Wild-type p53 controls cell motility and invasion by dual regulation of MET expression

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Recent observations suggest that p53 mutations are responsible not only for growth of primary tumors but also for their dissemination. However, mechanisms involved in p53-mediated control of cell motility and invasion remain poorly understood. By using the primary ovarian surface epithelium cell culture, we show that conditional inactivation of *p53* or expression of its mutant forms results in overexpression of MET receptor tyrosine kinase, a crucial regulator of invasive growth. At the same time, cells acquire increased MET-dependent motility and invasion. Wild-type p53 negatively regulates MET expression by two mechanisms: (*i*) transactivation of MET-targeting *miR-34*, and (*ii*) inhibition of SP1 binding to *MET* promoter. Both mechanisms are not functional in p53 absence, but mutant p53 proteins retain partial *MET* promoter suppression. Accordingly, MET overexpression, cell motility, and invasion are particularly high in p53-null cells. These results identify MET as a critical effector of p53 and suggest that inhibition of MET may be an effective antimetastatic approach to treat cancers with *p53* mutations. These results also show that the extent of advanced cancer traits, such as invasion, may be determined by alterations in individual components of p53/MET regulatory network.

Transcriptional factor p53 provides integrated responses to implement cell cycle arrest, senescence, differentiation, inhibition of cancer metabolism, or induction of the apoptotic cascade (1). Mutations of *p53* occur in about 50% of all cancers and result in loss of its function, either by null phenotype or dominant-negative effect. Additionally, some mutations result in new activities of p53, known as gain-of-function mutations (2). Recent observations indicate that p53 mutations affect cell motility and invasion, key features of metastasis (3–8). Better understanding of mechanisms of p53-dependent effects on cell motility and invasion should lead to development of approaches aimed toward correction of aberrant p53 signaling not only for suppressing growth of primary tumors but also for preventing their dissemination.

A signaling conduit known to play a critical role in invasion and metastasis is the MET pathway (9). The *MET* proto-oncogene encodes a transmembrane receptor-protein tyrosine kinase, whose overexpression is associated with poor prognosis in a broad variety of cancers (10, 11). Inhibition of MET functions has been shown to be effective in animal models and is among the most promising candidates for targeted therapy (10).

Previously it has been reported that MET is overexpressed in tumors of *p53*-deficient mice and in Li-Fraumeni patients (12). This observation is consistent with recent reports that MET represents one of the common targets for the miR-34 family (13–15). Genes encoding for the miR-34 family have been identified as direct targets of p53 transactivation (reviewed in ref. 16). At the same time, it has been reported that mouse *Met* promoter has a putative p53 responsive element and that *Met* promoter activity is activated by p53 through DNA binding to the p53 consensus sequence (17). Thus, the role of MET in p53-dependent suppression of invasion remains uncertain.

Because many cancers are genomically unstable and separation of critical alterations from "genetic noise" may be a daunting task in cells derived from advanced stages of the disease, we have used a model of conditional *p53* inactivation in the primary ovarian surface epithelium (OSE), transformation of which leads to epithelial ovarian cancer (EOC) (18, 19). This system is highly clinically relevant because *p53* mutations are by far the most frequent alterations in human high-grade serous adenocarcinoma of the ovary (20), are detected in the stage 1 of those cancers and in adjacent dysplastic lesions (21, 22), and their presence correlates with metastatic potential (23). MET overexpression is also associated with poor prognosis of EOC patients and targeting the MET pathway has been reported to suppress EOC in mouse models (11).

We report that MET is a critical player in p53-mediated control of motility and invasion, and show that such control includes miR-34–independent regulation of MET expression by p53, in addition to earlier described MET targeting by miR-34. Alterations in individual components of the p53/MET regulatory network may affect the extent of cancer invasion.

Results

***p53* Inactivation Leads to MET Overexpression.** To evaluate immediate transcriptome changes associated with *p53* inactivation, we conducted mRNA microarray analysis of primary OSE cells after acute inactivation of *p53*, *Rb*, or both *p53* and *Rb* concomitantly (Fig. 1*A* and Fig. S1). Interestingly, in addition to the expected targets of p53 and Rb/E2f signaling, up-regulation of the *Met* proto-oncogene was detected as a consequence of *p53* and *p53*/*Rb* inactivation, but not of inactivation of *Rb* alone (Fig. S1). According to qRT-PCR (Fig. 1*B*) and Western blot analysis (Fig. 1*C*), MET expression levels continued to rise for 72 h after gene inactivation and were particularly high in neoplastic OSE cell lines (over 40 passages) deficient for *p53* (OSN2) or *p53* and *Rb* (OSN1). Consistently, p53 knockdown in human ovarian cancer cells OVCA433 and colon cancer cells HCT116 carrying wild-type p53 resulted in increased MET expression (Fig. S2*A*). To examine whether MET overexpression would be detected in vivo early after *p53* inactivation, Ad-*Cre* was delivered to the OSE of $p53^{fl/fl}Rb^{fl/fl}$ Z/EG mice by transoviductal injection. Consistent with the cell-culture experiments, elevated levels of MET were detected in OSE cells that had Cre-*loxP*–mediated recombination according to expression of EGFP reporter 72 h after Ad-*Cre* administration, but Ad-Blank administration did not result in detectable MET or EGFP expression (Fig. 1*D*). No morphologically detectable differences were observed between mutant and wild-type OSE at that time (Fig. S2*B*).

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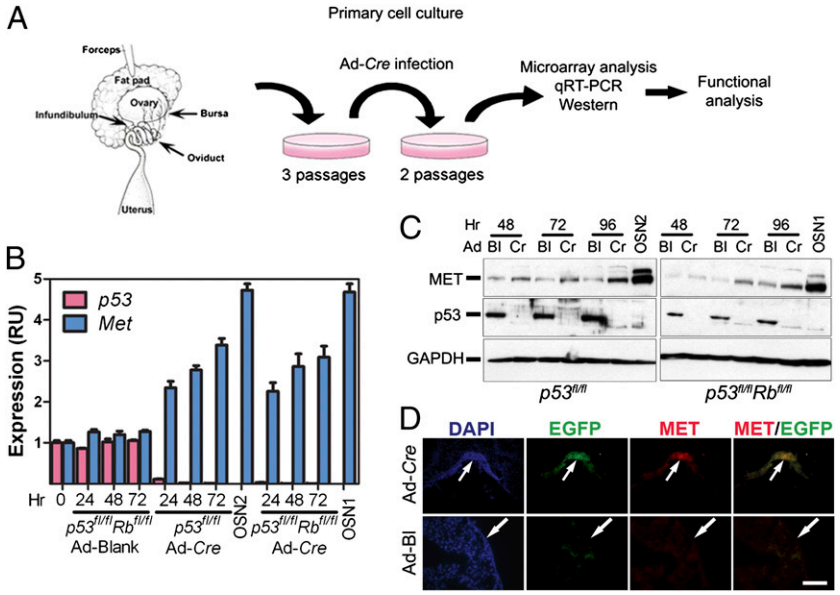

Fig. 1. Inactivation of *p53* leads to increase of MET expression. (*A*) Outline of experiments. After Ad-*Cre*–mediated *p53* inactivation, the mRNA expression profile was generated followed by qRT-PCR and Western blot validation of identified targets and elucidation of their functions by experimental testing. (*B* and *C*) Levels of MET mRNA and protein in primary OSE cells before and after *p53* inactivation or *p53* and *Rb* inactivation according to qRT-PCR and Western blot analysis, respectively. Neoplastic OSE cell lines (over 40 passages) deficient for p53 (OSN2), or p53 and Rb (OSN1), have particularly high levels of MET. qRT-PCR data: $n = 4$, mean ± SD. (*D*) Representative examples (*Upper* and *Lower*) of MET expression in the OSE of $p53^{fl/fl}Rb^{fl/f}$Z/EG mice 72 h after exposure to either Ad-*Cre* (*Upper*) or Ad-Blank (*Lower*) delivered by transoviductal intrabursal injection. Z/EG reporter indicates Cre-*loxP* mediated recombination by expression of EGFP. Note colocalization (arrows, yellow) of MET (red) and EGFP (green) in OSE cells that underwent Cre-mediated recombination. Arrows indicate OSE. Double immunofluorescence. DAPI counterstaining. (Scale bar, 100 μm.)

MET Is Essential for p53-Controlled Cell Motility and Invasion. Because increased motility and invasion are among the principal effects of up-regulated MET signaling, those features were tested in p53-deficient OSE cells. Compared to cells with wild-type p53, p53-null cells showed significantly increased cell motility in wound healing/time-lapse microscopy and migration assays, as well as increased propensity for invasion in Matrigel chambers (Fig. 2 *A*–*D*, Fig. S3*A*, and Movies S1 and S2). Moreover, treatment of cells with MET ligand HGF enhanced migration and invasion even further (Fig. 2 *A* and *B*). The increased motility and invasion was accompanied by elevation of levels of phosphorylated MET (Fig. S3*B*). To test the extent of *Met* contributions to these properties of p53-null cells, both genes were inactivated in OSE cells derived from $p53^{fl/fl}Met^{fl/fl}$ mice. *Met* inactivation abrogated the motility and invasion, but not proliferation phenotype associated with *p53* inactivation (Fig. 2*E* and Fig. S4). Thus, MET signaling is essential for p53-controlled motility and invasion.

p53 Has a miR-34-Independent Mechanism of MET Regulation. Consistent with regulation of MET expression by p53, expression of p53 at levels comparable to those of endogenous activation resulted in decreased amounts of MET protein in both mouse and human neoplastic ovarian cell lines, OSN2, and SKOV-3, respectively (Fig. 3*A* and Fig. S5).

To test the extent of MET expression dependence on miR-34, ovarian neoplastic cells were subjected to increasing amounts of miR-34 precursor molecules in transient transfection experiments. Maximum reduction of MET expression was observed at 30-nM concentration, with no apparent effect of further increases in amounts of miR-34a precursor molecule (Fig. S6 *A* and *B*) or combination of miR-34a, b, and c (15). No effect on *MET* promoter activity was observed after transfection of the full-length *MET* promoter reporter construct (pGL2-3.1MET) together with individual miR-34 family precursor molecules (Fig. S6*C*). These observations were in agreement with bioinformatics predictions and experimental evidences that miR-34 regulates MET expression principally by targeting MET 3′UTR (reviewed in ref. 16), However, cotransfection of miR-34a precursor together with wild-type *p53* resulted in further down-regulation of MET (Fig. 3*A*), suggesting that p53 may have a miR-34–independent mechanism of MET regulation. Similar effects of p53 and miR-34a precursor transfection on MET down-regulation were observed in p53-null lung cancer cells NCI-H1299, indicating potential significance of these observations for pathogenesis of other types of epithelial cancers (Fig. S6*D*).

To directly show the presence of miR-34–independent mechanism of MET regulation by p53, we isolated OSE cells from *mir-34a*$^{-/-}$*mir-34b/c*$^{-/-}$ (triple knockout, TKO) mice, lacking the entire *mir-34* family of genes. As expected, OSE cells from TKO

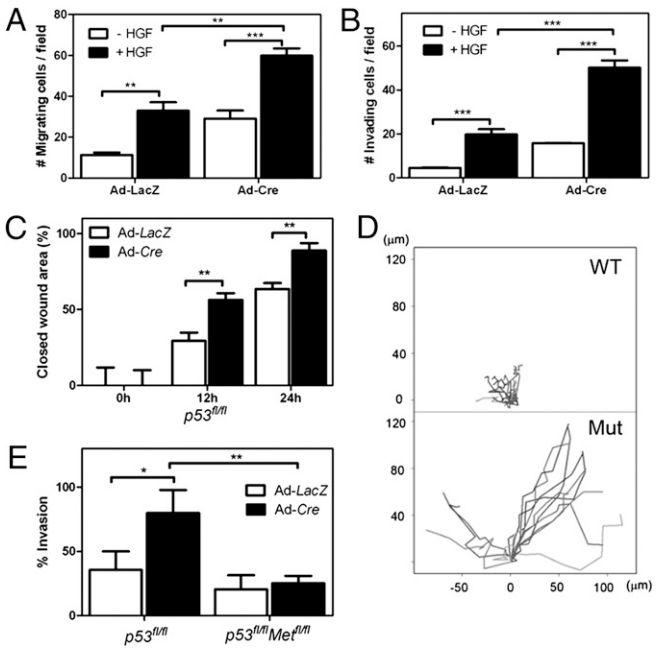

Fig. 2. MET is essential for *p53* inactivation-mediated cell motility and invasion. (*A* and *B*) Cell migration and invasion 24 h after *p53* inactivation either in absence or presence of 20 ng/mL HGF in the lower chamber. (*C*) Motility of primary $p53^{fl/fl}$ OSE cells 72 h after infection with either Ad-*LacZ* or Ad-*Cre*. Percentages (mean ± SD) of the closed-wound area 0, 12, and 24 h after the scratch were measured by TScratch software. (*D*) Tracking of individual wild-type (WT) and null p53 (Mut) cells ($n = 14$ each) in wound healing assay using time-lapse microscopy followed by analysis with ImageJ multitrack software. (*E*) Invasion properties of primary OSE cells isolated from $p53^{fl/fl}$ and $p53^{fl/fl}Met^{fl/fl}$ mice. Cells (2.5×10^4) were seeded into either control or Matrigel inserts 24 h after Ad-*LacZ* or Ad-*Cre* infection with HGF (20 ng/mL) in the lower chamber. Invading cells were counted after staining under the microscope 20 h afterward. Bars, SD ($n = 3$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

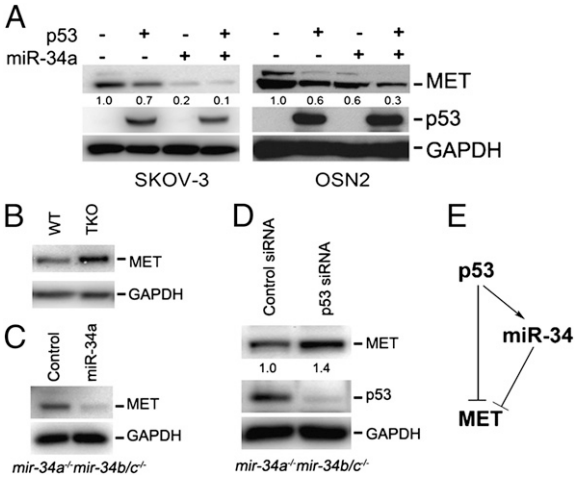

Fig. 3. p53 has a miR-34-independent mechanism of MET regulation. (*A*) MET expression after transient transfection of p53 expression vector (pORF-hp53) or miR-34a precursor molecule (30 nM) into SKOV-3 or OSN2 cells. (*B*) MET expression in OSE cells derived from either *mir-34a*$^{-/-}$*mir-34b/c*$^{-/-}$ (TKO) or age-matched wild-type (WT) mice. (*C*) MET expression in TKO OSE cells after transfection with either scrambled microRNA (control) or 30 nM miR-34a precursor molecules. (*D*) MET expression in TKO OSE cells 48 h after transfection with either control siRNA or p53 siRNA (20 nM). (*E*) A diagram of proposed miR-34–dependent and independent regulation of MET by p53.

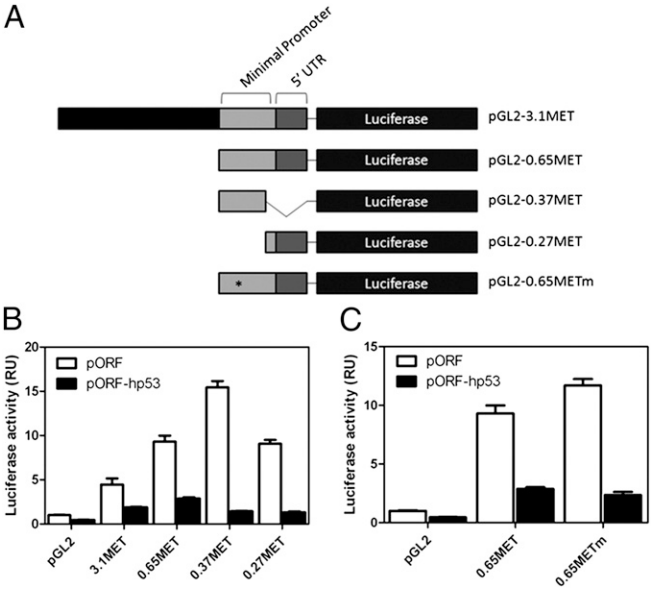

Fig. 4. p53 suppresses *MET* promoter activity. (*A*) Promoter constructs used in this study: 3.1 Kb human *MET* promoter sequence including 5′ UTR region (3.1MET) and its shorter fragments were cloned into pGL2-luciferase vector. p53 putative response element (star) was mutated in 0.65METm. (*B* and *C*) *MET* promoter activity after cotransfection of p53 expression vector (pORF-hp53) with individual promoter constructs into SKOV-3 cells. Cell lysates were harvested for estimation of luciferase activity 48 h after transfection. Note that p53 response element mutation does not abolish *MET* promoter suppression by p53 (*C*). All experiments were performed in triplicates. Bars, SD.

mice showed a higher level of MET compared with wild-type OSE cells (Fig. 3*B*). Reconstitution of miR-34 by transfecting miR-34a precursor molecules resulted in down-regulation of MET (Fig. 3*C*). Notably, p53 knockdown in TKO OSE cells led to an increase of MET expression (Fig. 3*D*), confirming that p53 down-regulates MET expression in a miR-34–independent manner, in addition to miR-34–dependent mechanism (Fig. 3*E*).

p53 Suppresses *MET* Promoter Activity. To test if *MET* promoter activity can be affected by p53 through binding of p53 consensus sequences in ovarian cells, reporter constructs containing −2619 to +353 fragments of *MET* upstream sequence (3.1MET) and its shorter fragments (24) were cotransfected with p53 expression vector into SKOV-3 (Fig. 4) and OSN2 cells (Fig. S7 *A* and *B*). Transfection of p53 significantly suppressed the promoter activity of the 3.1MET construct, as well as that of smaller fragments. Even when the putative p53 responsive element was mutated (0.65METm), p53 was still capable of repressing *MET* promoter activity comparably to the repression by corresponding wild-type promoter construct (0.65MET), indicating that the discrepancy between a previous report (17) and our findings may be a result of cell-type–specific effects.

To rule out the possibility that the observed effects of p53 on *MET* promoter activity were caused by global transcriptional repression by ectopically expressed p53, PG-13-luc and MG-15-luc reporter constructs, containing 13 copies of wild-type p53 consensus sequence and 15 copies of mutated p53 consensus sequence (25), respectively, were cotransfected with plasmids expressing either wild-type or DNA binding domain mutant p53. Only wild-type p53 could activate PG-13-luc promoter activity in SKOV3 (Fig. S7*C*) and OSN2 cells (Fig. S7*D*); all four mutant p53 constructs were unable to activate both promoter constructs.

Furthermore, p53 has been shown to inhibit hypoxia-inducible factor-stimulated transcription by destabilizing hypoxia-inducible factor 1α (HIF1A), one of the prominent regulators of *MET* promoter activity (26). However, *p53* inactivation did not lead to accumulation of HIF1A in OSE cells (Fig. S8*A*), likely because of uneven sensitivity of different cell types to hypoxia (27). Consistent with cell-type–specificity, OSE cells and ovarian cancer cell lines (OVCA432, OVCA433, and OVCAR-3) did not overexpress MET under hypoxic conditions regardless of HIF1A accumulation (Fig. S8*B*), unlike colon cancer HCT-116 cells (Fig. S8 *C* and *D*).

Another potential mechanism for p53-dependent regulation of MET is through CD44. CD44 is known to form a multimeric complex and cooperate with MET (28). CD44 has been also recently identified as a p53 (29) and miR-34 (30) target. However, CD44 was not among genes significantly up-regulated after *p53* inactivation in gene-expression microarrays (Fig. S1). Furthermore, ectopic p53 or miR-34 expression did not affect CD44 levels in ovarian carcinoma cells (Fig. S8*E*).

Taken together, these results show that *MET* suppression by p53 is unlikely to be to the result of global transcriptional repression, HIF1A, or CD44 expression.

p53 Inhibits SP1 Binding to *MET* Promoter. Because p53 was able to suppress activity of nonoverlapping promoter constructs 0.27MET and 0.37MET, transcription-factor binding sites common for both constructs were searched for by bioinformatics analysis. This search identified binding sites for the SP1 transcription factor (Fig. S7*E*). To test whether SP1 is required for *MET* promoter activity, SKOV-3 and OSN2 cells were treated with mithramycin A (mitA) to inhibit DNA binding of SP1 (31). MitA treatment suppressed luciferase expression under control of *MET* promoter in a dose-dependent manner, indicating that SP1 is required for *MET* promoter activity (Fig. 5*A* and Fig. S7*F*). This suppression was not increased by p53 expression, indicating that p53 and SP1 share a common molecular pathway to regulate *MET* promoter activity (Fig. 5*B*).

Coimmunoprecipitation experiments demonstrated that either ectopic or endogenous p53 physically interacts with endogenous SP1 in SKOV-3 and OVCA433 cells, respectively (Fig. 5 *C*–*E*). To test if p53 may inhibit SP1 DNA binding ability, ChIP experiments were performed with SP1 antibody and amplicons covering the distal and proximal region of the *MET* promoter, which contain and lack SP1 sites, respectively (Fig. 5*F*). SP1 binding was selectively enriched on the proximal region of the

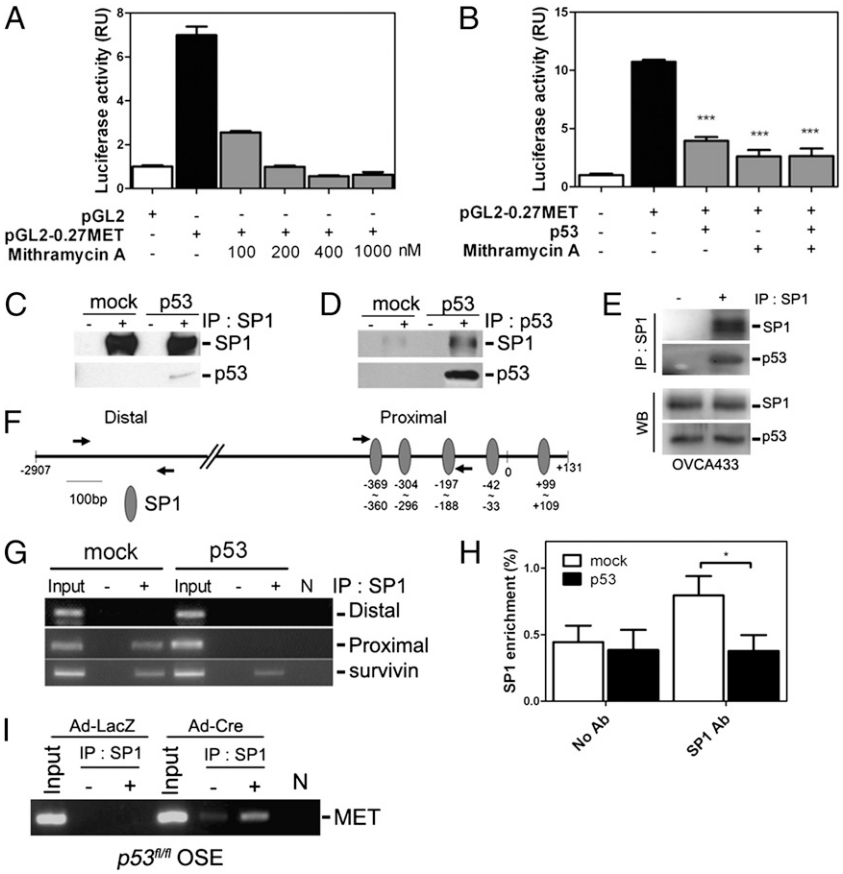

Fig. 5. p53 inhibits SP1 binding to *MET* promoter. (*A*) Effect of mithramycin A (mitA) on *MET* promoter activity. SKOV-3 cells were pretreated with mitA 1 h before transfection with 0.27MET promoter construct. (*B*) Individual and combined effects of mitA (100 nM) and p53 expression on 0.27MET promoter activity in SKOV-3 cells. Bars, SD ($n = 3$). ***$P < 0.001$. (*C–E*). Detection of p53 and SP1 binding by coimmunoprecipitation. Either control (pORF, mock) or p53 expression vector (pORF-hp53, p53) was transfected into SKOV-3 cells. Forty-eight hours after transfection, cell lysates were immunoprecipitated with SP1 (*C*) or p53 (*D*) antibodies followed by Western blotting with p53 or SP1 antibodies, respectively. (*E*) OVCA433 cell lysates were immunoprecipitated with SP1 antibodies, followed by Western blotting with p53 antibody (*Upper*, IP:SP1). Samples of the same lysates were used for Western blotting with SP1 or p53 antibody before precipitation (*Lower*, WB). (*F*) Design of ChIP assays. The proximal region of *MET* promoter contains three potential SP1 binding sites (gray oval shapes), but the distal region has none. Arrows, primers for PCR amplified regions. (*G* and *H*). Qualitative (*G*) and quantitative (*H*) analyses of ChIP assays with SP1 antibody and the distal and proximal region of *MET* promoter. ChIP assay was performed 48 h after transfection of p53 vector into SKOV-3 cells. Two-percent input control was loaded for comparison. The results are representative of three independent experiments. *$P = 0.0182$. Bars, SD. (*I*) Analysis of mouse *Met* promoter region corresponding to the proximal region of human *MET* promoter. ChIP assay was performed 48 h after either Ad-*LacZ* or Ad-*Cre* infection of OSE cells from $p53^{fl/fl}$ mice.

MET promoter, and p53 expression resulted in significant reduction of SP1 binding (Fig. 5 *G* and *H*). Conversely, as expected, p53 did not affect SP1 binding to the *survivin* promoter (Fig. 5*G*), which is repressed by p53 through recruitment of chromatin remodeling complexes (32). Consistently, in mouse OSE cells, *p53* inactivation led to an increase of SP1 binding to the corresponding mouse *Met* promoter proximal region (Fig. 5*I*). Taken together, these results show that p53 is likely to suppress *MET* promoter activity through inhibition of SP1 DNA binding.

Mutant p53 Proteins Interact with SP1 and Their Effects on OSE Motility and Invasion Depend on MET. To explore the role of mutant p53 in Met regulation, four expression vectors encoding DNA binding domain p53 mutants (V143A, R175H, R249S, and R273H) were transfected with *MET* promoter reporter constructs into SKOV-3 and OSN2 cells. All mutant p53 vectors suppressed 0.65MET and 0.27MET constructs, albeit less efficiently when compared with wild-type p53 (Fig. 6 *A* and *B*, and Fig. S9 *A* and *B*). Accordingly, the mutant p53 proteins were coimmunoprecipitated with SP1 (Fig. 6*C*), indicating that at least these common p53 mutants retain interactions with SP1.

Cells of human ovarian cancer cell lines OVCA432 and OVCAR-3 carrying mutant p53 did not show an increase of already high MET levels after p53 knockdown (Fig. S9*C*). To further explore the role of mutant p53 in motility and invasion, primary OSE cells were prepared from $p53^{+/LSLR172H}$ and $p53^{+/LSLR270H}$ mice, which contain a conditionally activated copy of mutant *p53* corresponding to human *p53* R175H and R273H hot-spot mutations, respectively. Despite overall increased p53 expression after Ad-*Cre* infection (Fig. S10*A*), *Met* expression increased only slightly (Fig. 6*D*). Although loss of both *p53* copies is sufficient to immortalize OSE cells (18), $p53^{+/R172H}$ and $p53^{+/R270H}$ OSE cells had a very limited proliferation potential and underwent senescence (Fig. S10*B*), consistent with a previously reported phenotype of mouse embryonic fibroblasts (MEFs) carrying the same *p53* mutations (33).

Similarly to MEF immortalization after the loss of remaining wild-type copy of *p53*, OSE cells from $p53^{fl/LSLR172H}$ and $p53^{fl/LSLR270H}$ mice were easily immortalized after Ad-*Cre* infection. In these cells, levels of *Met* expression were higher than those in cells heterozygous for mutant *p53* but less than those in a p53-null background (Fig. 6*E*). Cells carrying mutant R172H or R270H p53 also displayed increased cell invasion, although at a lesser extent than p53 null cells (Fig. 6*F*). Similarly to observations in p53-null cells, deletion of *Met* abrogated increased migration and invasion associated with mutant R172H or R270H p53 (Fig. 6*G* and Fig. S10*C*).

Discussion

Our work shows that p53 controls the expression of the proto-oncogene *MET* by two mechanisms, consisting of suppression of MET on the transcriptional level via promoter repression and on the posttranscriptional level via transactivation of miR-34. Contrary to a previous report (17), we were unable to find any canonical or novel p53 binding sites selectively responsible for either activation or repression of *MET* promoter. At the same time, the results of our promoter analysis, together with coimmunoprecipitation and ChIP assays, provide support for a mechanism of MET transcriptional repression based on inhibition of SP1 binding to DNA through physical interactions between p53 and SP1 rather than on direct promoter-binding by p53. Consistent with this possibility, it has been previously reported that SP1 activates *MET* promoter activity (34, 35) and interacts with wild-type (36, 37) and mutant p53 (2). Furthermore, p53 inhibits SP1 DNA binding to the *HIV-LTR* and *MGMT* promoter in vitro (38, 39).

Either lack of p53 or expression of its mutant forms abrogates *miR-34* transactivation of miR-34 (16). Therefore, both types of

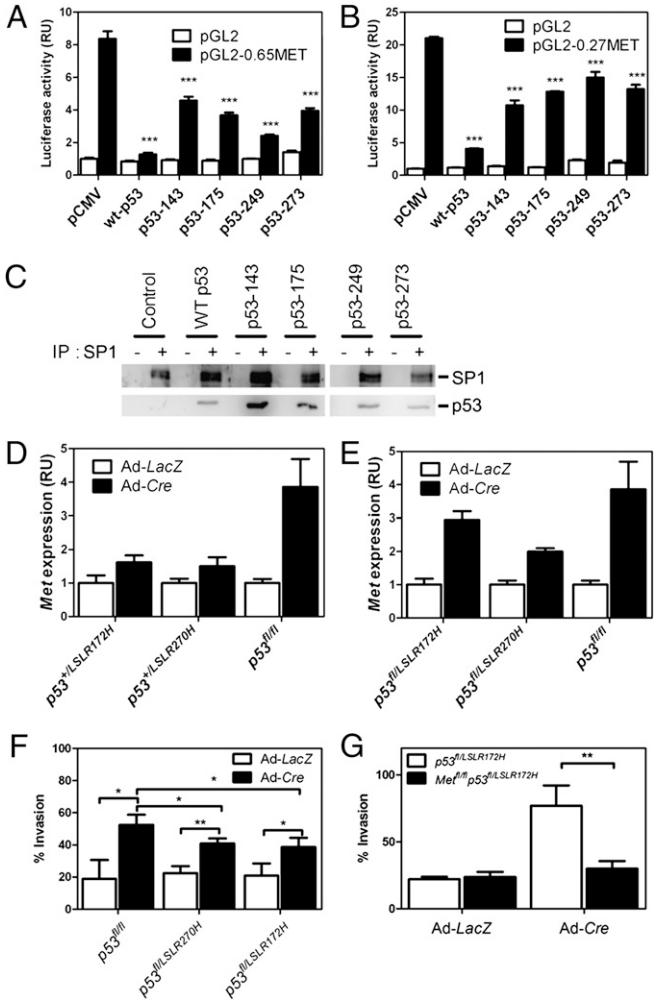

Fig. 6. Contribution of mutant p53 protein to OSE motility and invasion depends on MET. (*A* and *B*) Effects of wild-type and mutant p53 (V143A, R175H, R249S, and R273H) on the activity of 0.65MET (*A*) and 0.27MET (*B*) promoter fragments in SKOV-3 cells. (*C*) Detection of SP1 binding to wild-type and mutant p53 after transient transfection into SKOV-3 cells. (*D* and *E*) *Met* expression in OSE cells from $p53^{+/LSLR172H}$, $p53^{+/LSLR270H}$, $p53^{fl/LSLR172H}$, and $p53^{fl/LSLR270H}$ mice 48 h after Ad-*Cre* infection. qRT-PCR, $n = 3$, mean ± SD. (*F*) Invasion of OSE cells from $p53^{fl/fl}$, $p53^{fl/LSLR172H}$ and $p53^{fl/LSLR270H}$ mice 24 h after Ad-*Cre* infection. (*G*) *Met* inactivation abrogates increased invasion of OSE cells containing *p53* R172H mutation. Bars, SD ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

mutations result in elevation of miR-34–dependent MET expression. However, our study shows that unlike null mutations, mutant p53 protein retains *MET* promoter-suppressive function, albeit to a lesser degree than wild-type p53. Consistent with the lesser extent of MET expression, cells expressing mutant p53 display a lower MET-dependent motility and invasion compared with null mutants. These findings suggest that specific alterations in individual components of the p53/MET signaling network may modulate the course of pathological process. Consistent with this possibility, the poorest prognosis of *p53* null mutations has been reported in some types of neoplasms, including ovarian, lung, and breast cancers (40–42).

Recent studies indicate that lack of p53 versus expression of mutant p53 proteins may have a different impact on motility and invasion in the context of particular cell types, as well as additional genomic alterations. Our observations of increased motility and invasion by p53-null OSE cells are consistent with previous studies that have shown comparable effects in p53-null immortalized fibroblasts and MEFs (3, 4). Similarly, non-small-cell lung carcinoma line NCI-H1299 cells harboring a *p53*-null mutation have been reported to migrate faster than those expressing R175H mutant p53 (43).

At the same time, some investigators have reported that mutant p53 protein but not null *p53* mutations may drive cell motility and invasion by promoting integrin recycling (5), by forming a complex with Smad to oppose p63-mediated control of putative metastasis suppressors Sharp-1 and cyclin G2 (6) or by stabilizing the invasion promoter Slug (7). Consistent with cell-type specificity of p53 effects, mice expressing mutant p53 develop a more diverse spectrum of neoplasms compared with null mutants (33). Interestingly, in line with observations by Grugan et al. (44), knockdown of mutant p53 in ovarian carcinoma cells did not change levels of MET expression. This observation is at variance with our results on primary OSE cells carrying a conditional mutant *p53* allele, which may indicate that established cancer lines and neoplastic cells at advanced cancer stages develop additional mechanisms ensuring MET signaling in cells carrying mutant forms of p53 protein. Assessment of primary ovarian cancer cells should address this problem in future studies. It should be also of interest to evaluate p53/MET signaling in the fallopian tube epithelium, another potential cell of origin of serous adenocarcinoma of the ovary (45, 46).

There are indications that some additional genetic or epigenetic alterations are required for stabilization and accumulation of mutant p53, thereby leading to a gain-of-function phenotype (47). In agreement with this possibility, our results in primary OSE from $p53^{+/R172H}$ or $p53^{+/R270H}$ mice, as well as studies by others in MEFs with the same genotypes (33), show that cell immortalization is acquired only after the loss of the remaining wild-type copy of *p53*. Consistently, Adorno et al. (6) were able to observe increased migration of cells expressing mutant p53 only in combination with HRAS and TGF-β signaling. Notably, *RAS* mutations are extremely rare in high-grade serous ovarian carcinoma (23).

Development of therapies aimed at correction of the p53 pathway remains among the most coveted goals in cancer research (48). Importantly, our experiments have demonstrated that MET is a critical component of motility and invasion in cells either lacking p53 or expressing its mutant forms. Therefore, treatment of cancers with p53 mutations is likely to benefit from therapeutics aimed at MET, such as small targeting molecules (10). This finding is of particular significance because, despite successful outcomes in cell culture and animal in vivo experiments, direct reintroduction of the *p53* gene failed in clinical trials, including EOC (49). Our study also indicates that approaches aimed at indirect elimination of mutant p53 protein (e.g., by *p53* siRNA) should be avoided in cancers with an active p53/MET signaling network. The in-depth understanding of mechanisms by which p53 regulates MET in the context of different cell types, as well as specific p53 mutations, may be essential for future development of individualized therapeutics.

Materials and Methods

Experimental Animals. The origin and genotyping of mice with conditional alleles of wild-type and mutant *p53*, *Rb1*, *Met*, and reporter genes are described in the *SI Materials and Methods*. The *mir-34a*$^{-/-}$ mice generated in H.H.'s laboratory and *mir-34b/c*$^{-/-}$ mice generated in A.Y.N.'s laboratory were crossed to obtain *mir-34a*$^{-/-}$*mir-34b/c*$^{-/-}$ mice. All mice were maintained identically following recommendations of the Cornell Institutional Laboratory Animal Use and Care Committee.

Cell Culture. Derivation and culture conditions of primary mouse OSE cells, established mouse OSE cell lines (OSN1 and OSN2), and human cancer cell lines SKOV-3, OVCAR-3, OVCA432, OVCA433, NCI-H1299, and HCT116, as well as proliferation and senescence assays, are described in *SI Materials and Methods*.

Conditional Gene Inactivation and mRNA Microarray Profiling Studies. Primary OSE cells carrying conditional gene alleles were passaged three times and treated with recombinant adenoviruses essentially as described previously (18, 19). Cells were collected two passages after infection and processed for

mRNA isolation and assessment of mRNA profiles as described in *SI Materials and Methods*.

Transinfundibular Administration of Adenovirus, Collection of Histological Materials, and Immunohistochemistry. All procedures were performed as described previously (18). The detailed protocol MET/EGFP double immunofluorescence is provided in *SI Materials and Methods*.

Wound Healing, Time-Lapse Microscopy, Migration, and Invasion Assay. For wound-healing assay, OSE cells were infected with adenovirus and cultured in a 10-cm gelatin-coated dish to confluence. Cells were scraped with a p200 tip, and fresh medium supplemented. Digital images of the wound were taken at 0, 12, and 24 h after scraping. Analyses of the wound-closure and time-lapse microscopy are described in *SI Materials and Methods*. Migration and invasion assays were performed as described in ref. 15.

Promoter Activity Analysis and Transfection, Quantitative Real-Time RT-PCR, Coimmunoprecipitation, Western Blotting, ChIP Assays, and Statistics. All procedures were performed according to established methods as described in *SI Materials and Methods*.

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