10% FBS. H1299 and Clone-9 cells were maintained in DMEM supplemented with 2 mM L-glutamine and 10% FBS. Caki-2 cells were maintained in McCoy's 5A supplemented with 1.5 mM L-glutamine, 2.2 g/l sodium bicarbonate and 10% FBS. Cells were transiently transfected using 3 μl GeneJuice reagent (Novagen) per microgram of DNA, and empty vector was used to ensure equal DNA content in transfections. siRNA was delivered to cells by transfection with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

2.4. Western blotting

Cells were harvested by trypsinisation after the indicated times and pelleted by centrifugation. Cell pellets were lysed in SLIP buffer (50 mM HEPES pH 7.5, 10% glycerol, 0.1% Triton-X100, 150 mM NaCl) in the presence of the following protease inhibitors: aprotinin (2 μg/ml), leupeptin (0.5 μg/ml), pepstatin A (1 μg/ml), soybean trypsin inhibitor (100 μg/ml) and phenylmethylsulfonyl fluoride (PMSF) (1 mM). After 10 min incubation on ice, lysates were centrifuged at 20,000×*g* and protein concentrations in the supernatant were determined using Bradford reagent (BioRad). Typically, 50 μg samples of total protein in 1 × protein sample buffer (50 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 0.25% β-mercaptoethanol, bromophenol blue (1 mg/ml)) were separated by SDS–PAGE and transferred to Hybond ECL nitrocellulose membrane (GE Healthcare). Membranes were blocked in PBS-Tween-20 (0.1% v/v) containing non-fat dry milk (BioRad) (5% w/v) for 1 h at room temperature before incubation with primary antibodies (each at 3 μg/ml, except anti-p53 at 1 μg/ml). Membranes were washed three times for 15 min in PBS-Tween-20 before addition of HRP-conjugated anti-mouse (1:2500) secondary antibody (GE Healthcare) for 1 h at room temperature. Membranes were washed as before and signal was detected by Western Lightning Chemiluminescence Reagent (Perkin–Elmer) either on film or using a KODAK IS4000MM system.

2.5. Drug selection of transfected cells in hygromycin B and G418

Sub-clones of 117 cells transfected with plasmids conferring resistance to G418 (pCMVneobam [pCMV] and pCMVneobam-MDM2 [pMDM2]) and hygromycin B (pCEP4 and pCEP4-p53

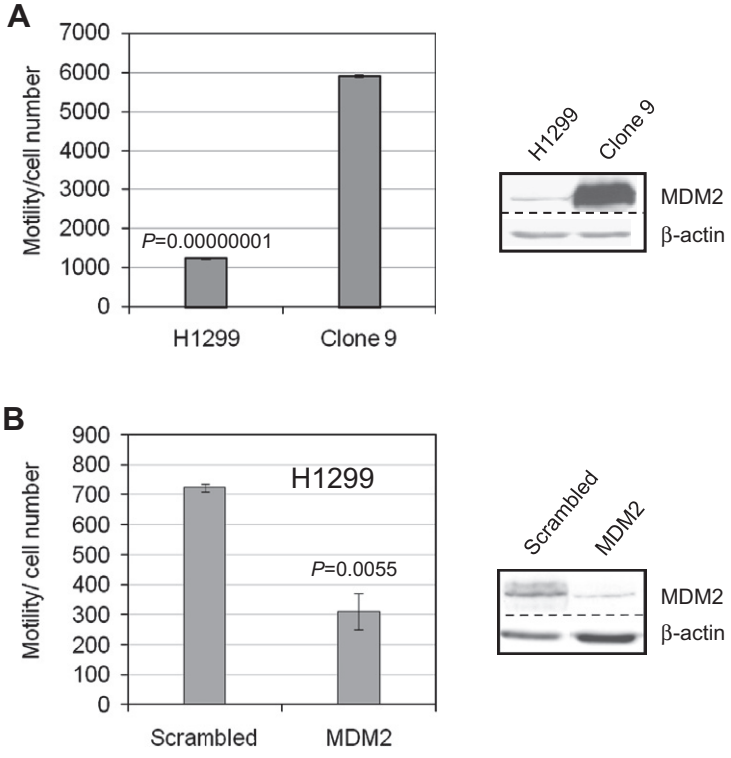

Fig. 2. MDM2 promotes cell motility in non-RCC cells. (A) H1299s and Clone 9 cells – an isogenic clonal derivative of these that stably expresses increased MDM2, were assayed for motility essentially as described in Fig. 1. (B) H1299 cells were transfected with either an siRNA for MDM2 or a scrambled control and their motility was assayed as described in Fig. 1. Results are mean ± S.E.M. Experiments shown are typical from a single experiment and for each condition three independent transfections were performed and analysed separately ($n = 3$). Statistical analysis performed using Student's *t*-test. Panels on the right display western blots of the proteins extracts from the indicated cells probed with the indicated antibodies from the same samples assayed for motility.

(R175H) [p53(R175H)]) were generated according to the scheme shown in Fig. 3C. Briefly, cells were transfected with the indicated plasmid and 24 h later were transferred into media containing either 800 μg/ml G418 (Calbiochem) and/or 250 μg/ml hygromycin B (Roche) as required. Media with fresh antibiotic was replaced every 2–3 days and 10–21 days later colonies were picked and expanded prior to analysis by western blotting.

2.6. Motility and invasion assays

Typically 1×10^4–5×10^4 cells were seeded into each Boyden chamber (VWR) that was inserted into a 24-well plate containing approximately 300 μl of cell culture media, essentially as described [15]. After 18 h, the inside of each chamber was scraped rigorously with cotton buds to ensure there were no cells adhering on the inner side of the chamber membrane. Cells were then fixed and stained with REASTAIN Quick-Diff Kit (Reagenta, Gamidon) according to the manufacturer's protocol. Briefly, the cells were fixed in REASTAIN Quick-Diff Fix for 10 min and thereafter stained with REASTAIN Quick-Diff Red followed by REASTAIN Quick-Diff Blue for 2 min each. The chambers were then rinsed in H_2O to remove any excess stain and allowed to dry for 30 min. Dried membranes were excised from the chamber and mounted onto microscopic slides with DPX (Sigma) mountant. To determine the number of cells that had passed through the chamber membrane, the membrane was divided into four or more grids. Digital images of each grid were counted manually. Three chambers were counted for each sample and the average number of cells that passed through to the outer chamber membrane has been represented in graphical format. For invasion assays, BD-Matrigel™ invasion chambers (Becton Dickinson) were used.

3. Results

In a recent study (Noon et al., submitted) we confirmed previous studies which had found that p53 and MDM2 are frequently

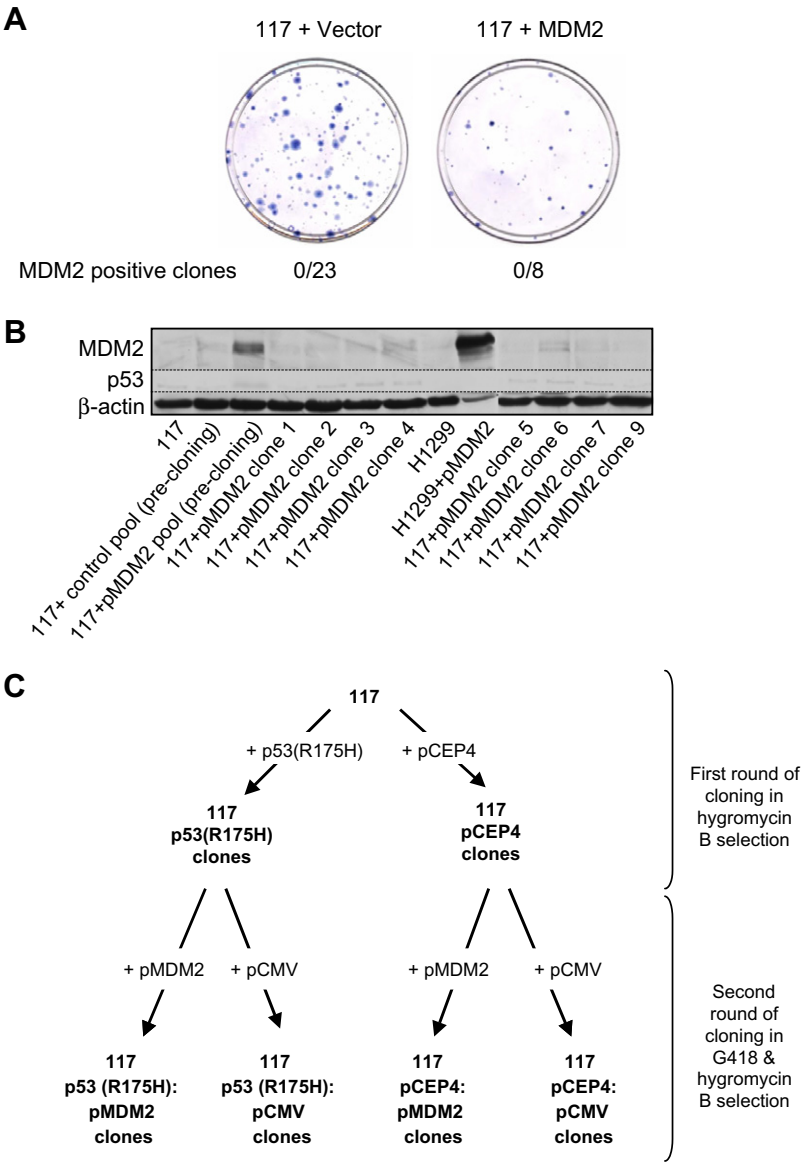

Fig. 3. Generation of cells that stably express higher levels of MDM2. (A) 117 (RCC) cells were transfected with expression construct for MDM2 or with an empty vector control as indicated. (B) Colonies obtained from (A) were picked, expanded and protein lysates from these were then examined by western blot for p53, MDM2 and actin as indicated. Note that the MDM2-transfected H1299 lysate was under-loaded to prevent the signal from obscuring adjacent samples. Whilst transient expression of MDM2 is readily achieved, stable expression was not detectable. (C) A scheme to generate 117 cell clones aimed at producing cells that express increased MDM2 (for discussion see text). Details of the cell clones produced are listed in Supplementary data Fig. 2 and a subset of these have been further examined in Figs. 4 and 5.

co-expressed in RCC [16,17]. We also confirmed that co-expression of p53 and MDM2 is linked with reduced disease-specific survival as Haitel et al. had previously shown [16]. In addition, we showed for the first time that the p53 co-expressed with MDM2 was frequently wild-type (in 86% of co-expressing cases, Polanski et al., submitted), which suggests that MDM2 up-regulation may be a consequence of p53 up-regulation as we have previously proposed [10,14]. Our working hypothesis is that co-expression of wild-type p53 and MDM2 promotes disease progression in RCC and thus we examined phenotypes in RCC cells that may contribute to this process. RCC patients with metastatic disease have an extremely short life expectancy and most patients who die from the disease do so as a consequence of metastatic spread [18]. Since motility and invasiveness are key cellular determinants of metastatic potential we have therefore examined the former processes in vitro. To determine whether p53 and/or MDM2 contribute to motility in renal cancer cells we transfected siRNAs for p53, MDM2 and for p53+MDM2 into two wt p53 renal carcinoma lines A498 and Caki-2 that co-express high levels of wild-type p53 and MDM2 (see Supplementary data Fig. 1) and which thus mimic the co-expression that occurs in a sub-set of poor prognosis RCC patients.

As Fig. 1 shows, reducing p53 and/or MDM2 expression with siRNA leads to significant reductions in motility in these cells. siRNA for MDM2 appears to be more effective at inhibiting motility than p53 siRNA transfection, but since targeting both p53 and MDM2 rescues this difference in A498 cells, this may be due to other effects of p53 induction in cells transfected only with MDM2 siRNA as we have previously described [14] (note that we have not observed evidence of reduced viability during these experiments, though these cells do display increased evidence of senescence). From these data we conclude that both p53 and MDM2 contribute to motility in RCC cells.

Whilst the focus of our study is on renal cancer cells, we wanted to determine both whether this effect is more general and if so, to take advantage of p53 null cancer cells from other tissues to examine the contribution of MDM2 in a p53-free environment. In a previous study we used a clone of H1299 cells (Clone 9) that stably express high levels of MDM2 from an integrated plasmid [7]. We tested the motility of these cells and found that Clone 9 cells expressing higher levels of MDM2 displayed increased motility as Fig. 2A shows. To address the question of whether endogenous MDM2 promotes motility and also to address concerns arising from the clonal selection process used to produce Clone 9 cells, we transfected MDM2 siRNA into H1299 cells which express low levels of MDM2 (as illustrated in Fig. 3B where on longer exposure the level of MDM2 in H1299 is comparable to the low level expressed in 117 cells, not shown). The result of this experiment is demonstrated in Fig. 2B. Remarkably, this experiment represents the first direct examination of endogenous MDM2 modulation and motility in cells. We conclude that MDM2 possesses cell motility promoting activity that is p53-independent.

A substantial subset of RCC patients appear to progress from a p53 low/MDM2 low phenotype to a more aggressive p53 high/MDM2 high phenotype. To study this we were required to generate RCC cells in vitro that would stably express higher levels of p53 and/or MDM2 (Supplementary data Fig. 1 shows the levels of p53 and MDM2 expression in a panel of renal cancer cell lines compared to U2OS cells which express comparable levels of MDM2 to H1299 cells). We selected the RCC cell line 117 which harbour low levels of both p53 (wild-type) and MDM2 for subsequent experiments. As Fig. 3A and B shows, transfecting an MDM2 expression plasmid into cells results in transient, but not stable expression of higher levels of MDM2. This phenomenon, namely that MDM2 an oncogene activated through up-regulation cannot readily be stably over-expressed in tumour cells, has been observed previously [19]. At the time that we performed this experiment it was not yet clear that the p53 co-expressed with MDM2 in RCC cells was wild-type. Therefore we performed an experiment to test whether expression of a wt or dominant negative mutant p53 could promote cells to tolerate high levels of MDM2. Not surprisingly we did not obtain any stable clones following wt p53 transfection, whereas numerous clones were obtained from cells transfected with the empty vector or vector expressing dominant negative p53 (R175H). We then took clones of cells that had been transfected with either the empty vector or with a dominant negative p53 (R175H) through a second round of transfection as shown in the scheme in Fig. 3C. The results of this cloning exercise are summarised in Supplementary data Fig. 2 and Table 1. Essentially these data show two things. RCC cells can spontaneously acquire increased p53 and MDM2 expression (even in cells that have received only empty vectors) and the in vitro results mimic the in vivo results; MDM2 up-regulation was again significantly associated with high p53 expression ($P < 0.01$).

A key prediction of our studies is that up-regulation of p53 and MDM2 promotes events leading to reduced disease-specific sur-

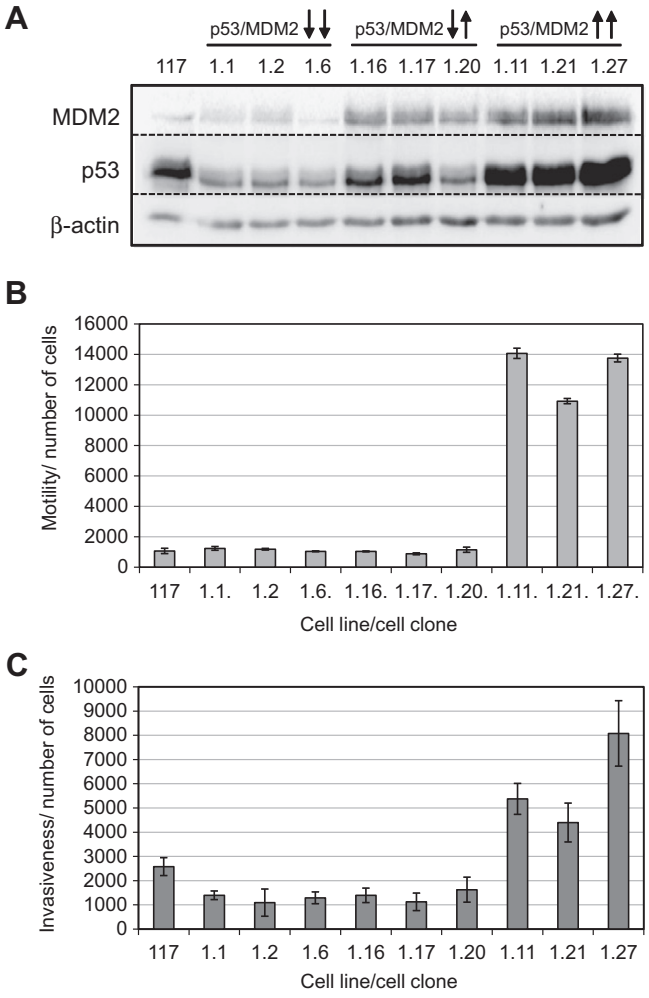

Fig. 4. Up-regulation of p53 and MDM2 promotes increased motility and invasion in RCC cells. (A) Cell clones derived from the 117 cell line following the experimental scheme described in Fig. 3C and selected from the clones listed/summarised in Supplementary data Fig. 2/Supplementary data Table 1, respectively, were analysed by western blotting for the indicated proteins. (B) The same cell lines were seeded into Boyden chambers for 18 h and were then stained and counted. (C) As for (B) but cells were seeded into Matrigel™ coated chambers. Results are mean ± S.E.M. Experiments shown are typical from a single experiment and for each condition three independent transfections were performed and analysed separately ($n = 3$). Statistical analysis performed using Student's *t*-test.

vival with metastatic potential being a likely determinant of this [18]. To investigate this we tested whether isogenic RCC cells differing in their p53 and MDM2 expression levels would exhibit altered motility. Fig. 4A and B shows that clones that express higher levels of p53 and MDM2 also display increased motility. In addition to motility, metastatic cells must acquire the ability to invade the extracellular environment. We therefore investigated the ability of the same clones shown in Fig. 4B to invade through Matrigel™. As Fig. 4C demonstrates, cells expressing up-regulated p53 and MDM2 were not only more motile (Fig. 4B), but were also more invasive. We noted however, that cells expressing intermediate levels of MDM2 (clones 1.16, 1.17 and 1.20), in the absence of up-regulated p53 (note 117 p53 level for comparison in Fig. 4A), were neither more motile, nor more invasive. These differences in motility and invasiveness are not due to differences in the in vitro growth rates of these cell clones as Supplementary data Fig. 3 shows. To determine whether the increased motility shown in Fig. 4B was due to p53 and/or MDM2 we used siRNA to modulate the expression of these genes in two highly motile clones 1.27 and 1.11. As Fig. 5 demonstrates for the highly motile clones 1.27 and 1.11, the motility of these cells is significantly reduced only when MDM2 siRNA is transfected. Note that these effects of MDM2 reduction by siRNA transfection are not due to any impact upon cell proliferation as Supplementary data Fig. 5 illustrates. Note also, that when p53 siRNA is used, MDM2 steady state levels of expression are reduced. Thus in RCC cells not only is MDM2 up-regulated in a p53-dependent manner as we showed elsewhere (Polanski et al., submitted), but up-regulation of both p53 and MDM2, significantly associated with reduced disease-specific survival in vivo, also promotes increased cellular motility and invasiveness in vitro.

Whilst the results presented may suggest that both p53 and MDM2 play a role in regulating cell motility and invasiveness, the evidence from p53 null cells (Fig. 2) and the data presented in Fig. 5 suggest that MDM2 may be the primary determinant of this process, with p53 performing an indirect function through its ability to promote MDM2 expression. This conclusion is further supported by analysis of one of the 117 clones that expresses mutant p53 (R175H) and exogenous MDM2 (from integrated pCMV-neobam-MDM2) and thus in these cells MDM2 expression is p53-independent. When these cells are transfected with siRNA for p53, p53 levels are reduced, but this has no impact on either motility or on MDM2 levels. In contrast, when siRNA for MDM2 is transfected into these cells (with or without concomitant transfection of p53 siRNA), then the cells display significantly reduced motility (illustrated in Supplementary data Fig. 4). Thus it appears that MDM2 is a key determinant of motility in these RCC cells and that any role for p53 in this process may depend upon its ability to regulate MDM2 levels.

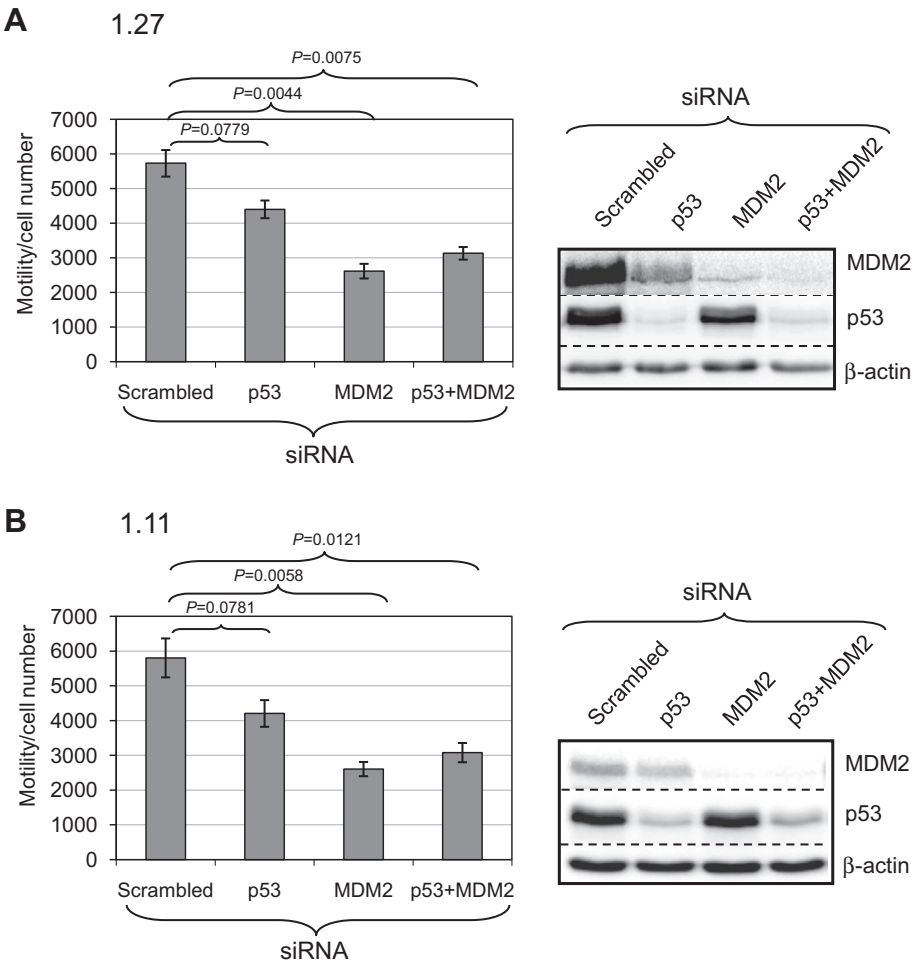

Fig. 5. MDM2 significantly promotes increased motility in RCC cells that spontaneously acquire increased p53/MDM2 expression. Histograms display results of motility experiments with the indicated cell lines (*A* = 1.27 and *B* = 1.11), transfected with the indicated siRNAs. Cells were transfected with siRNA and 24 h later were seeded into Boyden chambers. Eighteen hours later the membranes were stained prior to counting. Results are mean ± S.E.M. Experiments shown are typical from a single experiment and for each condition three independent transfections were performed and analysed separately ($n = 3$). Statistical analysis performed using Student's *t*-test. Panels to the right of the histograms display western blot analyses of samples from A and B probed with the indicated antibodies.

Two recent studies have examined aspects of MDM2 and cell motility, invasion and/or metastasis. One concluded that MDM2 regulated the levels of E-cadherin expression, and that this determined motility and invasiveness [9], whilst the other concluded that MDM2 promoted degradation of SLUG with the resulting increase in E-cadherin expression promoting increased invasiveness [8]. Neither study examined the effect of endogenous MDM2 suppression with siRNA on motility/invasion or metastasis. Moreover, since both studies proposed mechanisms that depend upon the ubiquitin ligase activity of MDM2, it seemed surprising that neither tested whether a mutant of MDM2 that cannot carry out ubiquitylation could promote motility.

To examine this we generated a panel of independent H1299 clones that express wild-type MDM2, a RING finger mutant of MDM2 (C464A) or harbour the empty vector alone and have performed motility assays on these as Fig. 6A illustrates. Clones that express either wild-type MDM2 or an ubiquitin ligase-dead mutant (C464A) all exhibit significantly increased cell motility. Since multiple clones displayed comparable motility phenotypes, it is unlikely that these effects are due to clonal selection. However, to further rule out this possibility we also transiently transfected H1299 cells with expression vectors for MDM2 and the same RING-finger mutant of MDM2 (C464A) and examined the motility of these cells. As Fig. 6B shows, cells that express either wt MDM2 or a RING-finger mutant also display significantly increased motility.

These results show that MDM2 increases motility, in a p53-independent manner, through a mechanism that does not depend upon the ubiquitin ligase activity of MDM2 [20]. We conclude that the ability of MDM2 to promote increased motility likely depends

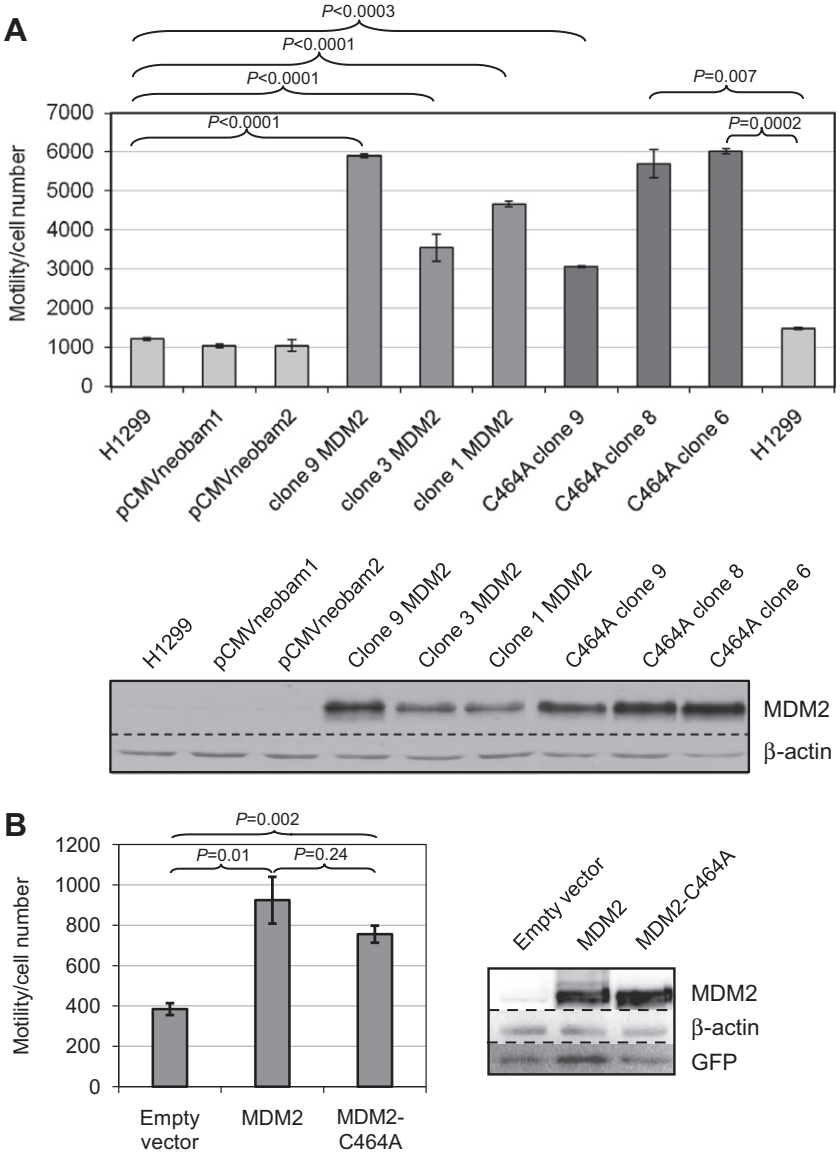

Fig. 6. MDM2 promotes increased motility in a p53-independent, RING finger-independent manner. (A) H1299 cells and stable clonal derivatives expressing either wild-type MDM2 or a RING finger mutant (C464A) were assessed for their motility using a Boyden chamber as described in the methods. The panel below shows western blot analysis of protein lysates from the same cell lines analysed with the indicated antibodies. (B) H1299 cells were transfected with the indicated expression plasmids and their motility was assessed as in (A). Note that for all experiments 15,000 cells were seeded/chamber. The panel on the right shows a western blot of protein lysates from the cells in (B) probed with the indicated antibodies. For each motility assay three individual cultures were analysed and for transfections three independent cultures were transfected and counted. Statistical analyses by Student's *t*-test ($n = 3$). Results are mean ± S.E.M. from a single typical experiment. Note that the H1299s were assayed on two separate occasions.

upon protein–protein interactions which may have important implications for drug targeting, particularly for RCC as discussed below.

4. Discussion

The initial focus of this study derived from an interest in the role of p53/MDM2 expression in determining phenotypes linked with poor outcome in RCC such as motility and invasiveness ([10] and Noon et al., submitted). However, the ensuing studies have also, for the first time, investigated two key questions arising from earlier studies that indicated an association between MDM2 ubiquitin ligase activity and motility/invasion/metastasis [8,9]: (i) does endogenous MDM2 promote cell motility and (ii) is the ubiquitin-ligase activity of MDM2 required for this? Neither of the cited studies examined the ability of endogenous MDM2 to promote motility as we have here using RNAi to down-regulate endogenous MDM2 (Figs. 1, 2 and 5). Furthermore, whilst these studies proposed a role for MDM2-mediated ubiquitylation in promoting motility/invasion and/or metastasis, neither tested an enzymatically inactive mutant as we have in Fig. 6. Our data clearly demonstrate that MDM2 can promote increased motility and invasiveness in cells and that this does not depend upon an intact RING-finger domain of MDM2. The RING-finger mutant that we have used, C464A has been extensively used and is well-documented to lack E3-ubiquitin ligase [20,21] activity. Also, an intact RING domain is required for NEDDylation activities [22]. Thus the ability of MDM2 to promote motility is independent of both MDM2-mediated ubiquitylation and NEDDylation. Recent studies in mice have proven that the RING domain of MDM2 is essential for MDM2-mediated regulation of p53 [23]. Nevertheless, there are a number of MDM2–protein interactions that may play important functional roles that either do not, or at least appear not to depend upon the enzyme activities (ubiquitylation and/or NEDDylation) encoded by the RING domain (reviewed in [1]). MDM2 interacts with a considerable number of proteins involved in a wide range of processes and these protein–protein interfaces are potentially amenable to drug targeting. To date, the best examples are drugs aimed at rescuing p53 from the negative regulation of MDM2 such as Nutlin-3 [24] and the MI-series of compounds [25]. It may be of interest in this regard that in initial experiments using Nutlin-3 we have observed an impact of Nutlin-3 on MDM2-mediated motility, and moreover that cells expressing higher levels of MDM2 appear to be resistant to this effect (see Supplementary data Fig. 6). Both Nutlin-3 and the MI compounds act as competitive inhibitors of p53–MDM2 interaction that bind to MDM2. Thus it seems that the role of MDM2 in promoting motility may also be amenable to similar drug targeting once the key target protein/s involved in this mechanism of action is/are discovered.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.10.049.

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