Divergent Roles of PAX2 in the Etiology and Progression of Ovarian Cancer

Ensaf M. Al-Hujaily, Yong Tang, De-Sheng Yao, Euridice Carmona, Kenneth Garson, and Barbara C. Vanderhyden

Abstract

PAX2 is an essential transcription factor for development. Aberrant PAX2 expression in adult tissues is associated with carcinogenesis and experimental evidence shows that PAX2 generally exhibits oncogenic properties. Although PAX2 is not expressed in normal ovaries, it is highly expressed in low malignant potential and low-grade epithelial ovarian tumors, suggesting that PAX2 induction in ovarian surface epithelium (OSE) may contribute to transformation. Herein, we provide evidence that expression of PAX2 in normal murine OSE cells (mOSE) enhances their proliferation and survival and, with loss of p53, induces tumorigenicity. PAX2 expression in murine ovarian cancer cells enhanced or inhibited tumorigenicity, depending on the model system. In RM cells (mOSE transformed by K-RAS and c-MYC), PAX2 expression inhibited p53 and induced pERK1/2 and COX2, resulting in enhanced angiogenesis and decreased apoptosis of tumors arising from these cells. However, in a murine model of high-grade serous ovarian cancer (STOSE), PAX2 expression improved animal survival by reducing proliferation and metastasis, which correlated with increased Htra1 and decreased COX2.

Thus, PAX2 may not be a classical oncogene or tumor suppressor but instead can act in either role by differential regulation of COX2 and/or HTRA1.

Corresponding Author: Barbara C. Vanderhyden, Ottawa Hospital Research Institute, Ottawa, Canada.

Introduction

Epithelial ovarian carcinoma (EOC) has been divided into two major subgroups with distinct molecular characteristics and clinical behavior (1). Type I tumors include low-grade serous (LGSC), low-grade endometrioid, clear cell, and mucinous carcinomas and are generally associated with mutations in KRAS, BRAF, ERBB2, PIK3CA, and CTNNB1 (2). Patients with type II tumors encompass 75% of all EOC cases and include high-grade serous (HGSC), endometrioid, and undifferentiated carcinomas (1). They frequently harbor TP53 mutations; 15% have BRCA1 or BRCA2 mutations and 20% have CCNE1 amplification (3).

Patients with type II tumors frequently harbor TP53 mutations; 15% have BRCA1 or BRCA2 mutations and 20% have CCNE1 amplification (3).

Aberrant expression of PAX2, as found in several carcinomas, promotes cell proliferation, survival, and chemoresistance (6, 9). PAX2 enhances tumor progression or chemoresistance in xenograft models for endometrial, colon, and renal cancers (10–12). PAX2 was proposed as a potential proto-oncogene, as ectopic expression of PAX2 led to transformation of rat fibroblast cells (13); however, constitutive expression of PAX2 in transgenic mice led to cyst formation in kidneys with no evidence of tumor incidence (14).

Aberrant expression of PAX2 in ovarian cancers has been extensively reported (4, 7, 9, 15), its role in ovarian tumorigenesis is uncertain, due to a limited number of experimental reports and their conflicting outcomes. Notably, PAX2 knockout in some ovarian cancer cell lines increased apoptosis and reduced cellular proliferation and/or migration (9, 15). However, overexpressing PAX2 in other cell lines suppressed cell proliferation (15). Although the mechanism behind the divergent roles of PAX2 is still unknown, comparison of cell lines suggests that PAX2 might act as a tumor suppressor in serous carcinoma with mutant TP53, whereas it may be oncogenic in cells with wild-type TP53. To determine whether induction of PAX2 expression is an early event in ovarian epithelial transformation, we used mouse ovarian surface epithelial (mOSE) cell lines with and without p53 expression.

The role of PAX2 in tumor progression was also evaluated in two different ovarian cancer model systems. To our knowledge, this is the first report that examines both in vitro and...
in vivo the consequence of PAX2 induction on the behavior of normal ovarian epithelial cells and ovarian cancer cells. Novel targets of PAX2 that are involved in tumor progression were also identified.

Materials and Methods

Cell lines

Two independent isolations of mOSE cells (M0505 and M1102) were obtained from 6-week-old FVB/N female mice as described previously (16). Long-term passage of M0505 cells led to spontaneous transformation (STOSE; ref. 16). An independent culture of mOSE cells was obtained from Tp53loxP/loxP mice (mOSE-fxp53). Treatment with the recombinant adenovirus Ad5CMVCre (AdCre; Vector Development Laboratory) results in the loss of functional p53 by excision of exons 2–10 (p5332–10, mOSE), confirmed as described by Clark-Knowles and colleagues (17). Mouse ovarian cancer cells (RM cells) were derived from mOSE cells obtained from Immortomice having temperature-permissive T-Antigen (Tag) expression. Following Tag-induced immortalization, cells were maintained at a nonpermissive temperature and transduced with retrovirus constructs to achieve expression of mutant K-Ras (KrasG12D) and Myc (18). All cell lines are of murine origin and were validated as syngeneic (STOSE) or confirmed to express RasG12D and Myc (RM cells). All cells were maintained as previously described (16).

Infection

The murine Pax2 cDNA, corresponding to Pax2b variant, was cloned from murine oviduct and used to generate a lentivirus expression vector (WPI-Pax2-ires-eGFP, hereafter Pax2). The empty vector, pWPI (Addgene plasmid 12254), was used as a control. Lentiviral vectors were prepared by cotransfection of vector plasmids (pWPI or pWPI-Pax2), packaging plasmid pCMVR8.74 (Addgene plasmid 22036), and the ecotropic envelope expression plasmid, pCAG-Eco (Addgene plasmid 35617) into 293T cells. After infecting the cells with either Pax2 or WPI, cells were passaged at least 3 times and sorted for GFP expression by fluorescence-activated cell sorting (Beckman Coulter, Inc.). Both the WPI and Pax2 integrates proviruses harbor loxP sites in both 5′ and 3′ LTRs allowing AdCre-mediated deletion. Deletion of both GFP and Pax2 was achieved by treating Pax2-expressing cells with AdCre and sorting them for GFP negativity (referred to as Pax2+/Cre). Except for the mOSE-fxp53 cells, infections were done twice for each cell line, with similar results.

Cell proliferation and survival assays

Cells were plated at 2 × 10⁵ cells per well in 12-well plates (mOSE) or 1 × 10⁶ cells per well in 6-well plates (p5332–10, mOSE, STOSE, and RM cells). Detached and attached cells were counted using a Vi-CELL XR Cell Viability Analyzer (Beckman Coulter, Inc.). For survival analysis, 24 hours after plating, cells were washed with PBS and refreshed with serum-free, growth factor-free media. After 48 hours in this starvation condition, cell viability was measured using a Vi-CELL XR Cell Viability Analyzer.

For chemosensitivity, 1 × 10⁶ cells were plated in 96-well plates and treated with cisplatin (12–50 μg/mL) the next day. Viability was assessed after 48 hours by Alam淀粉 Blue (Sigma-Aldrich), with absorbance measured using Fluoroskan Ascent FL (Thermo Scientific).

Animals

Animal experiments were performed in accordance with the Canadian Council on Animal Care’s Guidelines for the Care and Use of Animals and the University of Ottawa’s Animal Care Committee. Cells (1 × 10⁵ in 500 μL PBS) were injected into the peritoneal cavity of 8-week-old SCID or FVB/N mice (The Jackson Laboratory). Disease progression was monitored until humane endpoint (>15% weight gain, abdominal distension). Tumors were fixed in 10% buffered formalin for 24 hours, paraffin-embedded, and sectioned at 5 μm for histologic analysis or were snap-frozen for RNA or protein analysis.

Gene expression analysis

Gene expression was determined by quantitative PCR (QPCR), as previously described (16) using the primer pairs listed in Supplementary Table S1. Microarray analysis (accession number GSE69238) was performed on M0505 + WPI, M0505 + Pax2, and M0505 + Pax2 + Cre as described previously (16).

Western blot analysis

Proteins were extracted from cell lines and tumors as previously described (16). Blots were incubated with primary antibodies overnight (see Supplementary Table S2) and then incubated with the corresponding secondary antibodies and imaged on a FluorChem E gel documentation system (ProteinSimple).

Immunohistochemistry

Protein expression was visualized with anti-PAX2 (1:1,000, Abcam), anti-Ki-67 (1:300, Millipore), and anti-CD31 (1:100, Abcam) primary antibodies and Dako Envision system-HRP-labeled polymer anti-rabbit secondary antibodies (K4003, Dako), with detection using diaminobenzidine as a substrate (0.2% DAB, 0.001% H2O2; Sigma-Aldrich). Sections were counterstained with Harris hematoxylin (Fisher Scientific) and mounted with Permount (Fisher Scientific). Images were obtained with the ScanScope CS system and ImageScope software (Leica Microsystems Inc.) and 5 random areas for 3 to 4 tumors per group were analyzed for pixel positivity.

Statistical analyses

On the basis of the number of conditions tested, statistical significance was determined by t test, ANOVA (Tukey post-test), or log-rank test (Kaplan–Meier), performed by GraphPad Prism software (GraphPad).

Results

PAX2 enhances proliferation in mOSE cells

Similar to its expression in the human reproductive tract (4, 7), Pax2 was expressed in the murine oviduct and uterus, but not the ovary (Supplementary Fig. S1). To investigate the biologic consequences of Pax2 expression in normal mOSE cells, three independent isolations of mOSE cells were placed in primary culture (M0505, M1102, and mOSE-fxp53). Forced expression of Pax2 proteins was confirmed by immunoblotting (Fig. 1A; Supplementary Fig. S2A). In all three mOSE cell lines, Pax2 significantly enhanced cell proliferation (Fig. 1B; Supplementary Fig. S2B) without affecting viability (data not shown). When control cells were deprived of growth factors, their proliferation was inhibited but Pax2 was able to enhance proliferation with and without growth factors, supporting the notion that Pax2 has this...
PAX2 enhances survival in mOSE cells

To determine the effect of PAX2 on cell survival, cells were challenged by deprivation of growth factors, and the percentage of viable cells was normalized to viability of cells supplemented with growth factors. Only a limited decrease in cell viability (14%–18%) was observed in the parental lines (Fig. 1D; Supplementary Fig. S2D). PAX2 expression enhanced survival, albeit modestly, in M0505 cells, with a similar trend for enhanced survival in PAX2-expressing M1102 and mOSE-fxp53 cells, compared with non-expressing controls (P = 0.0725 and P = 0.0593, respectively; Supplementary Fig. S2D). Exposure to the DNA-damaging agent cisplatin for 48 hours decreased cell viability in a dose-dependent manner; this decrease was significantly inhibited by PAX2 expression (Fig. 1E; Supplementary Fig. S2E).

PAX2 inhibits p53 induction by cisplatin

Because our results showed that PAX2 enhances resistance to cisplatin and others have shown that PAX2 has a binding site in the first exon of Trp53 in murine cells (19), we investigated the effect of PAX2 on cisplatin-induced p53. Exposure to cisplatin increased p53 accumulation significantly in two mOSE cell lines (Fig. 2A), and this increase was suppressed in PAX2-expressing cells, reaching only 40% of the levels induced in control cells (Fig. 2B). However, PAX2 expression did not change the levels of Trp53 transcripts (Fig. 2C), indicating that PAX2 affects the translation and/or stabilization of p53 but not the transcription of Trp53.

PAX2 deregulates cancer-associated pathways in mOSE cells

To better understand the role of PAX2 in mOSE cells, microarray analysis was performed on M0505 + PAX2 and compared with M0505 + WPI and M0505 + PAX2 + Cre transcripts.
Changes were considered relevant only when a 2-linear-fold difference or more was evident between transcript levels in the PAX2-expressing cells compared with transcripts in both WPI and PAX2 + Cre cells. Using these criteria, there were 41 genes upregulated and 58 genes downregulated in PAX2-expressing cells. Ingenuity Pathway Analysis (IPA) showed changes in genes involved in cancer and cellular growth, predicting an activation of cancer-associated functions (hyperplasia and tumor size) and cell proliferation (Fig. 3). Supplementary Table S3 lists the top 10 most highly upregulated genes in the PAX2-expressing cells compared with the two control cell lines. Many of these genes have been reported to be involved in tumor growth and progression, which highlights the potential role of PAX2 in cancer initiation and development (Supplementary Table S4).

Figure 2.
PAX2 inhibits p53 induction in mOSE cells. Western blot analysis (A) and densitometric analysis (B) of p53 in M0505 and M1102 cells after cisplatin treatment (12.5 μg/mL; 48 hours), with UV-exposed mouse embryonic fibroblasts as a positive control. C, Trp53 mRNA levels after PAX2 expression. For all experiments, n = 3; *, P < 0.05; **, P < 0.01.

Figure 3.
IPA showing biologic functions associated with gene expression changes after PAX2 expression. Relationships between genes are color-coded by predicted interaction (see legend).
PAX2 exhibits oncogenic properties in normal mOSE cells

To determine whether PAX2 induction in mOSE facilitates oncogenic transformation, SCID mice were injected intraperitoneally with 10^7 cells of M0505 + WPI, M0505 + PAX2 (6 mice per group), or M1102 + PAX2 (5 mice). At 114 days postinjection, animals were sacrificed and none had developed tumors, indicating that PAX2 expression is not sufficient for transformation of ovarian epithelial cells.

TP53 mutation has been reported in 95% of HGSC (3), and null mutations in TP53 have been found in 24% of ovarian cancers (20). To determine whether PAX2 combined with p53 deficiency is tumorigenic, we generated mOSE cells lacking functional p53 (p53<sup>−/−</sup>-mOSE; Supplementary Fig. S3A). Although p53<sup>−/−</sup>-mOSE cells are not tumorigenic (21), they show anchorage-independent growth by forming colonies in soft agar and have enhanced proliferation and survival compared with normal mOSE cells (Supplementary Fig. S3B). PAX2 induction modestly increased proliferation and did not alter survival in these p53-deficient cells (Supplementary Fig. S3C), indicating that PAX2 enhances these functions, at least partially, in a p53-dependent manner.

To test the effects of PAX2 in p53-null cells, SCID mice were injected with either p53<sub>−/−</sub>-mOSE + WPI or p53<sup>−/−</sup>-mOSE + PAX2 cells (4 animals per group). Two mice injected with PAX2-expressing p53<sub>−/−</sub>-mOSE cells developed tumors, whereas there was no evidence of tumor in the p53<sub>−/−</sub>-mOSE + WPI group. Animals that did not develop tumors were kept for 6 months and examined for tumor incidence. None had developed tumors. Although the number of animals is small, these data indicate that loss of p53 confers oncogenic potency to PAX2 in mOSE cells.

PAX2 has contradictory effects on ovarian tumor progression

Our results are in accordance with reports showing that PAX2 enhances tumor progression in different cancer models (10, 11). To further determine whether this is the case in ovarian cancers, PAX2 was expressed in two murine ovarian cancer models generated in our laboratory (16, 18). The RM model was derived from immortalized mOSE cells transduced with retrovirus constructs to generate in our laboratory (16, 18). The RM model was derived from mOSE cells transduced with retrovirus constructs to generate in our laboratory (16, 18). The RM model was derived from immortalized mouse ovarian epithelial cells.
Figure 4.
PAX2 actions in RM and STOSE mouse ovarian cancer cells. A, Western blot analysis confirming PAX2 induction in both cell lines. B, proliferation of STOSE and RM cells with PAX2 induction (PAX2) versus controls (parental, WPI, PAX2-Cre). C, viability (left, alamar blue) and cleaved caspase-3 (right, Western blotting) were assessed after 48-hour cisplatin treatment ($n = 3; \times P < 0.01; \times \times \times \times P < 0.0001$). D, PAX2 expression decreased colony formation ability of STOSE but not RM cells ($n = 3; \times \times \times \times P < 0.0001$). E and F, Kaplan-Meier survival curves for mice injected with (E) RM + WPI or RM + PAX2 cells or (F) STOSE + WPI, STOSE + PAX2, or STOSE + PAX2 + Cre cells ($\times \times \times \times P < 0.0001$). G, PAX2 expression decreased STOSE tumor weight at endpoint ($\times P < 0.01; \times \times P < 0.05$).
Figure 5.
PAX2 inhibited p53 accumulation in RM but not in STOSE tumors. A, PAX2 expression abolished p53 and cleaved caspase-3 (Western blotting) in all RM + PAX2 tumors examined. Positive controls for p53 and cleaved caspase-3 were cisplatin-treated normal and p53-deficient mOSE, respectively. B, Trp53 mRNA levels in RM and STOSE tumors. C, STOSE tumors did not show p53 accumulation (Western blotting). (Continued on the following page.)
tumors express much lower levels of COX2 (0.14-fold), compared with controls (Fig. 6A). Therefore, although both derived from ovarian epithelial cells, PAX2 targets different genes in the two tumor models.

COX2 is associated with angiogenesis in some tumor types (24). To investigate whether the COX2 induction in RM tumors resulted in changes in tumor microvesSEL density (MVD), vascular endothelial marker (CD31) was detected by IHC analysis. PAX2–RM tumors have an increased MVD, as compared with control tumors (Fig. 6B). However, there is no difference in the MVD among STOSE tumors (with and without PAX2). These data indicate that in RM tumors, PAX2 may enhance angiogenesis, and therefore progression, through the induction of COX2.

Discussion

The aim of this study was to determine the role of the potential oncogene PAX2 in the etiology and progression of ovarian cancer. Because PAX2 is expressed in ovarian cancers but not in normal OSE, we developed mOSE cells expressing PAX2 to identify whether PAX2 induction is one of the initial events in transformation. Although PAX2 expression enhanced cell survival and proliferation, it was not sufficient to induce tumorigenicity, unless combined with loss of p53. PAX2 had opposing actions in two murine ovarian tumor models, accelerating or inhibiting tumor progression.

Although recent evidence shows that some HGSC originate from Mullerian cells (26), OSE cells remain a probable origin of several subtypes of EOCs (reviewed in ref. 27). OSE cells retain the potential to differentiate into epithelia resembling that of the Mullerian system (28, 29) by, for example, induction of HOX gene expression (30). HOX and PAX genes are required for Mullerian development and are expressed in a subset of EOC but not in normal OSE cells (4, 31). PAX2 is expressed in ciliated inclusion cysts, low-malignant potential, and LGSC (4), suggesting that gain of PAX2 expression promotes transformation (as shown in rat fibroblasts; ref. 13). We found that expression of PAX2 increased mOSE proliferation even when the cells were deprived of growth factors and increased survival during cisplatin treatment. Proliferation, self-sufficiency in growth signals, and apoptosis avoidance are key factors in tumor formation (32).

Microarray analysis showed that PAX2 disturbs signaling pathways involved in cancer, reproductive diseases, and cellular proliferation. Further experiments in OSE showed that PAX2 increased cancer-related characteristics, including inhibition of p53 accumulation, but was not sufficient to induce transformation. PAX2 has a potential binding site on exon 1 of the Trp53 gene in mice (19), but our results show that p53 downregulation by PAX2 is a posttranscriptional event.

Previous studies showed that Trp53 mutation is required for mOSE transformation by alterations in K-Ras, myc, and/or Akt (21). Because mutations in Trp53 have been found in almost all (96%) HGSC (3) and null mutations in 24% of ovarian cancers (20), we expressed PAX2 in p53-deficient mOSE cells. Some animals injected with p53-null PAX2-expressing cells formed tumors, but p53-null cells did not form tumors, as shown by others (21). This suggests that loss of p53 predisposes ovarian epithelial cells to transformation by PAX2 induction. Similarly, PAX2 sensitizes the cells to become tumorigenic following Trp53 mutation.

In vitro, PAX2 shows oncogenic behavior, as silencing PAX2 resulted in decreased tumor volume or enhanced cisplatin-induced tumor regression in xenograft models of human endometrioid, colon, and renal carcinoma cells (10–12). However, there is limited and conflicting evidence for its contribution to ovarian tumorigenicity. In human ovarian cancer cell lines, PAX2 can enhance or inhibit growth (9, 15). Although the mechanism was not identified in these studies, cancer subtype and/or TP53 status may explain these contradictory results.

To understand the role of PAX2 in ovarian tumor progression, we used two different mouse models reflecting LGSC and HGSC. The RM model was developed by inducing mutant K-RASG12D and MYC in immortalized mOSE cells. Activating mutations in K-RAS or B-Raf are present in two thirds of LGSC (33), and overexpression of c-MYC has been reported in up to 70% of ovarian cancers (34). LGSC cell lines and murine models are limited (35, 36), and the RM cells were used as a model for LGSC on the basis of their dependence on K-Ras mutation. Nevertheless, the RM cells form aggressive disease, in contrast to the slower disease progression of LGSC in women. Unlike STOSE tumors, RM tumors have stable p53 accumulation. This was not due toTAG expression, which is undetectable (Supplementary Fig. S4), but as mutant RAS or increased levels of c-MYC stimulate p19ARF–MDM2 complex formation, this may sequester MDM2 and stabilize p53 (37, 38).

RM cells expressing PAX2 became more proliferative in culture and more aggressive in vivo, showing increased angiogenesis and decreased apoptosis. Molecular analysis showed that PAX2 expression eliminated p53 and cleaved caspase-3 expression, decreased PTEN, and increased pAKT and pERK1/2. Therefore, it appears that enhanced angiogenesis and reduced apoptosis in the RM + PAX2 tumors account for the accelerated tumor growth and shorter survival.

Both the histotype and molecular profile for the STOSE ovarian cancer model mimic human HGSC. STOSE cells have a high degree of aneuploidy, increased expression of cyclin D1, and possibly increased NF-kB activity, but interestingly no mutations in the Trp53 gene (16). In contrast to the RM model, PAX2 improved survival and reduced STOSE tumor mass and dissemination in both immunodeficient and -competent mice, suggesting that PAX2 is a negative regulator of some aspects of ovarian tumorigenicity.

One possible explanation, as shown in Fig. 6C, is the regulation of Htra1 by PAX2 in these tumors. HTRA1 is a putative tumor suppressor gene that is downregulated in ovarian cancers (39) and enhances chemosensitivity by targeting the antiapoptotic protein XIAP for degradation (40). Downregulation of HTRA1 enhanced peritoneal dissemination in a xenograft study by increasing phosphorylation of EGFR, AKT, and ERK1/2 (22). We found that Htra1

(Continued) D, tumor proliferation was examined by immunohistochemistry for Ki67 (quantified in histograms). E, PAX2 expression reduced Htra1 transcripts in mOSE cells and RM tumors, but not STOSE tumors. F, PAX2 expression reduced PTEN levels in RM tumors (Western blotting). G, immunoblot detection of total and phospho-AKT following AKT immunoprecipitation in RM tumors. H, Western blot analysis shows PAX2-associated phosphorylation of ERK1/2 at Thr202 and Tyr204 in RM but not STOSE tumors; SCC25 head and neck carcinoma cells were used as a positive control for EGFR. EGF-treated SCC25 cells were used as positive and (with an EGF inhibitor) negative controls for pERK1/2.
Figure 6.
Effect of PAX2 on COX2 and angiogenesis. A, COX2 in ovarian tumors after PAX2 induction (Western blotting). B, MVD was examined using immunohistochemistry for CD31. Bottom, CD31-positive pixel selection, quantified in the histograms on the right (*, P < 0.05). Scale bar, 100 μm. C, a proposed model for PAX2 actions in normal and cancerous ovarian cells. Dotted lines indicate predicted functional effects.
transcripts were more abundant in STOSE + PAX2 tumors, but lower in RM + PAX2 tumors, as compared with the corresponding controls. In STOSE tumors, PAX2 did not alter apoptosis (cleaved caspase-3) or ERK1/2 phosphorylation, whereas PAX2-expressing RM tumors showed a robust increase in pAKT and pERK1/2, as compared with controls. This was likely not due to activation of the EGFR pathway, as EGFR was undetectable, but it is associated with the downregulation of PTEN, a known negative regulator of the PI3K pathway (41).

Another relevant PAX2 target with differential expression in RM versus STOSE tumors is PTGS2 (COX2). Aberrant upregulation of COX2 has been reported in ovarian tumors and is associated with chemoresistance (25). The COX2 inhibitor (celecoxib) suppressed growth of ovarian xenografts in mice, reduced angiogenesis, and increased apoptosis (24). In our study, PAX2 increased COX2 in RM tumors but reduced its expression in STOSE tumors. Interestingly, COX2 is induced by TGFβ1 (42) and HTRA1 may inhibit TGFβ signaling (43). TGFβ1 expression in human ovarian tumors is associated with poor prognosis (44), and we speculate that HTRA1 upregulation prevented potential deleterious effects of TGFβ1 in STOSE + PAX2 tumors. Therefore, PAX2 may confer or inhibit tumor progression at least partially through differential regulation of COX2 and/or HTRA1, depending on the tumor context.

This investigation identified novel targets dysregulated by PAX2 (i.e., COX2 and HTRA1), both of which are involved in cell proliferation (40, 45). Although apoptotic induction was enhanced by PAX2 in STOSE cells, no apparent apoptosis was evident in STOSE tumors, where PAX2 promoted proliferation. Interestingly, PAX2 expression reduced STOSE colony formation in soft agar, indicating a higher rate of anoikis (22). Thus, HTRA1 induction might cause the decreased metastasis seen in PAX2-expressing STOSE tumors.

This study confirms the differential role of PAX2 in ovarian tumor progression reported previously (9, 15) and identifies some molecular drivers that might be responsible. The differential role of PAX2 in ovarian cancer has been suggested previously by Song and colleagues (15). Compelling evidence that PAX2 might act as a tumor suppressor in well-differentiated epithelia comes from studies reporting that PAX2 is completely lost or markedly reduced in the early stages of tubal and endometrial intraepithelial carcinomas (46, 47), often in association with TP53 mutation or in the presence of co-existing pelvic serous cancer (48, 49). Interestingly, PAX2 transcription is stimulated by wild-type TP53 in kidney cells and this stimulation is inhibited by dominant-negative TP53 mutations (50), a common aberration in HGSC (3). Because HGSC have a low incidence of PAX2 expression (4), one could argue that the forced expression of PAX2 in STOSE cells, which generate HGSC-like tumors, allowed the demonstration of its tumor-suppressive function. In contrast, expression of K-RAS (as in RM cells) and PAX2 are both associated with LGSC (4, 33) and induction of PAX2 in this model enhanced tumor aggressiveness.

In summary, PAX2 induces carcinogenic features in OSE cells (enhanced proliferation, survival, and growth factor independence) and increases their sensitivity to transformation. In ovarian cancer cells, PAX2 enhances or inhibits their aggressiveness in vitro, depending on the molecular context. In tumors with K-Ras and Myc mutations, PAX2 enhances cell survival and angiogenesis, whereas in tumors resembling HGSC, PAX2 decreases proliferation and metastasis. Therefore, in mouse ovarian tumors, in agreement with results reported for human ovarian cancer cells (15), PAX2 seems to have contradictory effects on progression, depending on the context. The differential effects of PAX2 appear to be mediated by differential regulation of COX2 and/or HTRA1. It remains to be determined whether these molecular pathways are activated or repressed in a manner dependent on the molecular environment that leads to HGSC versus LGSC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: E.M. Al-Hujaily, Y. Tang, D.-S. Yao, K. Garson, B.C. Vanderhyden
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.M. Al-Hujaily, Y. Tang, D.-S. Yao, K. Garson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.M. Al-Hujaily, Y. Tang, D.-S. Yao, E. Carmona, B.C. Vanderhyden
Writing, review, and/or revision of the manuscript: E.M. Al-Hujaily, Y. Tang, D.-S. Yao, E. Carmona, K. Garson, B.C. Vanderhyden
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.C. Vanderhyden
Study supervision: B.C. Vanderhyden

Acknowledgments
The authors thank Olga Collins and Elizabeth Macdonald for excellent technical support. They are grateful to Dr. Didier Trono for providing plasmids pWtI and pCMV8.R7.4 and Dr. Arthur Nienhuis for providing plasmid pCAG-Eco.

Grant Support
This work was funded by grants from the Marsha Rivkin Center for Ovarian Cancer Research (B.C. Vanderhyden) and the Canadian Institutes of Health Research (MOP-111194; B.C. Vanderhyden), and a scholarship to E.M. Al-Hujaily from the King Abdullah International Medical Research Center.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 24, 2015; revised August 24, 2015; accepted September 8, 2015; published OnlineFirst September 15, 2015.

References


Cancer Prevention Research

Divergent Roles of PAX2 in the Etiology and Progression of Ovarian Cancer

Ensaf M. Al-Hujaily, Yong Tang, De-Sheng Yao, et al.

Cancer Prev Res  Published OnlineFirst September 15, 2015.

Updated version  Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-15-0121-T

Supplementary Material  Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2015/09/15/1940-6207.CAPR-15-0121-T.DC1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.