Published in final edited form as:

Int J Cancer. 2015 October 1; 137(7): 1528–1538. doi:10.1002/ijc.29528.

Mutant p53 expression in fallopian tube epithelium drives cell migration

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Abstract

Ovarian cancer is the fifth leading cause of cancer death among US women. Evidence supports the hypothesis that high-grade serous ovarian cancers (HGSC) may originate in the distal end of the fallopian tube. Although a heterogeneous disease, 96% of HGSC contain mutations in p53. In addition, the “p53 signature”, or overexpression of p53 protein (usually associated with mutation), is a potential precursor lesion of fallopian tube derived HGSC suggesting an essential role for p53 mutation in early serous tumorigenesis. To further clarify p53-mutation dependent effects on cells, murine oviductal epithelial cells (MOE) were stably transfected with a construct encoding for the R273H DNA binding domain mutation in p53, the most common mutation in HGSC. Mutation in p53 was not sufficient to transform MOE cells, but did significantly increase cell migration. A similar p53 mutation in murine ovarian surface epithelium (MOSE), another potential progenitor cell for serous cancer, was not sufficient to transform the cells nor change migration suggesting tissue specific effects of p53 mutation. Microarray data confirmed expression changes of pro-migratory genes in p53^{R273H} MOE compared to parental cells, which could be reversed by suppressing Slug expression. Combining p53^{R273H} with KRAS^{G12V} activation caused transformation of MOE into high-grade sarcomatoid carcinoma when xenografted into nude mice. Elucidating the specific role of p53^{R273H} in the fallopian tube will improve understanding of changes at the earliest stage of transformation and could help develop chemopreventative strategies to prevent the accumulation of additional mutations and reverse progression of the “p53 signature” thereby, improving survival rates.

Keywords

p53 mutation; high-grade serous cancer; fallopian tube

Introduction

Ovarian cancer is the fifth leading cause of cancer death and the most lethal gynecological malignancy¹. The initiating events associated with the disease are not fully understood. Historically it was hypothesized that ovarian cancer originated in the single layer of epithelial cells surrounding the ovary, known as the ovarian surface epithelium (OSE)². However, the epithelium of the fallopian tube fimbriae (FTE) has been proposed as an alternative origin for some high-grade serous cancers (HGSC), the most common and lethal histotype of the disease. This hypothesis is based on observational studies from tissue obtained from high-risk populations^{3–5}. Evidence has also been provided by recent *in vitro* and *in vivo* studies demonstrating that normal fallopian tube epithelium is capable of transforming into serous cancer^{6–8}. The main drawback with these models is that cell immortalization with SV40 functionally inactivates p53, precluding investigation into the role of mutant p53, which is common in HGSC⁹. Mice expressing mutant p53 develop an altered tumor spectrum (more carcinomas) than those harboring a p53 deletion¹⁰ suggesting that model systems inactivating p53 may not accurately recapitulate the human disease.

There are several pieces of evidence to support the fallopian tube hypothesis including morphologic resemblance of HGSC to cells of the fallopian tube¹¹. Women with *BRCA* mutations who are at increased risk of developing ovarian cancer often undergo prophylactic bilateral salpingo-oophorectomies⁴. Careful examinations of their reproductive tracts revealed lesions in the fimbriated end of the fallopian tube while the ovaries appeared morphologically normal suggesting initiating events occur in the FTE³. The focal neoplastic lesions were characterized by strong immunoreactivity for p53, increased Ki67 (MIB-1)¹² expression, and dysplastic appearance⁴. These serous tubal intraepithelial carcinomas (STICs) are observed in many of the women with advanced HGSC, regardless of *BRCA* status, and often both tubal and ovarian malignancies share identical p53 mutations suggesting a common origin⁵.

Almost all (96%) HGSC tumors contain a mutation in p53⁹. Missense p53 mutations affecting the DNA binding domain (exons 5 to 8) have previously been shown to be oncogenic with defects in wild-type p53 function as well as gain-of-function activities¹⁰ to enhance tumorigenicity, metastatic potential, and survival (called dominant negative)¹⁰. Wild-type p53 has a short half-life (20 min) and is difficult to detect using immunohistochemistry, while missense, mutant p53 often has stabilized expression¹³. This stabilization can be, in part, due to lowered concentrations of Mdm2, a transcriptional target of wild-type p53 and negative regulator of both wild-type and mutant p53¹⁴. Benign regions of the fallopian tube epithelium with over 12 consecutive secretory cells staining positive for p53 (deemed the “p53 signature”)¹⁵ has been suggested as a putative precursor lesion to STICs and HGSC formation, yet animal models harboring p53 mutation alone have not yet been characterized. It should be noted that some data also indicate that p53 overexpression is present in cycling women, independent of disease¹⁶.

Modeling transformation of fallopian tube epithelium is key to advancing our understanding of HGSC formation and confirming that putative preneoplastic lesions do indeed progress to carcinoma. *In vitro* cell cultures are flawed by immortalization strategies that do not mimic

human pathology¹⁷, while *ex vivo* systems are limited by senescence^{17, 18}. In this study, a murine oviductal epithelial cell line that did not require immortalization with hTERT or SV40 was developed and characterized. After stable transfection of mutant *TP53*, this cell line serves as the first tool to uncover downstream signaling of mutant p53 alone and in combination with other pathway alterations in oviductal epithelium. This tool will be invaluable in the study of early transformative events associated with HGSC formation.

Materials and Methods

Animals

All animals were treated in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the established Institutional Animal Use and Care protocol at the University of Illinois at Chicago (UIC). In addition, the Animal Care Committee approved the protocol A11-169. p53^{R270H/+} [129S4-*Trp53*^{tm3Tyj}] were obtained from the Mouse Models of Human Cancers Consortium Mouse Repository (National Cancer Institute, Rockville, MD). The p53^{R270H/+} mice were bred with FVB mice to generate a mixed strain and were genotyped as previously described (Jackson Laboratory, Bar Harbor, ME). Animals were housed in a temperature and light controlled environment (12 hours light, 12 hours dark) and were provided food and water *ad libitum*. All mice were euthanized by CO₂ inhalation followed by cervical dislocation. For xenograft experiments two million cells were suspended in a 50% PBS, 50% Matrigel (Corning Inc., Lake Forest, IL) mixture and injected subcutaneously into nude mice (n=6) (Taconic, Germantown, NY). 10 million cells suspended in PBS were injected into the peritoneal cavity via IP injection. All attenuated adenovirus was used at a concentration of 10¹⁰ plaque forming units (pfu)/ml (Iowa Gene Transfer Vector Core, Iowa City, IA) and *in vivo* administration was performed as previously described¹⁹.

Cell Culture

Murine oviductal cells (MOE) were obtained from Dr. Barbara Vanderhyden at the University of Ottawa and were maintained in MOSE media as previously described²⁰ with additional 20 ng/ml β -estradiol dissolved in ethanol (Sigma-Aldrich, St. Louis, MO). Stable cell lines were generated using antibiotic resistant plasmids containing the gene of interest. pCMV-Neo-BAM p53 R273H was a gift from Bert Vogelstein (Addgene plasmid # 16439)²¹. pLENTi-PGK-Hygro-KRAS4B(G12V) was a gift from Daniel Haber (Addgene plasmid # 35633)²². SV40 was a gift courtesy of Dr. Kathy Rundell (Northwestern University, Chicago, IL). pCMV6-XL4-Neo vector was obtained from OriGene Technologies (Rockville, MD) and shRNA Snai2 from Sigma-Aldrich. Cell lines were verified with Western blot or qRT-PCR analysis and maintained at 37°C in a 5% CO₂ incubator. Primer sequences are available upon request.

Generation of Primary MOSE and MOE Cell Lines

Ovaries and oviducts from 4 p53^{R270H/+} mice were obtained. To isolate the OSE, ovaries were incubated at 37°C with type IV collagenase (Worthington Biochemical, Burlington, CA). A series of vortexing for 2 minutes then dislodged the outer layer of cells. MOE cells were acquired through physical dissociation of oviductal tissue followed by culture for 48–

72 hours. During this time the epithelial cells lining the oviduct migrated out of the tube. Some of these MOSE and MOE cells were exposed to Ad5CMVCre-eGFP (Iowa Gene Transfer Vector Core)²³ *ex vivo* to activate expression of mutant p53 protein (R270H). GFP expression confirmed infection of cells. Unexposed MOSE and MOE from floxed p53^{R270H/+} mice served as the control.

Cell Characterization

Cells were fixed in 4% PFA, washed with PBS, and permeabilized with 0.2% Triton in PBS. 10% goat serum (Vector, Burlington, CA) block was added before incubation of primary antibodies at the following concentrations: acetylated tubulin 1:1,000 (Sigma-Aldrich), CK8 1:200 (TROMA-1, Developmental Studies Hybridoma Bank, Iowa City, IA), OVGP1 1:200 (Abcam, Cambridge, MA), p53 1:100 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and PAX8 1:100 (ProteinTech Group, Inc., Chicago, IL) for one hour. Following PBS washes, the fluorescent secondary (goat anti-rabbit Alexafluor 594, goat anti-mouse Alexafluor 488, goat anti-rat Alexafluor 594, and goat anti-rabbit Alexafluor 488; Invitrogen, Grand Island, NY) in PBS was added at 1:200. The mounting medium for the coverslip contained DAPI (Vector). Immunofluorescent images were acquired on a Nikon E600 microscope using a DS-Ri1 digital camera and NIS Elements software (Nikon Instruments, Melville, NY).

SRB Proliferation Assay

The sulforhodamine B (SRB) assay was used to measure cell viability. Cells were plated at 500 cells per well in a 96-well plate. Cell lines were cultured for 1, 3, and 5 days. In addition, a day 0 measurement was taken 2 hours following cell plating. Colorimetric assay was performed to determine cell density as previously described²⁴. Absorbance at 505 nm was measured on a BioTek Synergy 2 microplate reader (BioTek, Winooski, VT). Each absorbance value was normalized to the corresponding day 0 absorbance value to determine fold increase. Significance was determined by 2-way ANOVA.

Soft Agar Colony Formation Assay

Soft agar colony formation assay was performed and imaged as previously described²⁰ except images were taken after 14 days in culture. Alisertib (25 nM) treatment was replaced every four days. Wells were blinded and colonies counted using ImageJ NIH software. Significance was determined by one-way ANOVA.

Migration Assays

Cells were plated to confluence (1.5×10^5 cells/well) in a 24-well plate. A uniform wound was created through the cell monolayer and pictures were taken at 0 and 8 hours after scratching using an AmScope MU900 with Toupview software (AmScope, Irvine, CA). The area of the scratch was analyzed with ImageJ NIH software. Percentage of closure was determined by measuring the final volume of the wound relative to the initial volume of the scratch. Significance was determined by one-way ANOVA.

Migration was also measured with the xCELLigence® DP system (Acea Biosciences, Inc., San Diego, CA) as previously described²⁵. 4×10^4 cells were suspended in serum-free

media and plated in the upper chamber of the CIM plate. 10% FBS media was used as an attractant. Cells were allowed to settle for 30 minutes before measurements were taken every 15 min for 50 hours. Significance was determined by t-test.

Western Blot Analyses

Protein concentration was determined by BCA assay (Pierce, Rockford, IL). Protein lysate (30 µg) was analyzed by 12% SDS-PAGE and transferred to nitrocellulose. Blots were then blocked with 5% milk or BSA in TBS-T and probed overnight with primary antibodies at 4°C. The following primary antibodies were used at a concentration of 1:1000: actin (Sigma-Aldrich), HA Tag (Cell Signaling Technology, Inc., Beverly, MA), p53 (Santa Cruz), Slug (Abcam), SV40 (Santa Cruz), and Vimentin (Sigma-Aldrich). Mdm2 (Abcam) antibody concentration was used at 1:500. Anti-mouse and anti-rabbit horseradish peroxidase (HRP)-linked secondary antibodies (Cell Signaling Technology, Inc.) were used at a concentration of 1:1000 for all blots except for actin, which was used at 1:10,000 (Promega, Madison, WI) in blocking buffer. Membranes were washed and incubated in SuperSignal West Femto substrate (Thermo Scientific, Rockford, IL) before imaging on a FlourChem™ E system (ProteinSimple, Santa Clara, CA). Densitometric analysis was performed using NIH ImageJ and significance determined by t-test.

RNA Isolation, cDNA Synthesis, and qRT-PCR

RNA extraction was performed using Trizol (Life Technologies, Grand Island, NY) and chloroform with isopropanol precipitation followed by ethanol washes and DNase step. RevertAid (Thermo Scientific) and SYBR (Roche, Madison, WI) were used according to manufacturer's instructions to synthesize cDNA and quantify DNA, respectively. All qRT-PCR measurements were performed using the ABI ViiA7 (Life Technologies).

Microarray

RNA was isolated from 3 different passages each of MOE cells transfected with either pCMV6-XL4-Neo vector (empty control) or pCMV-Neo-BAM p53 R273H using an RNeasy kit with spin columns (Qiagen, Germantown, MD). The Mouse 2.0 transcription array was performed using the Affymetric GeneChip System. Fold change of gene expression in p53^{R273H} compared with control MOE was calculated and analyzed by ANOVA by the Core Genomic Facility at the University of Illinois at Chicago (GSE62694). p-values less than 0.00643 were considered statistically significant. Gene Ontology "GO" analysis was performed by the Biomedical Informatics Core at the University of Illinois at Chicago using DAVID software.

Tumor Histology

Tissues were prepared for paraffin sectioning and immunohistochemistry as previously described¹⁹. Tissues were incubated with the following primary antibodies at 4°C overnight: CK8 1:200 TROMA-1 (Developmental Studies Hybridoma Bank), Ki67 1:100 (Abcam), p53 1:25 (Santa Cruz), PAX8 1:150 (Proteintech), and WT1 1:100 (Abcam). In all cases, omission of the primary antibody was used as a negative control. Images were acquired on a

Nikon E600 microscope using a DS-Ri1 digital camera and NIS Elements software (Nikon Instruments).

Statistical Analysis

Data are presented as mean $\pm$ standard error, with significance determined utilizing GraphPad Prism software (GraphPad, La Jolla, CA) for $p < 0.05$. All data sets were analyzed for significant outliers by Grubbs' test of deviation.

Results

Mutation of p53 in murine oviductal epithelium (MOE) increases migration without altering cell proliferation or ability to grow in soft agar

Positive staining for cytokeratin 8 (CK8) and oviductal glycoprotein 1 (OVGP1) confirmed the oviductal origin of the MOE cells. The cells are of the secretory subtype, staining positive for PAX8. No ciliated cells are present as evidenced by the absence of acetylated tubulin staining (Figure 1A). MOE were stably transfected with human mutant *TP53* with the point mutation of an arginine to histidine at amino acid 273 in the DNA binding domain (p53^{R273H}), the most common mutation found in human ovarian tumors⁹. MOE stably transfected with an empty vector served as the control while MOE stably transfected with SV40 served as a positive control because oviduct specific promoter driven expression of SV40 has previously been shown to drive tumor formation in a murine model^{26, 27} (Figure 1A). Proliferation of the p53^{R273H} MOE was similar to that of control MOE, while SV40 MOE had significantly higher proliferation than control on day 3 and day 5 (Figure 1B). No significant increase in colony formation was measured in p53^{R273H} MOE compared to control, suggesting that the R273H p53 mutation does not transform MOE cells (Figure 1C). SV40 MOE formed an average of 38.6 colonies in soft agar. This significant increase compared to control is evidence of transformation. Both p53^{R273H} and SV40 MOE displayed a significant increase in migration in an 8 hour wound closure assay compared to control MOE (Figure 1D). Increased cell migration of p53^{R273H} was further confirmed in an xCELLigence® electric impedance assay with significantly different cell index (CI) measurements between p53^{R273H} MOE and control for 50 hours (Figure 1E). To confirm our results, an additional MOE cell line was generated harboring a R270H mutation in p53 (the murine equivalent of R273H). These cells were generated from floxed p53^{R270H/+} mice (Supplemental Figure 1A) and exhibited no change in proliferation or ability to grow in soft agar but displayed a significant increase in migration similar to MOE cells harboring the human R273H p53 mutant protein (Supplemental Figure 1B–D).

Mutation of p53 in murine ovarian surface epithelium (MOSE) does not alter migration, proliferation, or ability to grow in soft agar

p53^{R270H} MOSE cells were generated from OSE enzymatically isolated from the ovaries of floxed p53^{R270H/+} mice exposed to Cre recombinase *in vitro*. Unexposed OSE from floxed p53^{R270H/+} mice served as the control (Figure 2A). No significant change was noted in proliferation, ability to grow in soft agar, or migration between the p53^{R270H} MOSE compared to control MOSE (Figures 2B–D). A previous publication showed that SV40 transforms MOSE²⁰. These results were confirmed *in vivo* with injection of adenovirus

expressing Cre recombinase into the bursa of p53^{R270H/+} mice. Previous studies have shown this technique specifically targets Cre expression in the OSE¹⁹. Mice with conditional expression of mutant p53 in the OSE did not develop an altered phenotype 3 (n=11), 6 (n=5), or 10 months (n=6) following activation (Figure 2E).

Microarray reveals migratory gene signature in p53^{R273H} MOE

To examine the transcriptome changes associated with p53 mutation in MOE and the increased migratory phenotype, a murine, cDNA microarray was performed. A total of 5,317 genes were found to have expression that was significantly different in p53^{R273H} MOE compared to control MOE. Previous ChIP experiments have shown p53 occupies DNA response elements in Mdm2, Btg2, and Dhfr9²⁸. Microarray data revealed significant down-regulation of Mdm2 and Btg2 and significant up-regulation of Dhfr9 in p53^{R273H} MOE cells (Figure 3A). The findings were validated through qRT-PCR for Mdm2, Btg2, and Dhfr9 in p53^{R273H} and p53^{R270H} MOE (Figure 3B and Supplemental Figure 1E). Western blotting analysis also identified decreased Mdm2 protein in p53^{R273H} MOE (Figure 3C). Gene Ontology (GO) analysis was performed using DAVID to understand the biological pathways most significantly altered within the genes differentially expressed. Among the top ten most significantly altered GO categories (lowest FDR, false discovery rate), five were related to cell migration (Cell Motion, Morphogenesis of Branching Structure, Cell Migration, Cell Adhesion, and Biological Adhesion) (Figure 3D).

Slug contributes to the migratory phenotype observed in p53^{R273H} MOE

GO terms from the microarray data comparing p53^{R273H} to control MOE cells suggested a pro-migratory reprogramming. Specifically, up-regulation of extracellular matrix genes *Snai2* (the gene that encodes for Slug), *Col6A*, and *Lama4* and down-regulation of adhesion molecules *Vcam1* and *Entpd5* were observed in the p53^{R273H} MOE microarray (Figure 4A) and are associated with migration, metastasis, and carcinoma progression^{29, 30}. Increased expression of Slug and Vimentin, a mesenchymal marker³¹ and down-stream target of Slug, was measured using qRT-PCR and western blot analysis in MOE containing either R273H or R270H mutation (Figure 4B–D and Supplemental Figure 1E–F). Further, knockdown of Slug with *Snai2* shRNA in p53^{R273H} MOE (Figure 4E) was able to significantly inhibit migration as measured by wound closure and electrical impedance assay (Figure 4E–F). Slug expression was not enhanced in p53^{R270H} MOE (Figure 4G), which was previously shown to have unaltered migration (Figure 2B).

R273H p53 mutation combined with G12V KRAS activation transforms murine oviductal epithelium in vitro and in vivo

Transformation of MOE with mutation in p53 was further investigated with additional genetic manipulations. Cell lines with mutated p53 and activated KRAS pathway alteration via the G12V mutation resulted in decreased proliferation at day 5 and no change in migration (Supplementary Figure 2A–B), however, a significant increase in soft agar colonies was detected (Figure 5A).

Previous microarray analyses performed on MOE cells lacking p53 suggested that Aurora kinase A, Aurora kinase B, Met, Polo-like kinase 4, and Wt1 were up-regulated³² in the

absence of p53. In order to directly compare loss and mutation of p53 in MOE, qRT-PCR for these targets was used to quantify expression. Significant up-regulation of Aurora kinase A and Met was measured in KRAS^{G12V}/p53^{R273H} MOE compared to Control MOE (Supplementary Figure 2C). Interestingly, Wt1 expression was significantly down-regulated (the opposite result compared to p53 deletion). Aurora kinase A specific inhibitors are currently in clinical trials for the treatment of high-grade serous ovarian cancer³³ however, treatment with the Aurora kinase A specific inhibitor (Alisertib) was not sufficient to block colony formation (Figure 5A). To examine the relevance of these findings in human tumors, data generated by the TCGA Research Network (<http://cancergenome.nih.gov/>)⁹ was analyzed using cBioPortal for Cancer Genomics. Input of the genes TP53 and KRAS revealed that 304 HGSC tumors out of 316 samples studied (96.2%) contain alterations (gene amplification, homozygous deletion, mutation, mRNA down-regulation or mRNA up-regulation) in one or both of these genes. Interestingly, patient tumors containing alterations in KRAS and TP53 were shown to have significantly increased survival compared to tumors with other alterations (Figure 5B)^{34, 35}.

To determine if the genetically altered MOE cells were tumorigenic, 2 million cells were xenografted subcutaneously and 10 million cells injected intraperitoneally into nude mice (n=6/group). All mice with KRAS^{G12V}/p53^{R273H} xenografts were sacrificed prior to the 6-month time point due to loss of wellness from tumor burden and had a mean survival of 147.3 days $\pm$ 10.5 (Figure 5C). The average subcutaneous tumor mass was 363.0 mg $\pm$ 140 (Table 1). No tumors formed in the mice with xenografts of parental, p53^{R273H}, or KRAS^{G12V} MOE (data not shown), which were sacrificed at six months post xenograft.

Histologic analysis (Figure 5D) showed that the tumors were poorly differentiated with mostly spindle cell and areas of epithelioid cell proliferation. Tumors were high grade, characterized by high-grade nuclei, increased mitotic activity (Ki-67 index is about 30%), and areas of tumor necrosis. Further immunostains showed tumor cells were positive for CK8, consistent with sarcomatoid carcinoma. These tumors proved to be of oviductal origin and serous type differentiated as indicated by immunoreactivity for PAX8 and WT1, respectively (Figure 5D). In addition, expression of p53 was observed, as is associated with mutation in p53.

Intraperitoneal injection of 10 million cells was performed to measure the metastatic capacity of the genetically altered MOE. Widespread intraperitoneal explants were observed in only one of the six mice (~17% of animals) and included nodules on the uterus, muscle of the peritoneal wall, and diaphragm. The explants displayed similar histology to the subcutaneous tumors (Figure 5D). The mouse harboring peritoneal explants also presented with an enlarged spleen and pale kidneys. Enlarged lymph nodes were noted in 4 of the 6 mice with KRAS^{G12V}/p53^{R273H} xenografts (~67% of animals) (data not shown).

Discussion

Large scale analysis by the Cancer Genome Atlas Network has revealed that p53 is the most common mutation in HGSC, occurring in 96–100% of tumors⁹. Fallopian lesions, termed as the p53 signature and STICs, have altered p53 expression and have been proposed as

potential precursors to HGSC³⁶ suggesting that mutation of p53 is an early and essential event in tumorigenesis. This study has utilized a model of fallopian (oviductal) tube epithelium with mutation in p53 to further clarify the functional significance and downstream signaling specific to p53 mutation in the fallopian tube. Mutation of p53 alone was insufficient to transform murine oviductal epithelium (MOE). Similar results were obtained for mutant p53 expression in OSE³⁷. MOE cells expressing mutant p53 did display increased cell migration, which correlated to increased expression of the pro-migratory factor Slug. Microarray analysis confirmed a pro-migratory gene signature in the p53^{R273H} MOE cell line. Interestingly, increased cell migration was not observed in MOE containing a similar mutation in p53, providing evidence that the effects of the mutation are cell-type specific. Further clarification of the aberrant signaling downstream of p53 mutation in FTE can help to inform prevention and therapeutic strategies, which have not improved in several decades¹.

While several models have been developed to study mutant p53 expression in ovarian surface epithelium^{37–39}, no model has addressed how mutant p53 alone effects fallopian tube epithelium. The effect of R270H p53 mutation has been previously reported only in combination with inactivation of *Brca1* (or 2) and *Pten*⁷ while the R172H mutation of p53 was combined with *Apc* and *Pten* inactivation⁴⁰, thus obscuring understanding of the role of p53 mutation alone. In addition, other murine models have utilized promoters, such as MISRII, that lack specificity for the oviductal epithelium^{27, 41} and adenoviral injections into the oviduct do not result in efficient infection and gene manipulation¹⁹. Human cell lines often rely on immortalization with SV40, which precludes investigation into the role of mutant p53 by functionally inactivating it. In addition, microarrays have previously been performed on the two potential progenitor cells of HGSC, oviductal and ovarian surface epithelium; however, the cells utilized in these studies have silenced p53^{27, 32}. Given that mutations in the DNA binding domain (gain of function mutations, rather than loss or truncation) are the most common found in serous cancer, a clear determination of the transcriptional reprogramming, both activating and repressing, of this essential genetic event could provide an opportunity to impact cancer formation and progression.

The microarray in this study revealed p53^{R273H} MOE cells exhibit a significant increase in the expression of *Snai2*, the gene that encodes for Slug. Slug plays a role in cell migration and invasion by increasing the transcription of mesenchymal markers including Vimentin⁴². Slug expression has been reported in all stages of serous cancer, providing evidence that up-regulation could be an early event⁴³. The *Snai2* gene has p53-responsive elements and *in vitro* experiments have demonstrated that p53 directly regulates Slug transcription⁴⁴. p53 is also known to regulate Slug in a transcription-independent manner. Cooperation between p53 and p21 have been shown to mediate Mdm2 degradation of Slug in non-small cell lung cancer^{45,46}. Further, the R273H mutant p53 is still capable of cooperating with p21 to induce Slug degradation, however, the decreased levels of Mdm2, a transcriptional target of wild-type p53, may cause the increased Slug expression observed in the p53^{R273H} MOE. Mutation of p53 has previously been associated with regulation of cell invasion⁴⁷; however, this is the first report of the R273H mutation of p53 driving invasion of oviductal epithelium. Overexpression of Slug may contribute to the spread of fallopian tube

epithelium to organs in the peritoneal cavity, including the ovary. Activation of additional genetic mutations in these newly colonized sites may give rise to HGSC or additional metastases.

Mutation of *TP53* combined with *KRAS* mutation led to anchorage independent growth, which agrees with previous reports that p53 mutation combined with RAS activation induces a cancer-related gene signature⁴⁸. Mutations in *KRAS* are observed in 24% of HGSC and components of the RAS pathway are altered in up to 45% of tumors⁹. This transformation may be due to increased genetic instability caused from the increase in Aurora kinase A expression. Aurora kinase A is a member of the serine/threonine kinase family and is involved in centrosome separation, bipolar spindle assembly, and chromosome segregation ensuring the equal distribution of genetic material during cell division. The protein is commonly amplified in HGSC⁴⁹ and is correlated to overall decreased survival in ovarian cancer patients⁴⁹. p53 negatively regulates Aurora A expression by transcriptional (E2F3 binds promoter) and post-translational modification (Fbw7α)⁵⁰. Treatment with 25nM Alisertib, an Aurora kinase A specific inhibitor, was insufficient to prevent formation of colonies in soft agar. This dose was previously shown to be effective in OVCAR5³³, an ovarian cancer cell line, but a higher concentration may be needed to block transformation of KRAS^{G12V}/p53^{R273H} MOE. The decreased proliferation of KRAS^{G12V}/p53^{R273H} MOE correlates with human tumor data from the cBioPortal showing improved survival and could be evidence that KRAS^{G12V}/p53^{R273H} MOE tumors are less aggressive than other HGSC. This theory is further strengthened by the xenograft data showing limited peritoneal dissemination of the cells. KRAS mutations are commonly observed in low-grade serous or mucinous carcinomas in human tumors, which are characterized with low malignant potential and are associated with higher survival². Further, these data provide evidence that loss of p53 when combined with KRAS activation does not recapitulate mutation, as cells with SV40 and KRAS demonstrated enhanced proliferation^{6, 8}.

In summary, a novel model of fallopian (oviductal) epithelium has been generated to understand the role of mutant p53 in early events leading to HGSC formation. Increased Slug expression observed in p53^{R273H} MOE could explain how high-grade serous tumors move to the ovary and are not only localized to the fallopian tube. Targeting Slug-expressing cells could prevent mutant p53 fallopian tube epithelium from migrating to the ovary where they could potentially acquire the additional mutations necessary for transformation. This is the first model to allow direct investigation into p53 mutations in oviductal epithelium and can help uncover critical targets for treatment of early disease or pathways that can be used in prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank Mark Maienschein-Cline and the UIC Center for Research Informatics (CRI), supported in part by NIH grant UL1TR000050 (University of Illinois CTSA), for assistance with gene ontology and systems biology analysis. We gratefully acknowledge contributions from the UIC Core Genomics Facility staff, particularly

Nina S. Los, for consultation and skillful technical help. This research was supported by the Department of Defense OC110133, Nested Teal Pre-Doctoral Scholar Award, American Cancer Society RSG-12-230-01-TBG, and the UIC Graduate College Medical Research Fellowship.

References

1. Society AC. Cancer Facts and Figures 2014. American Cancer Society. 2014
2. Landen CN Jr, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. *J Clin Oncol*. 2008; 26:995–1005. [PubMed: 18195328]
3. Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, Garber JE, Cramer DW, Crum CP. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am J Surg Pathol*. 2006; 30:230–236. [PubMed: 16434898]
4. Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, Gille JJ, Jongsma AP, Pals G, Kenemans P, Verheijen RH. Dysplastic changes in prophylactically removed fallopian tubes of women predisposed to developing ovarian cancer. *J Pathol*. 2001; 195:451–456. [PubMed: 11745677]
5. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, Callahan MJ, Garner EO, Gordon RW, Birch C, Berkowitz RS, Muto MG, et al. Intraepithelial Carcinoma of the Fimbria and Pelvic Serous Carcinoma: Evidence for a Causal Relationship. *The American Journal of Surgical Pathology*. 2007; 31:161–169. 10.1097/01.pas.0000213335.40358.47. [PubMed: 17255760]
6. Karst AM, Levanon K, Drapkin R. Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. *PNAS*. 2011; 108:7547–7552. [PubMed: 21502498]
7. Perets R, Wyant GA, Muto KW, Bijron JG, Poole BB, Chin KT, Chen JY, Ohman AW, Stepule CD, Kwak S, Karst AM, Hirsch MS, et al. Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca;Tp53;Pten models. *Cancer cell*. 2013; 24:751–765. [PubMed: 24332043]
8. Jazaeri AA, Bryant JL, Park H, Li H, Dahiya N, Stoler MH, Ferriss JS, Dutta A. Molecular requirements for transformation of fallopian tube epithelial cells into serous carcinoma. *Neoplasia*. 2011; 13:899–911. [PubMed: 22028616]
9. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011; 474:609–615. [PubMed: 21720365]
10. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T. Mutant p53 Gain of Function in Two Mouse Models of Li-Fraumeni Syndrome. *Cell*. 2004; 119:847–860. [PubMed: 15607980]
11. Dubeau L. The Cell of Origin of Ovarian Epithelial Tumors and the Ovarian Surface Epithelium Dogma: Does the Emperor Have No Clothes? *Gynecol Oncol*. 1999; 72:437–442. [PubMed: 10053122]
12. Gross AL, Kurman RJ, Vang R, Shih Ie M, Visvanathan K. Precursor lesions of high-grade serous ovarian carcinoma: morphological and molecular characteristics. *Journal of oncology*. 2010; 2010:126295. [PubMed: 20445756]
13. Morgan SE, Kim R, Wang PC, Bhat UG, Kusumoto H, Lu T, Beck WT. Differences in mutant p53 protein stability and functional activity in teniposide-sensitive and -resistant human leukemic CEM cells. *Oncogene*. 2000; 19:5010–5019. [PubMed: 11042688]
14. Terzian T, Suh YA, Iwakuma T, Post SM, Neumann M, Lang GA, Van Pelt CS, Lozano G. The inherent instability of mutant p53 is alleviated by Mdm2 or p16INK4a loss. *Genes Dev*. 2008; 22:1337–1344. [PubMed: 18483220]
15. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol*. 2007; 211:26–35. [PubMed: 17117391]
16. Cass I, Walts AE, Barbuto D, Lester J, Karlan B. A cautious view of putative precursors of serous carcinomas in the fallopian tubes of BRCA mutation carriers. *Gynecol Oncol*. 2014; 134:492–497. [PubMed: 25026639]
17. Karst AM, Drapkin R. Primary culture and immortalization of human fallopian tube secretory epithelial cells. *Nature protocols*. 2012; 7:1755–1764. [PubMed: 22936217]

18. Levanon K, Ng V, Piao HY, Zhang Y, Chang MC, Roh MH, Kindelberger DW, Hirsch MS, Crum CP, Marto JA, Drapkin R. Primary ex vivo cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. *Oncogene*. 2010; 29:1103–1113. [PubMed: 19935705]
19. Quartuccio SM, Lantvit DD, Bosland MC, Burdette JE. Conditional inactivation of p53 in mouse ovarian surface epithelium does not alter MIS driven Smad2-dominant negative epithelium-lined inclusion cysts or teratomas. *PloS one*. 2013; 8:e65067. [PubMed: 23741457]
20. King SM, Quartuccio SM, Vanderhyden BC, Burdette JE. Early transformative changes in normal ovarian surface epithelium induced by oxidative stress require Akt upregulation, DNA damage and epithelial-stromal interaction. *Carcinogenesis*. 2013; 34:1125–1133. [PubMed: 23299406]
21. Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science*. 1990; 249:912–915. [PubMed: 2144057]
22. Singh A, Sweeney MF, Yu M, Burger A, Greninger P, Benes C, Haber DA, Settleman J. TAK1 inhibition promotes apoptosis in KRAS-dependent colon cancers. *Cell*. 2012; 148:639–650. [PubMed: 22341439]
23. Berman SD, Calo E, Landman AS, Danielian PS, Miller ES, West JC, Fonhoue BD, Caron A, Bronson R, Boussein ML, Mukherjee S, Lees JA. Metastatic osteosarcoma induced by inactivation of Rb and p53 in the osteoblast lineage. *Proc Natl Acad Sci U S A*. 2008; 105:11851–11856. [PubMed: 18697945]
24. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst*. 1990; 82:1107–1112. [PubMed: 2359136]
25. Hickok JR, Sahni S, Mikhed Y, Bonini MG, Thomas DD. Nitric oxide suppresses tumor cell migration through N-Myc downstream-regulated gene-1 (NDRG1) expression: role of chelatable iron. *J Biol Chem*. 2011; 286:41413–41424. [PubMed: 21976667]
26. Miyoshi I, Takahashi K, Kon Y, Okamura T, Mototani Y, Araki Y, Kasai N. Mouse transgenic for murine oviduct-specific glycoprotein promoter-driven simian virus 40 large T-antigen: Tumor formation and its hormonal regulation. *Mol Reprod Dev*. 2002; 63:168–176. [PubMed: 12203826]
27. Sherman-Baust CA, Kuhn E, Valle BL, Shih Ie M, Kurman RJ, Wang TL, Amano T, Ko MS, Miyoshi I, Araki Y, Lehmann E, Zhang Y, et al. A genetically engineered ovarian cancer mouse model based on fallopian tube transformation mimics human high-grade serous carcinoma development. *J Pathol*. 2014; 233:228–237. [PubMed: 24652535]
28. Garritano S, Inga A, Gemignani F, Landi S. More targets, more pathways and more clues for mutant p53. *Oncogenesis*. 2013; 2:e54. [PubMed: 23817466]
29. Vainionpää N, Lehto VP, Tryggvason K, Virtanen I. Alpha4 chain laminins are widely expressed in renal cell carcinomas and have a de-adhesive function. *Lab Invest*. 2007; 87:780–791. [PubMed: 17533363]
30. Cheon DJ, Tong Y, Sim MS, Dering J, Berel D, Cui X, Lester J, Beach JA, Tighiouart M, Walts AE, Karlan BY, Orsulic S. A collagen-remodeling gene signature regulated by TGF-beta signaling is associated with metastasis and poor survival in serous ovarian cancer. *Clin Cancer Res*. 2014; 20:711–723. [PubMed: 24218511]
31. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest*. 2009; 119:1429–1437. [PubMed: 19487819]
32. Hwang CI, Matoso A, Corney DC, Flesken-Nikitin A, Korner S, Wang W, Boccaccio C, Thorgeirsson SS, Comoglio PM, Hermeking H, Nikitin AY. Wild-type p53 controls cell motility and invasion by dual regulation of MET expression. *Proc Natl Acad Sci U S A*. 2011; 108:14240–14245. [PubMed: 21831840]
33. Do TV, Xiao F, Bickel LE, Klein-Szanto AJ, Pathak HB, Hua X, Howe C, O'Brien SW, Maglaty M, Ecsedy JA, Litwin S, Golemis EA, et al. Aurora kinase A mediates epithelial ovarian cancer cell migration and adhesion. *Oncogene*. 2014; 33:539–549. [PubMed: 23334327]
34. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer discovery*. 2012; 2:401–404. [PubMed: 22588877]

35. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science signaling*. 2013; 6:p11. [PubMed: 23550210]
36. Folkins AK, Jarboe EA, Saleemuddin A, Lee Y, Callahan MJ, Drapkin R, Garber JE, Muto MG, Tworoger S, Crum CP. A candidate precursor to pelvic serous cancer (p53 signature) and its prevalence in ovaries and fallopian tubes from women with BRCA mutations. *Gynecol Oncol*. 2008; 109:168–173. [PubMed: 18342932]
37. Szabova L, Yin C, Bupp S, Guerin TM, Schlomer JJ, Householder DB, Baran ML, Yi M, Song Y, Sun W, McDunn JE, Martin PL, et al. Perturbation of Rb, p53 and Brca1 or Brca2 cooperate in inducing metastatic serous epithelial ovarian cancer. *Cancer Res*. 2012
38. Xing D, Orsulic S. A mouse model for the molecular characterization of brca1-associated ovarian carcinoma. *Cancer Res*. 2006; 66:8949–8953. [PubMed: 16982732]
39. Connolly DC, Bao R, Nikitin AY, Stephens KC, Poole TW, Hua X, Harris SS, Vanderhyden BC, Hamilton TC. Female mice chimeric for expression of the simian virus 40 TAG under control of the MISIR promoter develop epithelial ovarian cancer. *Cancer Res*. 2003; 63:1389–1397. [PubMed: 12649204]
40. Wu R, Baker SJ, Hu TC, Norman KM, Fearon ER, Cho KR. Type I to type II ovarian carcinoma progression: mutant Trp53 or Pik3ca confers a more aggressive tumor phenotype in a mouse model of ovarian cancer. *Am J Pathol*. 2013; 182:1391–1399. [PubMed: 23499052]
41. Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM, Matzuk MM. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proc Natl Acad Sci U S A*. 2012; 109:3921–3926. [PubMed: 22331912]
42. Kurrey NK, K A, Bapat SA. Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. *Gynecol Oncol*. 2005; 97:155–165. [PubMed: 15790452]
43. Kim MK, Kim MA, Kim H, Kim YB, Song YS. Expression profiles of epithelial-mesenchymal transition-associated proteins in epithelial ovarian carcinoma. *BioMed research international*. 2014; 2014:495754. [PubMed: 24800235]
44. Wu WS, Heinrichs S, Xu D, Garrison SP, Zambetti GP, Adams JM, Look AT. Slug antagonizes p53-mediated apoptosis of hematopoietic progenitors by repressing puma. *Cell*. 2005; 123:641–653. [PubMed: 16286009]
45. Kim J, Bae S, An S, Park JK, Kim EM, Hwang SG, Kim WJ, Um HD. Cooperative actions of p21WAF1 and p53 induce Slug protein degradation and suppress cell invasion. *EMBO reports*. 2014
46. Wang SP, Wang WL, Chang YL, Wu CT, Chao YC, Kao SH, Yuan A, Lin CW, Yang SC, Chan WK, Li KC, Hong TM, et al. p53 controls cancer cell invasion by inducing the MDM2-mediated degradation of Slug. *Nature cell biology*. 2009; 11:694–704. [PubMed: 19448627]
47. Muller PA, Caswell PT, Doyle B, Iwanicki MP, Tan EH, Karim S, Lukashchuk N, Gillespie DA, Ludwig RL, Gosselin P, Cromer A, Brugge JS, et al. Mutant p53 drives invasion by promoting integrin recycling. *Cell*. 2009; 139:1327–1341. [PubMed: 20064378]
48. Solomon H, Buginim Y, Kogan-Sakin I, Pomeranec L, Assia Y, Madar S, Goldstein I, Brosh R, Kalo E, Beatus T, Goldfinger N, Rotter V. Various p53 mutant proteins differently regulate the Ras circuit to induce a cancer-related gene signature. *J Cell Sci*. 2012; 125:3144–3152. [PubMed: 22427690]
49. Lassus H, Staff S, Leminen A, Isola J, Butzow R. Aurora-A overexpression and aneuploidy predict poor outcome in serous ovarian carcinoma. *Gynecol Oncol*. 2011; 120:11–17. [PubMed: 20937525]
50. Wu CC, Yang TY, Yu CT, Phan L, Ivan C, Sood AK, Hsu SL, Lee MH. p53 negatively regulates Aurora A via both transcriptional and posttranslational regulation. *Cell cycle*. 2012; 11:3433–3442. [PubMed: 22894933]

Novelty and Impact Statement

This is the first study to explore the role of p53 mutation alone, the most common alteration found in high-grade serous cancer, in fallopian tube epithelium (FTE). While mutation is insufficient to drive transformation, migration of FTE significantly increases, which is not observed in mutant p53 expressing ovarian surface epithelium. The finding presented here could explain why the bulk of high-grade serous tumors are identified in the ovary and other distant peritoneal sites.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Cell Line	Survival (days)	Subcutaneous Tumor Weight (mg)	Peritoneal Disease (%)
MOE	No mortality	0	0
p53 ^{R273H}	No mortality	0	0
KRAS ^{G12V}	No mortality	0	0
KRAS ^{G12V} /p53 ^{R273H}	147.3 ± 10.5	363.3 ± 140.1	16.7 (1/6)