Modeling high-grade serous ovarian carcinogenesis from the fallopian tube

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Edited* by William G. Kaelin, Harvard Medical School, Boston, MA, and approved March 31, 2011 (received for review November 18, 2010)

High-grade serous ovarian carcinoma (HGSOC) is a lethal disease for which improved screening and treatment strategies are urgently needed. Progress in these areas is impeded by our poor understanding of HGSOC pathogenesis. Most ovarian cancer research is based on the hypothesis that HGSOC arises from ovarian surface epithelial cells. However, recent studies suggest that >50% of high-grade serous carcinomas involving the ovary likely arise from fallopian tube epithelium. Therefore, limiting HGSOC research to modeling based on ovarian surface epithelium alone is inadequate. To address the need for a fallopian tube–based model of HGSOC, we have developed a system for studying human fallopian tube secretory epithelial cell (FTSEC) transformation. Our model is based on (*i*) immortalization of FTSECs isolated from primary samples of normal, nondiseased human fallopian tubes, (*ii*) transformation of FTSECs with defined genetic elements, and (*iii*) xenograft-based tumorigenic assays. We use our model to show that FTSECs immortalized with human telomerase reverse transcriptase (hTERT) plus SV40 large T and small T antigens are transformed by either oncogenic Ras (H-Ras^{V12}) or c-Myc expression, leading to increased proliferation, clonogenicity, and anchorage-independent growth. Additionally, we demonstrate that FTSECs remain susceptible to c-Myc–mediated transformation in the absence of viral oncoproteins, by replacing SV40 large T and small T antigens with sh-p53, mutant CDK4 (CDK4^{R24C}), and sh-PP2A-B56γ. Importantly, all transformed FTSECs gave rise to high-grade Müllerian carcinomas that were grossly, histologically, immunophenotypically, and genomically similar to human HGSOC. With this model, we will now be able to assess the transformative effects of specific genetic alterations on FTSECs in order to characterize their respective roles in HGSOC development.

Fimbria | Cell of origin | Secretory cell | TP53

Ovarian cancer is the fifth deadliest cancer among American women (1). It has a disproportionately high mortality rate, attributed primarily to difficulties in diagnosing early stage disease and to the development of drug resistance in tumors that were initially chemosensitive (2). To improve ovarian cancer screening and treatment strategies, we must better understand the cancer's origin and pathogenesis. The most common histologic subtype, accounting for >50% of ovarian epithelial malignancies, is serous ovarian carcinoma (3–5). Because of inadequate early detection tools, the vast majority of serous ovarian carcinomas (>80%) are diagnosed at late stage [International Federation of Gynecology and Obstetrics (FIGO) stages III–IV], for which the 5-y survival rate is only 9–34% (6). Most of these (>50%) are classified as "high-grade" based on their degree of nuclear atypia and high mitotic index (7). High-grade serous ovarian carcinoma (HGSOC) stands out from other subtypes both for its aggressive nature and because it harbors unique genetic alterations. For example, clear cell, endometrioid, low-grade serous, and mucinous ovarian carcinomas typically present as indolent low-grade neoplasms [type I tumors (8)] with somatic mutations in genes such as *KRAS*, *BRAF*, *ERBB2*, *PTEN*, *CTNNB1*, and *PIK3CA* (8). In contrast, highly aggressive HGSOCs [type II tumors (8)] are characterized by mutations in *TP53* and the DNA-damage repair genes *BRCA1* and *BRCA2* (9, 10). These stark differences in tumor phenotype and genetic aberrations have led many to question whether HGSOC pathogenesis differs fundamentally from that of low-grade ovarian carcinomas. To answer this question, it is critically important that we verify the anatomic site and specific cell type from which HGSOC arises. Type I tumors are thought to develop from ovarian surface epithelial (OSE) cells because they are typically associated with cortical inclusion cysts or low-grade precursor lesions ("borderline tumors") in the ovarian cortex. In contrast, type II tumors are not clearly linked to an ovarian precursor lesion, and their origin remains unclear (8, 11). However, recent studies suggest that a large proportion of HGSOCs may arise from fallopian tube epithelium, specifically, the fallopian tube secretory epithelial cell (FTSEC) (4, 12, 13). This hypothesis was founded on studies of women predisposed to HGSOC because of inherited *BRCA1/2* mutations. Fallopian tubes from these women contain putative serous carcinoma precursor lesions in the fimbriated end, immediately next to the ovary (4, 14–16). The putative precursor, termed "p53 signature," is defined as ≥12 consecutive FTSECs that appear morphologically benign in H&E-stained sections but exhibit intense nuclear p53 immunostaining. Many p53 signatures share identical somatic *TP53* mutations with coexisting serous tubal intraepithelial carcinomas and HGSOCs, suggesting that all three entities have a common origin (15). Incidentally, p53 signatures are rarely observed in OSE cells (17). These observations have since been supported by independent studies, lending credence to the hypothesis that many HGSOCs arise from the fallopian tube. Although compelling, these studies are limited by their descriptive nature and do not actually demonstrate that FTSECs can be transformed into HGSOCs. To study FTSEC transformation, an experimental model is required. Our laboratory recently developed an ex vivo model system for fallopian tube epithelium that preserves epithelial architecture, polarity, and cell differentiation (18). However, its small scale does not lend itself well to large-scale in vitro studies. Here, we have established a complementary model suitable for studying FTSEC transformation and tumorigenesis based on (*i*) immortalization of primary human FTSECs, (*ii*) transformation by defined genetic elements, and (*iii*) xenograft-based tumorigenic assays. Importantly, our model does not require the use of viral oncogenes, which are physiologically irrelevant to human ovarian cancer and have ill-defined global effects on the cell.

Author contributions: A.M.K., K.L., and R.D. designed research; A.M.K. and K.L. performed research; A.M.K. and R.D. analyzed data; and A.M.K. and R.D. wrote the paper.

Conflict of interest statement: R.D. is a consultant to Novartis Pharmaceuticals.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1017300108/-/DCSupplemental.

Results

FTSECs Immortalized with Human Telomerase Reverse Transcriptase (hTERT) and SV40 Large T Plus Small T Antigens (SV40 TAg) Retain a Lineage-Specific Phenotype. The fallopian tube epithelium is composed of two cell types: secretory and ciliated cells. When primary fallopian tube epithelial cells are seeded onto plasticware, only the secretory cells readily adhere and proliferate, presumably because ciliated cells are terminally differentiated. We exploited this phenomenon to isolate primary secretory cells from fresh normal fimbriae obtained from patients with benign gynecological conditions not affecting their fallopian tubes. Epithelial cells were enzymatically dissociated (described previously in ref. 18), seeded onto collagen-coated plates, and allowed to grow for 3–5 d (Fig. 1*A*). To confirm that cultures contained FTSECs, we performed immunofluorescent staining for Paired box gene 8 (Pax8), a Müllerian lineage marker expressed by secretory but not ciliated cells (18, 19). Adherent cells expressed Pax8 but not FoxJ1, a ciliated cell marker (Fig. 1*B*). We have used this method to isolate FTSECs from more than 100 different patient samples. Primary FTSECs growing on plasticware typically senesce or enter a state of crisis within two to three passages (Fig. S1) and must be immortalized for large scale in vitro studies. We generated immortal cells (FT33) by transducing first-passage FTSECs with a retroviral vector encoding *hTERT*, the catalytic subunit of telomerase, which maintains telomere ends and stalls replicative senescence (20). Elevated *hTERT* transcript levels were detected in FT33 cells by quantitative RT-PCR (Fig. S2). Although hTERT alone prevented senescence for ∼10 passages, it did not promote growth to an extent that allowed for cell-line expansion. This finding likely reflects tumor suppressor–mediated restrictions on cell growth by the p53 and pRb pathways. To overcome these barriers, we transduced FT33 cells with SV40 large T plus small T antigens, herein referred to as SV40 TAg (Fig. 1*C*). SV40 large T binds to and functionally inactivates p53 and pRb proteins, facilitating unchecked cellular proliferation (21). Accordingly, expression of SV40 TAg in FT33 cells (FT33-TAg) enhanced growth and passaging ability but did not transform the cells. Western blot analysis showed that FT33-TAg cells retained expression of Müllerian (Pax8) and epithelial (Cytokeratin 7, CK-7) lineage markers (Fig. 2*A*).

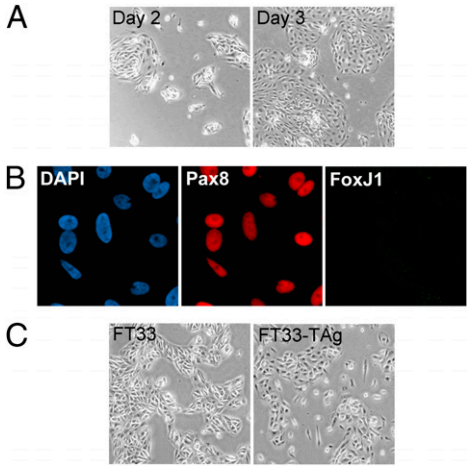

Fig. 1. Isolation and immortalization of primary human FTSECs. (*A*) Dissociated fallopian tube epithelial cells proliferate rapidly in culture (magnification: 40×). (*B*) Proliferative cells express secretory (Pax8) but not ciliated (FoxJ1) lineage markers, identifying them as FTSECs (magnification: 400×). (*C*) FT33 cell morphology before (*Left*) and after (*Right*) immortalization with SV40 TAg (magnification: 40×).

H-Ras^{V12} or c-Myc Expression Transforms SV40 TAg-Immortalized FTSECs. FTSEC transformation, to our knowledge, has not been demonstrated experimentally. To determine whether FTSECs are susceptible to transformation by oncogene activation, we transduced FT33-TAg cells with either *H-Ras^{V12}* or *c-Myc*. H-Ras^{V12} is a constitutively active form of the GTPase H-Ras that activates the MAPK, PI3K, and Ral signaling pathways and can efficiently transform various human cell types (22). However, because *H-Ras* mutations are not seen in HGSOC, we also attempted to transform FT33-TAg cells with *c-Myc*, a transcription factor and proto-oncogene whose locus (8q24) is frequently amplified in HGSOC (23–25). The resulting cells lines, FT33-TAg-Ras and FT33-TAg-Myc, were analyzed by Western blotting to confirm ectopic gene expression and retention of lineage markers (Fig. 2*A*). To determine the effects of H-Ras^{V12} and c-Myc on FTSEC proliferation, we used the CellTiter-Glo viability assay. Over 72 h, immortal FT33-TAg cell number increased only 2.4-fold (± 0.5), whereas FT33-TAg-Ras and FT33-TAg-Myc cell numbers increased 5.1-fold (± 0.4) and 8.0-fold (± 0.3) respectively (Fig. 2*B*). Growth rates were on par with that of a highly proliferative ovarian carcinoma cell line, OVCAR-8, which increased by 6.7-fold (± 0.3). We also assessed clonogenicity after introduction of H-Ras^{V12} or c-Myc. When sparsely seeded cells were grown for 10 d, FT33-TAg formed just 2 (± 1) colonies (≥1 mm) per 10^3 cells seeded (Fig. 2*C*). However, expression of H-Ras^{V12} or c-Myc increased the colony-formation rate by 14-fold (29 ± 1 colonies per 10^3 cells) and eightfold (16 ± 2 colonies per 10^3 cells), respectively. OVCAR-8 formed 11-fold more colonies (22 ± 2 colonies per 10^3cells) than immortal FTSECs. These results together indicate that expression of either H-Ras^{V12} or c-Myc dramatically enhances FTSEC growth in vitro. We next tested whether FTSECs expressing H-Ras^{V12} or c-Myc had acquired a capacity for anchorage-independent growth, a key indicator of epithelial cell transformation. Cells seeded in soft agar were grown for 2 wk, then examined microscopically (20×) for colony formation. Under these conditions, FT33-TAg formed no colonies, whereas both FT33-TAg-Ras and FT33-TAg-Myc exhibited robust colony formation, generating 74 (± 9) and 42 (± 11) colonies per microscopic field, respectively (Fig. 2*D*). OVCAR-8 formed 116 (± 15) colonies per field. These data suggest that introduction of either H-Ras^{V12} or c-Myc is sufficient to transform immortal FTSECs in vitro. Finally, we tested whether FTSECs transformed in vitro would be tumorigenic in vivo. First, we implanted 10^7 FT33-TAg-Ras cells into Nude mice (n = 5) by i.p. injection, supported by Matrigel basement-membrane matrix. Tumors arose in all five mice within 57 (± 7) d, accompanied by abdomen distention and weight loss (Fig. S3). To ensure that tumorigenicity was not attributable to a genetic aberration uniquely associated with parental FT33 cells, we generated a second line of H-Ras^{V12}–transformed FTSECs (FT65-Tag-Ras) using normal fimbria from different a patient. These cells were also tumorigenic in Nude mice (Fig. S3), demonstrating that human FTSECs can be reproducibly transformed by using the same genetic elements. Next, we implanted 10^7 FT33-TAg-Myc cells into NOD.Cg-*Prkdc^{scid}*/*Il2rg^{tm1Wjl}*/SzJ (NSG) mice (n = 5) by i.p. injection. To observe the pattern of tumor growth, we did not use Matrigel for these (or subsequent) injections. Two additional mice were implanted with FT33-TAg-Ras cells as a positive control. As expected, H-Ras^{V12}–expressing cells rapidly produced tumors (2/2) within 52 (± 3) d (Fig. 3*A*). Two of the five mice injected with FT33-TAg-Myc cells developed tumors within 150 (± 64) d. All tumors were widespread throughout the abdomen, adhered to serosal membranes, and exhibited local invasion, consistent with the typical presentation of human HGSOC (Fig. 3*A* and Figs. S4 and S5). These results indicate that, although both H-Ras^{V12} and c-Myc can transform immortal FTSECs, H-Ras^{V12} drives tumor progression more efficiently. Despite differing growth rates, H-Ras^{V12}– and

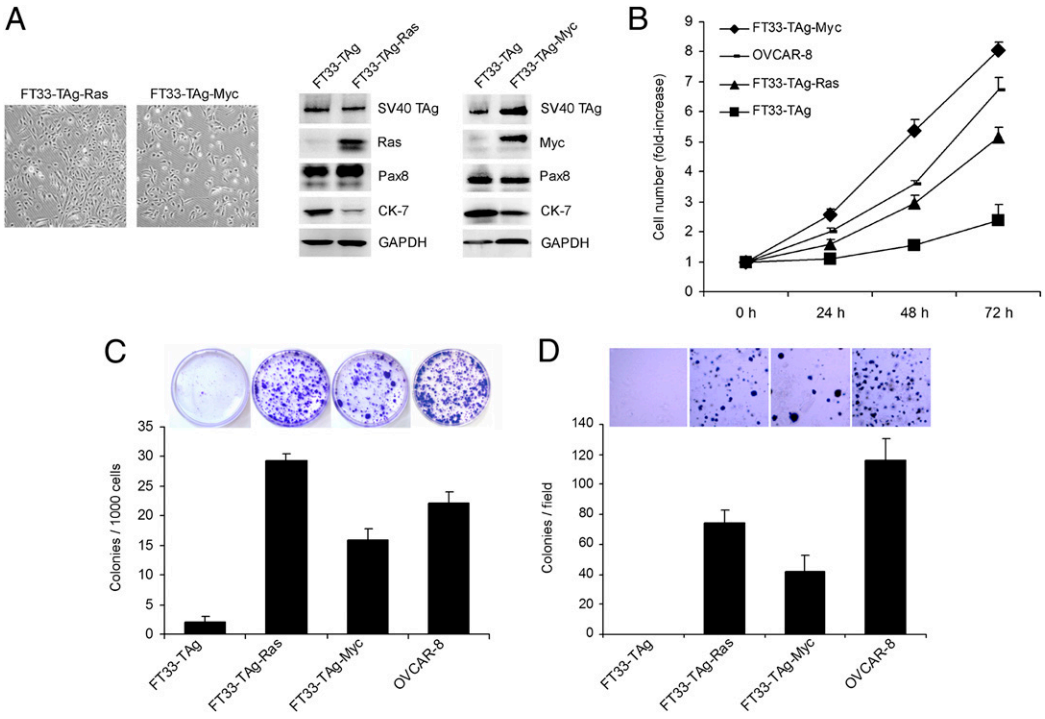

Fig. 2. Transformation of SV40 TAg-immortalized FTSECs by H-Ras^{V12} or c-Myc. (*A*) Immortal FT33-TAg cells transduced with H-Ras^{V12} or c-Myc (*Left*; magnification: 40×) were analyzed by Western blotting (*Right*) to validate ectopic gene expression (Ras and Myc) and the retention of lineage markers (Pax8 and CK-7). (*B–D*) Expression of H-Ras^{V12} or c-Myc dramatically accelerated FT33-TAg cell proliferation (*B*), enhanced colony formation (*C*), and enabled anchorage-independent growth in soft agar (*D*). The ovarian carcinoma cell line OVCAR-8 was used as a positive control.

c-Myc–expressing tumors were histologically indistinguishable. Both cell lines gave rise to high-grade epithelial neoplasms that were pleomorphic, exhibited large hyperchromatic-to-vesicular nuclei, had prominent nucleoli, and contained multiple atypical mitotic figures (Fig. 3*B*). Areas of necrosis and inflammation, accompanied by neutrophil infiltration, were also observed. Tumor histology was consistent with that of poorly differentiated HGSOC. Immunohistochemical analysis confirmed that all tumors retained ectopic gene expression in vivo and expressed Pax8 and keratin proteins, verifying Müllerian and epithelial origin, respectively (Fig. 3*C*). They also expressed WT-1, a marker that is used clinically to identify serous ovarian carcinoma in metastatic lesions, pleural effusions, and ascites. In addition, tumors expressed CA-125 and HE-4, both of which are approved by the Food and Drug Administration as HGSOC serum biomarkers (Fig. S6). The tumors were highly proliferative, evidenced by high Ki-67 indices, and strongly expressed p53, as expected, because SV40 large T binds to p53 and prevents its

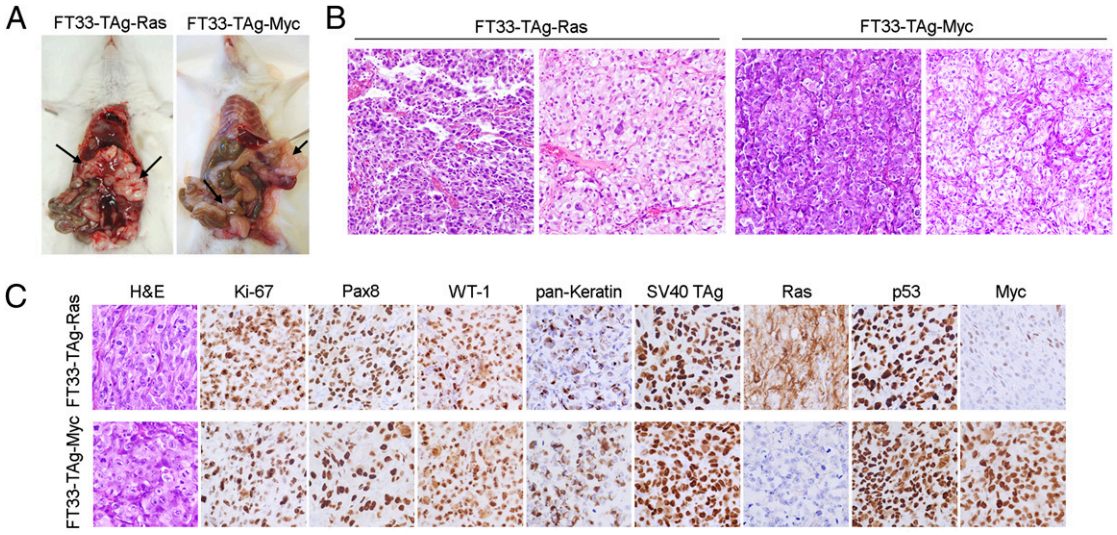

Fig. 3. H-Ras^{V12} and c-Myc–transformed FTSECs produce high-grade serous tumors. (*A*) i.p. injection of either FT33-TAg-Ras or FT33-TAg-Myc cells produced widespread tumor formation in immunodeficient mice. (*B*) Both cell lines gave rise to high-grade Müllerian carcinomas, determined by H&E staining (magnification: 200×). (*C*) Tumors were analyzed by immunohistochemistry to validate ectopic and lineage-specific gene expression (magnification: 600×).

degradation, resulting in nuclear accumulation (21). In summary, our findings indicate that H-Ras or c-Myc oncogene activation on a background of hTERT, p53, and pRb pathway dysfunction is sufficient to transform FTSECs into high-grade serous carcinoma.

FTSECs Can Be Immortalized and Transformed Without Viral Oncogenes. After verifying that FTSECs are amenable to oncogene-induced transformation, we improved our model by eliminating the use of viral oncogenes. To replace SV40 large T–mediated inactivation of p53 and pRb, we transduced FT33 cells with shRNA targeting p53 and mutant cyclin-dependent kinase 4 ($CDK4^{R24C}$). The R24C mutation renders CDK4 insensitive to p16 inhibition and thus phenocopies loss of pRb function (26). Expression of sh-p53 and $CDK4^{R24C}$ was sufficient to immortalize FT33 cells and generate a passageable cell line (FT33-shp53-R24C) (Fig. 4*A*). To transform these cells, we transduced them with shRNA targeting the B56γ subunit of protein phosphatase 2A (PP2A-B56γ) plus *c-Myc*. PP2A is the major Ser/Thr phosphatase in mammalian cells. Its activity is regulated by variable "B subunits," including B56γ (27). SV40 small T interacts with B56γ to deregulate PP2A function, thus contributing to the derailment of cellular machinery (27). We therefore replaced SV40 small T with sh-PP2A-B56γ. The resulting cells (shp53-R24C-shPP2A-Myc) were analyzed by Western blotting to confirm ectopic gene expression (Fig. 4*A* and Fig. S7). In a cell-proliferation assay, shp53-R24C-shPP2A-Myc cells exhibited highly accelerated growth, their numbers increasing 13.2-fold (± 0.8) over 72 h, whereas immortal FT33-shp53-R24C cells increased only 2.3-fold (± 0.1) (Fig. 4*B*). Likewise, in a clonogenic assay, FT33-shp53-R24C-shPP2A-Myc demonstrated robust colony-formation ability, generating 68 (± 3) colonies per 10^3 cells seeded compared with only 3 (± 1) colonies per 10^3 cells for FT33-shp53-R24C (Fig. 4*C*). When tested for anchorage-independent growth in a soft agar, FT33-shp53-R24C formed no colonies over a 2-wk period, whereas FT33-shp53-R24C-shPP2A-Myc produced abundant large colonies, averaging 159 (± 54) per microscopic field (Fig. 4*D*). These very high rates of cell proliferation, colony formation, and anchorage-independent growth exceeded those of FT33-TAg-Ras, FT33-TAg-Myc, and OVCAR-8 (Fig. 2 *B–D* and 4 *B–D*). Overall, our data show that FTSECs harboring hTERT, p53, and pRb dysfunction (nonvirally mediated) can be transformed in vitro by a combination of PP2A-B56γ inactivation and c-Myc expression.

FTSECs Transformed with Nonviral Genetic Elements Are Tumorigenic. To determine whether nonviral genetic alterations also induce FTSEC tumorigenicity, we implanted 10^7 FT33-shp53-R24C-shPP2A-Myc cells into NSG mice ($n = 4$) by i.p. injection. Indeed, 2/4 mice developed tumors in 147 (± 31) d. Tumors presented with diffuse peritoneal involvement and extensive studding of the omentum and serosal surfaces of the bowel (Fig. 5*A* and Fig. S8). Histological features, including high grade, pleomorphism, severe nuclear atypia, large nucleoli, numerous mitotic figures, necrosis, and fibrovascular cores, were nearly identical to those of $H\text{-}Ras^{V12}$– and c-Myc–expressing tumors, suggesting that cell lineage may be more influential in determining tumor histology than the specific genetic alterations used for transfor-

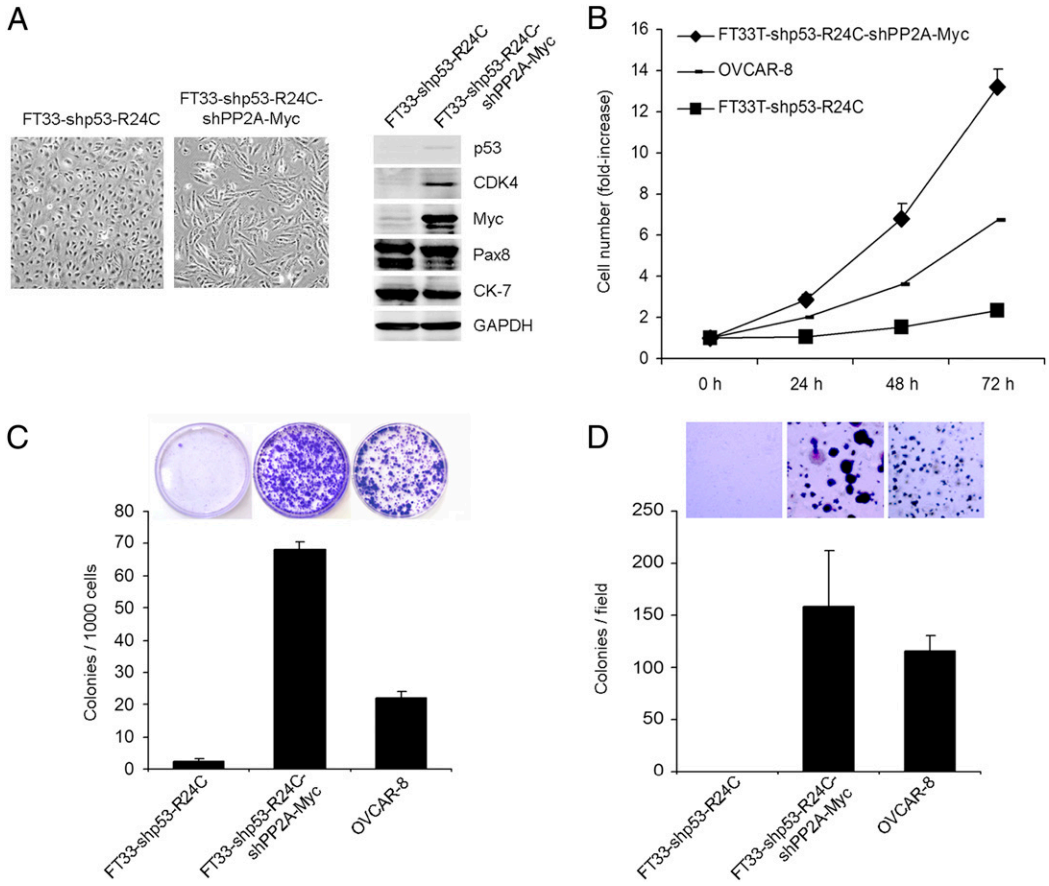

Fig. 4. FTSECs can be immortalized and transformed in the absence of viral oncogenes. (*A*) Immortal FT33-shp53-R24C cells transduced with sh-PP2A-B56γ and c-Myc (*Left*; magnification: 40×) were analyzed by Western blotting (*Right*) to confirm expression of exogenous genetic alterations (p53, CDK4, and Myc) and endogenous lineage markers (Pax8 and CK-7). (*B–D*) Transduction of FT33-shp53-R24C cells with sh-PP2A-B56γ and c-Myc dramatically accelerated cell proliferation (*B*), increased colony formation (*C*), and enabled anchorage-independent growth (*D*). OVCAR-8 was used as a positive control.

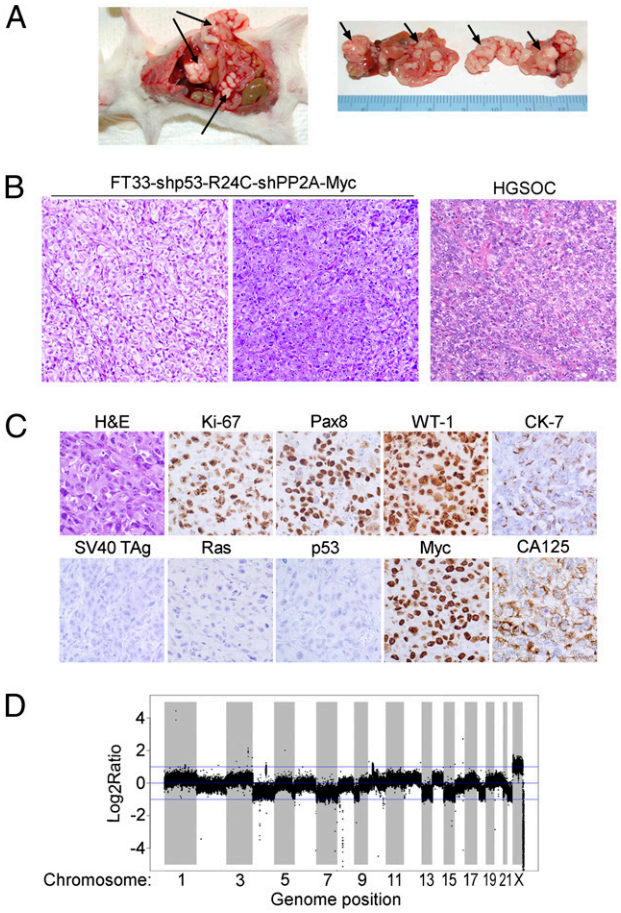

Fig. 5. FTSECs transformed with nonviral genetic alterations form high-grade serous carcinomas. (*A*) i.p. injection of FT33-shp53-R24C-shPP2A-Myc led to extensive tumor formation in immunodeficient mice. (*B*) Tumors were high-grade with epithelial features (*Left* and *Center*) and histologically consistent with HGSOC (*Right*) as determined by H&E staining (magnification: 200×). (*C*) Expression of ectopic and lineage-specific gene expression in tumor tissue was validated by immunohistochemistry analysis (magnification: 600×). (*D*) Tumors exhibited severe genomic instability as indicated by changes in DNA copy number. The *y* axis represents gain (positive) or loss (negative) of copy number along the length of each chromosome (shown on the *x* axis) as determined by aCGH analysis. Additional data are given in Fig. S10.

mation (Figs. 3*B* and 5*B*). FT33-shp53-R24C-shPP2A-Myc xenografts invaded the pancreas and musculature and metastasized to the liver, a common site of HGSOC metastasis (Fig. S8). Tumors were poorly differentiated high-grade Müllerian carcinomas and therefore consistent with a subset of human HSGOC (Fig. 5*B*). Immunohistochemical analysis indicated that tumor cells were highly proliferative (Ki-67), exhibited both Müllerian (Pax8, WT-1, CA125, and HE4) and epithelial phenotypes and retained high-level Myc expression in vivo (Fig. 5*C* and Fig. S9). We also confirmed that the tumor cells lacked p53, SV40 TAg, and Ras expression. Lastly, array comparative genomic hybridization (aCGH) was performed to determine whether FT33-shp53-R24C-shPP2A-Myc tumors resembled human HGSOC at the genomic level. Strikingly, the xenografts exhibited severe chromosomal instability, with multiple regions of DNA gain and loss, highly reminiscent of human HGSOC (Fig. 5*D* and Fig. S10). Overall, these results show that primary human FTSECs can be immortalized and transformed into high-grade genomically unstable serous carcinoma in the complete absence of viral oncoproteins. Moreover, this result was accomplished by using a limited number of genetic alterations equivalent to those commonly seen in HGSOC.

Discussion

The pathogenesis of HGSOC has remained ill-defined for many years, leading to considerable debate surrounding its site of origin. A recent series of paradigm-shifting studies has suggested that >50% of these neoplasms arise from fallopian tube epithelium and thus may be more accurately described as high-grade "pelvic" serous carcinoma (4, 5, 12). Consequently, there is an unprecedented need to study FTSEC biology, with few existing tools to do so. The aim of our study was to develop an experimental model of FTSEC transformation and with this model answer three basic questions: (*i*) Can FTSECs be transformed in vitro? (*ii*) What genetic alterations are required for FTSEC transformation? and (*iii*) Do transformed FTSECs give rise to high-grade tumors resembling HGSOC? The first step in developing our model was to immortalize primary FTSECs. Using the classic immortalization method pioneered by Hahn et al. (20), we found that FTSECs immortalized with hTERT and SV40 TAg were able to fend off replicative senescence and avert a state of crisis. However, they proliferated slowly, formed few colonies, and could not grow in soft agar, indicating that combined dysfunction of hTERT, p53, pRb, and PP2A-B56γ is not sufficient for FTSEC transformation. Introduction of H-Ras^{V12}, however, transformed these cells, suggesting that FTSECs additionally require at least one mitogenic signal to achieve transformation. Although H-Ras^{V12} efficiently induces FTSEC tumorigenicity, *H-Ras* mutations are not characteristic of HGSOCs. In fact, aside from *TP53* and *BRCA1/2* mutations, the mutational spectrum of HGSOC is quite narrow. Compared with other malignancies, HGSOC is most strongly characterized by its unusually high degree of genomic instability, resulting in numerous DNA copy-number alterations (28, 29). Genomic analyses have consistently identified region 8q24, containing the *c-Myc* locus, as the most recurrently gained genomic region (≥60%) in HGSOC, which is often accompanied by high-level *c-Myc* gene amplification (23, 29, 30). To more accurately model HGSOC genetics, we replaced H-Ras^{V12} with c-Myc and, in doing so, demonstrated that a clinically relevant oncogene such as *c-Myc* can also transform FTSECs immortalized with hTERT and SV40 TAg. Despite the utility of this HGSOC transformation scheme, a major caveat lies in the fact that SV40 TAg expression exerts pleiotropic effects on the cell that may confound the results of experiments designed to test the transformative effects of specific genetic alterations. Recognizing this possibility, we improved our model by eliminating viral oncogenes and replacing SV40 TAg with equivalent nonviral genetic elements: sh-p53, CDK^{R24C}, and sh-PP2A-B56γ. In this setting, c-Myc expression was still able to transform immortal FTSECs into high-grade serous tumors. It is important to note that the genetic alterations we used are highly relevant to human HGSOC and are therefore appropriate for building an FTSEC-HGSOC transformation model. For example, functional inactivation of p53 (represented by sh-p53) occurs in nearly all HGSOCs and is the earliest detectable change in premalignant FTSEC lesions (p53 signatures) (4, 5). Similarly, loss of *RB1* on genomic region 13q14 (phenocopied by CDK4^{R24C}) is the most frequently observed homozygous deletion (10.6%) in HGSOC (28). Although expressing c-Myc on a background of hTERT, p53, pRb, and PP2A dysfunction led to tumor formation in our model, the long latency and incomplete tumor take associated with c-Myc expressing tumors (particularly in the absence of SV40 TAg) strongly suggests that additional genetic events led to full transformation. Accordingly, aCGH analysis of tumor DNA revealed severe genomic aberrations in xenograft cells, highly reminiscent of those seen in human HGSOC, suggesting that the genetic elements we introduced in vitro may have primed FTSECs for subsequent development of genomic instability in vivo. Analysis

of the DNA copy-number gains and losses will provide critical insight into the mechanisms of Myc-mediated serous tumorigenesis. Finally, we demonstrated that our FTSEC transformation model produces tumors that are grossly, histologically, and immunophenotypically consistent with HGSOC. Notably, they all express Müllerian lineage markers Pax8 and WT-1, which are characteristic of both FTSECs and the vast majority of HGSOCs (19, 31, 32) but not OSE cells. Few models of human ovarian cancer can produce high-grade serous carcinomas using clinically relevant genetic alterations. Although two groups have reported high-grade serous tumor formation with human OSE-based models (33–35), their studies either did not demonstrate a Müllerian phenotype or relied on SV40 TAg expression to achieve transformation.

In summary, we have presented a model of human HGSOC that uses nonviral, clinically relevant genetic alterations to transform normal human FTSECs into high-grade serous carcinomas. Our study provides proof-of-principle evidence that FTSECs may indeed be a cell of origin for HGSOC and demonstrates a framework for defining their contribution to HGSOC development. Further work should focus on the transformative effects of other genetic alterations strongly associated with HGSOC, such as *BRCA1/2* mutations and genes that recurrently undergo DNA copy-number changes such as *CCNE*, *EVI1*, *PTK2*, *ERBB2*, *PRKCI*, *NF1*, and *PTEN* (23, 29, 30). Our model also serves as a platform for investigating the many candidate oncogenes and tumor suppressors now being identified by The Cancer Genome Atlas and other large-scale genomic analyses of HGSOC (29, 36, 37).

Materials and Methods

Fresh fallopian tube fimbriae were obtained from the Brigham and Women's Hospital Department of Pathology with institutional review board approval. Specimens were resected from patients with benign gynecological conditions not affecting the fallopian tubes. FTSECs were isolated, immortalized, and transformed as described in *SI Materials and Methods*.

ACKNOWLEDGMENTS. Special thanks to the faculty and staff of the Brigham and Women's Hospital Department of Pathology for allocation of tissues, to Alexei Protopopov and Lynda Chin of the Belfer Institute for Applied Cancer Science (Dana-Farber Cancer Institute) for performing aCGH analysis, and to Drs. William Hahn and Michelle Hirsch for their feedback on the manuscript. This work was supported by a Canadian Institutes of Health Research Fellowship (to A.M.K.), a Marsha Rivkin Foundation Scientific Scholar Award (to K.L.), the American Association for Cancer Research/George and Patricia Sehl Fellowship for Cancer Genetics Research (to K.L.), the American Physicians Fellowship for Medicine in Israel/Claire and Emmanuel G. Rosenblatt Foundation Grant (to K.L.), National Institutes of Health Specialized Programs of Research Excellence (SPORE) Grant P50 CA105009, the Ovarian Cancer Research Fund (R.D.), Novartis Pharmaceuticals (R.D.), the Robert and Debra First Fund (R.D.), the Randi and Joel Cutler Ovarian Cancer Research Fund (R.D.), and The Mary Kay Foundation (R.D.).

1. Jemal A, et al. (2009) Cancer statistics, 2009. *CA Cancer J Clin* 59:225–249.
2. Bast RC, Jr., Hennessy B, Mills GB (2009) The biology of ovarian cancer: New opportunities for translation. *Nat Rev Cancer* 9:415–428.
3. Seidman JD, et al. (2004) The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin. *Int J Gynecol Pathol* 23:41–44.
4. Karst AM, Drapkin R (2010) Ovarian cancer pathogenesis: A model in evolution. *J Oncol* 2010:932371.
5. Levanon K, Crum C, Drapkin R (2008) New insights into the pathogenesis of serous ovarian cancer and its clinical impact. *J Clin Oncol* 26:5284–5293.
6. Vang R, Shih IeM, Kurman RJ (2009) Ovarian low-grade and high-grade serous carcinoma: Pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. *Adv Anat Pathol* 16:267–282.
7. Malpica A, et al. (2004) Grading ovarian serous carcinoma using a two-tier system. *Am J Surg Pathol* 28:496–504.
8. Kurman RJ, Shih IeM (2010) The origin and pathogenesis of epithelial ovarian cancer: A proposed unifying theory. *Am J Surg Pathol* 34:433–443.
9. Landen CN Jr., Birrer MJ, Sood AK (2008) Early events in the pathogenesis of epithelial ovarian cancer. *J Clin Oncol* 26:995–1005.
10. Lynch HT, et al. (2009) Hereditary ovarian carcinoma: Heterogeneity, molecular genetics, pathology, and management. *Mol Oncol* 3:97–137.
11. Dubeau L (2008) The cell of origin of ovarian epithelial tumours. *Lancet Oncol* 9: 1191–1197.
12. Przybycin CG, Kurman RJ, Ronnett BM, Shih IeM, Vang R (2010) Are all pelvic (nonuterine) serous carcinomas of tubal origin? *Am J Surg Pathol* 34:1407–1416.
13. Crum CP (2009) Intercepting pelvic cancer in the distal fallopian tube: Theories and realities. *Mol Oncol* 3:165–170.
14. Medeiros F, et al. (2006) The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am J Surg Pathol* 30:230–236.
15. Lee Y, et al. (2007) A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol* 211:26–35.
16. Kindelberger DW, et al. (2007) Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol* 31:161–169.
17. Folkins AK, et al. (2008) A candidate precursor to pelvic serous cancer (p53 signature) and its prevalence in ovaries and fallopian tubes from women with BRCA mutations. *Gynecol Oncol* 109:168–173.
18. Levanon K, et al. (2010) Primary ex vivo cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. *Oncogene* 29:1103–1113.
19. Bowen NJ, et al. (2007) Emerging roles for PAX8 in ovarian cancer and endosalpingeal development. *Gynecol Oncol* 104:331–337.
20. Hahn WC, et al. (1999) Creation of human tumour cells with defined genetic elements. *Nature* 400:464–468.
21. Ali SH, DeCaprio JA (2001) Cellular transformation by SV40 large T antigen: Interaction with host proteins. *Semin Cancer Biol* 11:15–23.
22. Karnoub AE, Weinberg RA (2008) Ras oncogenes: Split personalities. *Nat Rev Mol Cell Biol* 9:517–531.
23. Haverty PM, Hon LS, Kaminker JS, Chant J, Zhang Z (2009) High-resolution analysis of copy number alterations and associated expression changes in ovarian tumors. *BMC Med Genomics* 2:21.
24. Darcy KM, et al. (2009) Prognostic relevance of c-MYC gene amplification and polysomy for chromosome 8 in suboptimally-resected, advanced stage epithelial ovarian cancers: A Gynecologic Oncology Group study. *Gynecol Oncol* 114:472–479.
25. Dimova I, Raitcheva S, Dimitrov R, Doganov N, Toncheva D (2006) Correlations between c-myc gene copy-number and clinicopathological parameters of ovarian tumours. *Eur J Cancer* 42:674–679.
26. Rane SG, Cosenza SC, Mettus RV, Reddy EP (2002) Germ line transmission of the Cdk4 (R24C) mutation facilitates tumorigenesis and escape from cellular senescence. *Mol Cell Biol* 22:644–656.
27. Chen W, et al. (2004) Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell* 5:127–136.
28. Kuo KT, et al. (2009) Analysis of DNA copy number alterations in ovarian serous tumors identifies new molecular genetic changes in low-grade and high-grade carcinomas. *Cancer Res* 69:4036–4042.
29. Gorringe KL, et al.; Australian Ovarian Cancer Study (2010) Copy number analysis identifies novel interactions between genomic loci in ovarian cancer. *PLoS ONE* 5: e11408.
30. Nowee ME, et al. (2007) DNA profiling of primary serous ovarian and fallopian tube carcinomas with array comparative genomic hybridization and multiplex ligation-dependent probe amplification. *J Pathol* 213:46–55.
31. Laury AR, et al. (2010) PAX8 reliably distinguishes ovarian serous tumors from malignant mesothelioma. *Am J Surg Pathol* 34:627–635.
32. Madore J, et al. (2010) Characterization of the molecular differences between ovarian endometrioid carcinoma and ovarian serous carcinoma. *J Pathol* 220:392–400.
33. Sasaki R, et al. (2009) Oncogenic transformation of human ovarian surface epithelial cells with defined cellular oncogenes. *Carcinogenesis* 30:423–431.
34. Liu J, et al. (2004) A genetically defined model for human ovarian cancer. *Cancer Res* 64:1655–1663.
35. Zheng J, et al. (2010) Induction of papillary carcinoma in human ovarian surface epithelial cells using combined genetic elements and peritoneal microenvironment. *Cell Cycle* 9:140–146.
36. Khalil I, Brewer MA, Neyarapally T, Runowicz CD (2010) The potential of biologic network models in understanding the etiopathogenesis of ovarian cancer. *Gynecol Oncol* 116:282–285.
37. Ramakrishna M, et al. (2010) Identification of candidate growth promoting genes in ovarian cancer through integrated copy number and expression analysis. *PLoS ONE* 5: e9983.