Abstract

Development of mouse models representing human spontaneous ovarian cancer has been hampered by the lack of understanding of the etiology of this very complex disease. Mouse models representing the different types of ovarian cancer are needed to understand how epithelial ovarian cancer differs from granulosa cell tumors. Many different methods have been used to generate a viable genetic model with limited success. This review focuses on the methods of various investigators and the limitations of each model in establishing a reproducible and inheritable line to study this disease.

Introduction

Ovarian cancer (OC) is the most lethal malignancy of the female reproductive system and the fifth leading cause of cancer death in women [1]. Ninety percent of OC are thought to arise from the epithelium and its inclusion cysts [2] due to multiple genetic changes [3]. However, the etiology of spontaneous epithelial (E)OC is poorly understood, partially due to a lack of an appropriate experimental model. While many approaches have been used, model development has been hampered by the absence of a specific promoter for the ovaries, as many promoters are sufficiently leaky. Numerous investigators have sought to develop a model that would effectively represent spontaneous human EOC. This review focuses on the methods various investigators have employed and the limitations of each murine model in establishing a reproducible, inheritable line to study this disease.

Carcinogen induced tumor models

As early as 1969, ovarian tumors were induced by direct application of chemical carcinogens [4]. While 7,12-Dimethylbenz(a)anthracene (DMBA) had been used in 1970 to induce tumorigenesis in guinea pigs [5], a DMBA-coated suture was used in 1984 to induce ovarian tumorigenesis with only one of thirty-five mice developing an epithelial carcinoma [6]. However, despite these discouraging results, Nishita et al. [7] replicated this experiment by directly applying DMBA to the rat ovary using a coated suture. Nearly fifty percent of the rats developed ovarian tumors in 36 weeks, most of which were carcinomas. Unfortunately, DMBA also stimulated the epithelial surface of the fallopian tube, endometrium, and cervix to induce neoplastic transformation.

Other chemical carcinogens used to induce ovarian tumorigenesis include 20-methylcholanthrene, 1,3-butadiene, formic acid 2- [4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide, a nitrofuran antibiotic, and N-methyl-N'-nitrosourea, a direct-acting alkylating agent [8-10]. To date, chemical carcinogens have not been associated with OC etiology [11].

Syngeneic ovarian epithelial tumor models

Syngeneic models combine in vitro and in vivo methods to generate a tumor model. Briefly, mouse ovarian surface
epithelial (MOSE) cells are isolated from the ovaries of virgin wildtype mice and cultured in vitro before transplantation into recipient mice [12]. Development of the mouse model was predicated on the work by Godwin et al. [13] and Testa et al. [14] on the spontaneous transformation of surface epithelial cells isolated from rats.

Roby et al. [12] established the technique of isolating and culturing MOSE cells, showing that MOSE cells can spontaneously transform in vitro with repeated passages and have tumorigenic capacity as they formed tumors and hemorrhagic ascitic fluid upon injection into athymic and C57B1/6 receipt mice. This technique has been used by numerous investigators for subsequent studies [15-20].

Perhaps one of the most revealing MOSE studies was conducted by Roberts et al. [15], who compared the alterations of the actin cytoskeleton as well as expression of cellular adhesion proteins versus the number of passages to study the progression of ovarian carcinoma, showing that MOSE cells spontaneously transform with repeated passages. Late passage cells injected intraperitoneally into immunocompetent C57BL6 mice formed tumors in numerous organs, showing the transformation from a premalignant to a highly malignant phenotype with downregulation of E-cadherin and connexin-43.

Greenaway et al. [16] injected a spontaneously tumorigenic MOSE cell line, ID8, into the ovarian bursal cavity of C57Bl6 mice. The ID8 cells formed direct contact with the ovarian stroma, resulting in primary tumor formation, secondary peritoneal carcinomatosis, and extensive ascites fluid production between 80 to 90 days post-exposure. The cytological and architectural features resembled serous carcinoma. Interaction between ID8 cells and the ovarian stroma resulted in increased expression of proliferative and survival markers, including phosphorylated Akt, proliferating cell nuclear antigen (PCNA), and Bcl-2. Vascular endothelial growth factor (VEGF) levels were also increased in the serum and ascitic fluid. In conjunction, the pro-apoptotic factor Bax was decreased. The study supports the theory that the ovarian surface epithelium (OSE) can undergo invaginations and form inclusion cysts capable of undergoing neoplastic transformation [21].

**Genetically induced ovarian epithelial tumor models**

One of the first reports to test genetic changes was made by Orsulic et al. [22], who used an avian retroviral delivery system. Transgenic mice were established to express the TVA virus receptor making them susceptible to infection to a subgroup of replication-competent avian leukemia viral-derived vectors (RCAS), thus allowing for the introduction of oncoproteins that would integrate newly reverse-transcribed DNA into the host genome and allow long-term expression. The TVA receptor was placed under control of the keratin 5 promoter to direct expression to the ovarian epithelium or under control of the β-actin promoter to direct expression to all cells of the ovary. TVA-transgenic mice were crossed with p53-/-mice to generate TVA/p53-/-, which were used to study the oncoproteins c-myc, K-ras, and Akt individually and in combination. However, the keratin 5 promoter is constitutively active in the basal layer of stratified and simple epithelia in several organs [23]; therefore, it was necessary to isolate the expression of the virally delivered oncoproteins. The ovaries were removed from the TVA/p53-/- mice, cultured, and infected in vitro before introduction into recipient mice either by subcutaneous or intraperitoneal injection or by transplantation under the ovarian bursa. Once infected, the mammalian cells would not produce detectable levels of infectious viral particles, which limited spreading to the surrounding tissue. Introduction of any two oncoproteins in keratin 5-TVA/p53-/- ovarian cells was sufficient to drive tumorigenesis. While providing valuable insight into the genetics of tumorigenesis, this methodology is cumbersome at best. Because transplantation and in vitro manipulation are required, it is not possible to generate a stable transgenic line with an inheritable form of EOC.

Connolly et al. [24] used a novel approach to target simian virus 40 T antigen (SV40 TAg) to the epithelial ovarian surface by using the Mullerian Inhibitory Substance Type II Receptor (MISIIR) promoter. The Mullerian duct in the 8-week old embryo gives rise to female reproductive organs, including the fallopian tubes, uterus, and upper vagina. By linking the MISIIR promoter to the SV40 TAg, they were able to target expression of SV40 TAg to the epithelium of the female reproductive tract by microinjection of this construct into the male pronucleus of 0.5-day old embryos to generate transgenic animals. While 18 of 36 (50%) transgenic mice developed bilateral ovarian tumors resembling serous carcinomas by 6 to 13 weeks of age, the aggressiveness of this formation inhibited reproduction, making it extremely difficult to establish a transgenic line via the female founders. Two individual transgenic mice also developed a uterine mass and enlarged polycystic kidneys, respectively, possibly due to recombination events during transgenic mouse production. Not unexpectedly, 7 of 25 (28%) transgenic animals developed testicular cancer. Intrapleural invasion of tumors into the omentum, the mesentery, and visceral and parietal pleura was also observed, possibly due to the invasiveness of the ovarian tumors. However, SV40 TAg is not known to be a genetic contributor to ovarian carcinogenesis [3,25,26]. Yet despite these limitations, this model has been used for further experiments by establishing a transgenic line through the male founder [27,28].

Models that require either ex vivo manipulation or expression of a transgene during embryonic development do not accurately represent human EOC, which tends to be spon-
taneous in post-menopausal women. In an effort to mimic spontaneous EOC development, Flesken-Nikitin et al. [29] obtained mice from Anton Berns [30,31] with LoxP sites containing p53 and Rb alleles to assess gene inactivation in the initiation of EOC. Mice were homozygous for the mutation and crossbred to generate p53\textsuperscript{loxP/loxP}Rb\textsuperscript{1floxP/loxP}. To assess the efficiency of Cre recombinase (Cre) expression derived by the cytomegalovirus (CMV) promoter, the ovaries were removed and cultured prior to exposure to adenovirus infection. Adenoviruses carrying CMV-enhanced fluorescent green protein (AdCMVEGFP) were used as a control against adenoviruses carrying CMV-Cre (AdCMVCre). Administration of AdCMVCre resulted in increased cell proliferation assessed by BrdU incorporation. To detect the feasibility in targeting the ovarian bursal cavity in the mouse, AdCMVEGFP was administered. It was detected only in the OSE for 21 days, as expected with a transient adenovirus infection. As a result of both p53 and Rb inactivation, 33 of 34 mice succumbed to ovarian tumors at a median of 227 days. However, administration of an adenovirus to achieve the desired results is cumbersome without generating a reproductive line that would spontaneously form tumors. Targeting the ovarian bursal cavity is difficult at best, making this model not a feasible choice for large-scale applications.

While the previous models developed tumors resembling human serous adenomas, Dinulescu et al. [32] generated mice that have a transcriptionally silent oncogenic allele of K-ras (LSL-K-ras\textsuperscript{G12D/+}) as first developed by Tyler Jacks [33-35], which can be conditionally expressed through administration of an adenovirus containing Cre. While the LSL-K-ras\textsuperscript{G12D/+} mice formed benign endometrosis-like lesions and benign lesion within the OSE upon K-ras activation, the mice did not form ovarian carcinomas. However, when the LSL-K-ras\textsuperscript{G12D/+} mice were crossed with PTEN\textsuperscript{loxP/loxP} mice, they developed invasive primary ovarian endometrioid adenocarcinomas (OEA), a subtype of EOC, suggesting that phosphate and tensin homologue deletion on chromosome 10 (PTEN) plays a role in tumorigenesis when combined with other oncogenes. This finding is consistent with PTEN deletion or mutation in other cancer types including endometrium, breast, thyroid, intestines, prostate, lung, liver, and T-cell lymphomas [36-40]. Concurrent K-ras and PTEN mutations have also been found in complex endometrial hyperplasias, the precursor type of uterine endometrioid adenocarcinomas [41].

Wu et al. [42] used similar methods to conditionally delete PTEN and adenomatous polyposis coli (APC) tumor suppressor gene upon administration of an adenovirus carrying Cre. APC has been shown to regulate Wnt/β-catenin signaling [43]. Wu et al. cross-bred PTEN\textsuperscript{loxP/loxP} with APC\textsuperscript{loxP/loxP} transgenic mice to determine if there was an interaction between the two pathways. The PTEN\textsuperscript{-/-} APC\textsuperscript{-/-} animals developed tumors within 6 weeks upon inactivation, with death occurring at 19 weeks. These tumors resembled human OEA, with increased signaling through Akt. Loss of E-cadherin and cytokeratins indicated that these tumors were undergoing epithelial-mesenchymal transition (EMT), which is consistent with Wnt/β-catenin and PI3K/Akt activation [44,45]. Both the studies by Dinulescu et al. [32] and Wu et al. [42] rely on adenovirus administration and are therefore subject to the same limitations.

Chodanker et al. [46] crossbred mice with follicle stimulating hormone (FSH) receptor promoter fused to Cre recombinase (FSHR-Cre) to mice carrying Brca1\textsuperscript{loxP/loxP} to conditionally knockout Brca1 in the granulosa cells. Loss of Brca1 resulted in multiple cyst formation in 40 of 59 animals (58%) attached to the ovarian wall and interior or exterior surface of the uterine horns, which resembled human serous cystademons, the benign form of ovarian serous carcinomas. One animal formed a solid tumor. Although the FSHR promoter targeted the granulosa cells, the cysts resembled an epithelial morphology as they expressed keratins.

Clark-Knowles et al. [47] used Brca1\textsuperscript{loxP/loxP} mice, which upon administration of AdCre would remove introns 5 through 13 (Brca1\textsuperscript{55-13}). Conditional deletion of Brca1 resulted in morphological changes, such as surface epithelium hyperplasia and formation of inclusion cysts, which was not due to increased proliferation. The incidence of these changes increased over time as observed from 60 days post-infection to 240 days. Interestingly, the genes involved in cancer initiation and progression p53 [48], E-cadherin [49], and Collagen IV [50] were altered in Brca1\textsuperscript{55-13} ovaries compared to other tumor models. In Brca1\textsuperscript{55-13} ovaries, p53 was absent compared to SV40-induced tumors. E-cadherin was also downregulated, consistent with preneoplastic transformation. Collagen IV expression was found in the basement membrane, regardless of morphological changes of the OSE.

Building on the report by Connolly et al. [24], El-Naggar et al. [51] used the MISIIR promoter linked to the pituitary tumor-transforming gene (PTTG) to target expression to the OSE. This construct was microinjected into the male pronucleus of CD2F1 embryos to produce transgenic founders. The founders were crossbred with wildtype animals to produce the F1 generation. Positive male and female F1’s from the same line were crossbred to produce the F2 generation. While the transgenic females failed to generate any visible tumors, there was an increase in the corpus luteum mass in the transgenic ovaries, which was accompanied by the increase in serum luteinizing hor-
mone (LH) and testosterone levels. The transgenic females also displayed a generalized hypertrophy of the endometrium. This study showed that by using the MISIIR promoter, 3 different tissues could be targeted: OSE, granulosa cells, and pituitary.

More recently, Liang et al. [52] used the MISIIR promoter to drive expression of murine phosphatidylinositol 3-kinase catalytic subunit p110-alpha (PIK3CA) in transgenic mice. Although over-expression of PIK3CA resulted in increased phosphorylated Akt as its downstream target and in OSE hyperplasia, after 18 months post-birth of the F1 generation, tumorigenesis did not occur. Interestingly, the authors cultured isolated ovaries from non-transgenic mice and co-transfected them with both PIK3CA and mutant K-ras or c-myc to assess OSE transformation in vitro. Concurrent over-expression of PIK3CA and mutant K-ras led to increased anchorage-independent growth of cultured OSE cells. Liang et al. [52] acknowledged that producing a "bigenic" animal by crossing the transgenic PIK3CA mouse with a transgenic mutant K-ras remains a technical challenge because mutant K-ras animals develop tumors that inhibit reproduction. However, they suggested that a Cre-lox system of K-ras expression may provide an alternative method of generation.

**Genetically induced granulosa cell tumor (GCT) models**

Granulosa cell tumors (GCT) represent 2-5% of all OCs [53] arising from the granulosa cells of the ovary, which are responsible for estradiol production. Therefore, GCT are also called sex cord-stromal tumors. One of the first GCT models was produced by Kananen et al. [54], who fused the inhibin α-subunit promoter to SV40 TAg to generate transgenic founders. Three lines were established from these founders with all transgenic offspring developing tumors. The thymus also showed enlargement demonstrating that OSP-1 was sufficiently leaky. Male founders also expressed TAg in a variety of tissues including testes, liver, and lung, but failed to produce any tumors.

Boerboom et al. [57] showed that constitutive activation of β-catenin in granulosa cells of transgenic mice (Catnb-flox(ex3)Amhr2cre/+*) produced GCT. Cre knocked into the anti-Mullerian hormone receptor, type II (AMHR2) gene, designated AMHR2cre, to localize its expression. Exon 3 of β-catenin encodes for multiple phosphorylation sites that are necessary for its degradation, while its removal maintains the protein’s functionality. However, the excision of exon 3 of Catnb by Cre was a relatively inefficient process as few Catnb-flox(ex3)Amhr2cre/+* mice displayed abnormal expression of β-catenin. Histochemical analysis showed that the ovaries of 3 to 24-week-old transgenic mice developed abnormal follicle-like structures consisting of pleiomorphic granulosa cells without the presence of an oocyte, resulting in sub-fertility due to an impaired follicular response that could be overcome with age at the end of the third month. GCT were seen at 19 weeks with the incidence of formation over time to 57% at 7.5 months.

Building upon the previous study, Lague et al. [58] conditionally deleted PTEN in the granulosa cells by cross-breeding PTENfl/fl with AMHR2cre/+ mice to create PTENfl/flAMHR2cre/+ mice. Most PTENfl/flAMHR2cre/+ mice failed to generate any ovarian abnormalities; while these animals could establish pregnancies, they failed to carry the litter to term or had small litters due to fetal death. However, 5 of 70 (~ 7%) female PTENfl/flAMHR2cre/+ developed ovarian tumors. Four of the 5 were bilateral tumors developing between 7 weeks and 7 months that were identified as GCT. PTENfl/flAMHR2cre/+ mice also developed tumor cell emboli and metastases in the lungs. PTENfl/flAMHR2cre/+ GCT showed altered PI3K/Akt signaling, with increases in both phosphorylated Akt and mammalian target of rapamycin (mTOR) levels compared...
to normal granulosa cells. Furthermore, to determine if the PI3K/Akt pathway could cross-talk with the WNT/CTNNB1 (encoding β-catenin) pathway, they constitutively activated both pathways using the mouse model PTEN\textsuperscript{flox/flox}CTNNB1\textsuperscript{flox(ex3)}AMHR\textsuperscript{cre/+}. These mice developed bilateral ovarian tumors with 100% penetrance at an early age. Dysplastic cells were seen in the ovaries of newborn mice and 20.5-day embryos suggesting that this occurs perinatally. The ovarian tumors visibly distended the abdomen by 5 weeks of age with death occurring before 9 weeks, possibly due to severe anemia. Pulmonary emboli were also seen in PTEN\textsuperscript{flox/flox}CTNNB1\textsuperscript{flox(ex3)}AMHR\textsuperscript{cre/+} mice.

Conclusion
The syngeneic model has shown that MOSE cells are capable of spontaneously transforming into a tumorigenic phenotype with repeated passages, indicating that repeated repair of the OSE as a result of excessive ovulation could be a cause of tumorigenesis. The manipulation of MOSE cells and subsequent injection may form a tumor, but the tumor could form solely from the MOSE cells and not the host OSE cells as MOSE cells could undergo mesenchymal-epithelial transition (MET) to imbed in the host tissue. The limitation of extracting MOSE cells and culturing them before transplantation allows for only a limited number of animals to be produced and does not establish an inheritable line that would spontaneously form EOC.

A summary of the genetically induced ovarian epithelial tumor models can be found in Table 1. These models have provided valuable information regarding gene dysfunction necessary for tumorigenesis, including p53 and Rb deletion, as well as over-expression of known oncogenes c-myc, Kras, and Akt. Models that use transgene expression during embryonic development do not accurately represent spontaneous EOC, which tends to occur in post-menopausal women, and yet gene deletion by adenoviruses would spontaneously form EOC.

Table 2 summarizes the genetically-induced GCT models. OSP-1 and inhibit α-subunit promoters are not specific to the ovaries, although sufficiently strong to drive tumorigenesis. While knocking Cre into the AMHR2 locus was a clever design, the efficiency of the targeted gene deletion was relatively ineffective, as gene expression was maintained, possibly due to Cre acting on only the cis chromosome so ovarian abnormalities were not observed.

Many models have used SV40 TAg, a monkey virus belonging to the polyomavirus family, to initiate tumorigenesis. In a breast cancer model, SV40 TAg was shown to inactivate p53 and Rb to initiate tumorigenesis [59]. While SV40 TAg has been reported in several types of human cancer including breast, brain, osteocarcinomas, lymphomas, hepatocellular carcinomas, papillary thyroid carcinomas, and pleural mesothelioma [60-66], it has not been reported in OC. At best, SV40 TAg has been used widely to immortalize OC cell lines [67-69]. Moreover, SV40 TAg immortalization of cultured human OSE cells eliminated the presence of CA-125 [69], one of the current diagnostic markers for EOC [70].

To understand the complexity of OC, a mouse model representing each subtype is needed. From the current transgenic models, we have learned that different pathways are used for tumorigenesis. For EOC, p53 mutations/inactivation plays a role, as seen in high-grade tumors [26], while GCT have intact p53 but dysregulated PTEN and Wnt/β-catenin signaling occurring perinatally [42,57,58].

List of Abbreviations
α-LHβCTP: inhibin α-subunit promoter, LH gene with a carboxy-terminal peptide of human chorionic gonadotropin β subunit attached; AdCMVCre: adenoviruses contain-

Table 1: Summary of promoters and targeted genes for ovarian epithelial tumorigenesis.

| Authors               | Promoter     | Targeted gene       | Tumorigenesis | Limitation          |
|-----------------------|--------------|---------------------|---------------|---------------------|
| Orsulic et al. (2002) | keratin-5, RCAS | TVA, p53\textsuperscript{c-}, oncogenes | Yes | External manipulation |
| Connolly et al. (2003) | MISIR        | SV40 TAg            | Yes | Inhibited female reproduction |
| Flesken-Nikkita et al. (2003) | AdCre | p53\textsuperscript{c-} & Rb\textsuperscript{c-} | Yes | Transient expression |
| Dinulescu et al. (2005) | AdCre | K-ras & PTEN\textsuperscript{c-} | Yes | Transient expression |
| Wu et al. (2007) | AdCre | PTEN\textsuperscript{c-} & APC\textsuperscript{c-} | Yes | Transient expression |
| Chandran et al. (2005) | FSHR | Cre, BRCA1\textsuperscript{15-13} | No | No |
| Clark-Knowles et al. (2007) | AdCre | BRCA1\textsuperscript{15-13} | No | No |
| El-Naggar et al. (2007) | MISIR | PTTG | No | No |
| Liang et al. (2009) | MISIR | PIK3CA | No | No |
Table 2: Summary of promoter and targeted genes of granulosa cell tumors (GCT).

| Authors                          | Promoter                                      | Targeted gene     | Tumorigenesis | Limitation                  |
|----------------------------------|-----------------------------------------------|-------------------|---------------|------------------------------|
| Kananen et al. (1995)            | α-subunit of inhibin                          | SV40 TAg          | Yes           | Females unable to reproduce |
| Nilson et al. (2000)             | α-subunit of inhibin                          | LH/JICTP          | Yes           | Developed tumors            |
| Garson et al. (2003)             | OSP-1                                         | SV40 TAg          | Yes           | Transient expression        |
| Boerboom et al. (2005)           | MISIRIR/Cre                                    | mutant β-catenin  | Yes           | Transient expression        |
| Lague et al. (2008)              | MISIRIR/Cre                                    | PTEN nuances & CTNNB1 nuances | Yes |                          |

ing CMV promoter and Cre gene; AdCMVVEGF: adenoviruses containing CMV promoter and EGF gene; AMHR2: anti-Mullerian hormone receptor, type II; APC: adenomatous polyposis coli; Catnblox(ex3)Amhr2cre/+: transgenic mice thatCre knocked into the AMHR2 gene to produce constitutive activation of β-catenin; CMV: cytomegalovirus; Cre: Cre recombine; DMBA: 7,12-Dimethylbenz(a)anthracene; EMT: epithelial-mesenchymal transition; EOC: epithelial ovarian cancer; FSHR: follicle stimulating hormone receptor; GCT: Granulosa cell tumors; LH: luteinizing hormone; LSL-K-rasG12D/+: mice that have a transcriptionally silent, oncogenic allele of K-ras; MET: mesenchymal-epithelial transition; MISIRIR: Mullerian Inhibitory Substance Type II Receptor; MOSE: mouse ovarian surface epithelium; OEA: ovarian endometrioid adenocarcinomas; OSE: ovarian surface epithelium; OSP-1: ovarian-specific promoter-1; PTGT: putative tumor-transforming gene; RCAS: replication-competent avian leukosis viral-derived vectors; SV40 TAg: simian virus 40 T antigen; TVA/p53/+: transgenic mice expressing TVA receptor and are null for p53.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MYF drafted the manuscript. SSK participated in substantial contribution to conception and revising of the manuscript. All authors read and approved the final manuscript.

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References
1. National Cancer Institute: A snapshot of ovarian cancer. [http://planning.cancer.gov/disease/Ovarian-Snapshot.pdf].
2. Scully RE: Pathology of ovarian cancer precursors. J Cell Biochem 1995; 60:208-18.
3. Aunoble B, Sachsra R, Didier E, Bignon YJ: Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer (review). Int J Oncol 2000; 16:567-76.
4. Kcrap T: Oocyte destruction and ovarian tumorigenesis after direct application of a chemical carcinogen (7,8-dime-thyl-1,2-benzanthrene) to the mouse ovary. Int J Cancer 1969, 4:61-75.
5. Toth B: Susceptibility of guinea pigs to chemical carcinogens: 7,12-Dimethylbenz(a)anthracene and urethan. Cancer Res 1970, 30:2533-9.
6. Jacobs AJ, Curtis GL, Newland JR, Wilson RB, Ryan WL: Chemical induction of ovarian epithelial carcinoma in mice. Gynecol Oncol 1984, 18:177-80.
7. Nishida T, Suguina T, Kazuoka A, Ushijima K, Yakuishi J, Moriguchi J: Histologic characterization of rat ovarian carcinoma induced by intraovarian insertion of a 7,12-dimethylbenz[a]anthracene-coated suture: common epithelial tumors of the ovary in rats. Cancer 1998, 83:965-70.
8. Kato T, Yakuishi J, Tanawaki A, Ide K: [Experimental ovarian tumor]. 1. Experimental ovarian tumor produced in rats by a chemical carcinogen, 20-methylcholanthrene. Igaku Kenkyu 1973, 43:270-6.
9. Tunca J, Erturk E, Bryan GT: Chemical induction of ovarian tumors in rats. Gynecol Oncol 1985, 21:1-4-64.
10. Melnick RL, Huff JE, Roycroft JH, Choai DJ, Miller RA: Inhalation toxicity and carcinogenicity of I,3-butadiene in B6C3F1 mice following 65 weeks of exposure. Environ Health Perspect 1990, 86:27-36.
11. Bast RC Jr, Hennessy B, Mills GB: The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 2009, 9:415-28.
12. Roby KF, Taylor CC, Sweetwood JP, Cheng Y, Pace JL, Tafvifk O, Persons DL, Smith PG, Terranova PF: Development of a syngeneic mouse model for ovarian cancer. Cancer 2000, 21:585-91.
13. Godwin AK, Testa JR, Handel LM, Liu Z, Vanderveer LA, Tracey PA, Hamilton TC: Spontaneous transformation of rat ovarian surface epithelial cells: association with cytogenetic changes and implications of repeated ovulation in the etiology of ovarian cancer. J Natl Cancer Inst 1992, 84:592-601.
14. Testa JR, Getts LA, Salazar H, Liu Z, Handel LM, Godwin AK, Hamilton TC: Spontaneous transformation of rat ovarian surface epithelial cells results in well to poorly differentiated tumors with a parallel range of cytogenetic complexity. Cancer Res 1994, 54:2778-84.
15. Roberts PC, Motiff EP, Baxa AC, Heng HH, Dayon-Reale N, Greigore L, Lancaster WD, Rabah R, Schmelz EM: Sequelal tumoral and cellular events during neoplastic progression: a mouse syngeneic ovarian cancer model. Neoplasia 2005, 7:444-56.
16. Greenaway J, Moorehead R, Shaw P, Petrak J: Epithelial-stromal interaction increases cell proliferation, survival and tumorigenicity in a mouse model of human epithelial ovarian cancer. Gynecol Oncol 2008, 108:385-94.
17. Urzua U, Frankenberg C, Ganger L, Mayer S, Burket S, Munroe DJ: Microarray comparative genomic hybridization profile of a murine model for epithelial ovarian cancer reveals genomic imbalances resembling human ovarian carcinomas. Tumour Biol 2005, 26:236-44.
18. Janat-Amsbury MM, Yockman JW, Anderson ML, Kieback DG, Kim SW: Combination of local, non-viral IL12 gene therapy and systemic paclitaxel chemotherapy in a syngeneic ID8 mouse model for human ovarian cancer. Anticancer Res 2006, 26:3223-8.
19. Janat-Amsbury MM, Yockman JW, Anderson ML, Kieback DG, Kim SW: Comparison of ID8 MOSF and VEGF-modified ID8 cell lines in an immunocompetent animal model for human ovarian cancer. Anticancer Res 2006, 26:2785-9.
20. Urzua U, RobyKF, Gangi LM, Cherry JM, Powell JI, Munroe DJ: Transcriptomic analysis of an in vitro murine model of ovarian carcinoma: functional similarity to the human disease and identification of prospective tumoural markers and targets. J Cell Physiol 2006; 206:594-602.

21. Auersteg P, Wang A, Choi K, Kang S, Leung P: Ovarian surface epithelium: Biology, endocrinology, and pathology. Endocr Rev 2001; 22:224-249.

22. Orslic S, Li Y, Solow RA, Vitale-Cross LA, Gutkind JS, Varmus HE: Induction of ovarian cancer by defined multiple genetic changes in a mouse model system. Cancer Cell 2002; 1:53-62.

23. Marks F, Furstenberger G, Muller-Decker K: Tumor promotion as a target of cancer prevention. Recent Results Cancer Res 2007; 174:37-47.

24. 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 PEBP2 promoter develop epithelial ovarian cancer. Cancer Res 2003; 63:1389-97.

25. Tammela J, Odunsi K: Gene expression and prognostic significance in ovarian carcinoma. Minerva Ginecol 2004; 56:495-502.

26. Landen C Jr, Birrer M, Sood A: Early events in the pathogenesis of epithelial ovarian cancer. J Clin Oncol 2008; 26:1005-1009.

27. Hensley H, Quinn BA, Wolf RL, Litwin SL, Mabuchi S, Williams SJ, Williams C, Hamilton TC, Connolly DC: Magnetic resonance imaging for detection and determination of tumor volume in a genetically engineered mouse model of ovarian cancer. Cancer Biol Ther 2007; 6:1717-25.

28. Mabuchi S, Altmare DA, Connolly DC, Klein-Szanto A, Litwin S, Hoezle MK, Hensley HH, Hamilton TC, Testa JR: RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. Cancer Res 2007, 67:2408-13.

29. Flesken-Nikitin A, Choi KC, Eng JP, Shmidt EN, Nikitin AY: Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium. Cancer Res 2003, 63:1334-43.

30. Marino S, Voojis M, Gulden H van der, Jonkers J, Berns A: Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. Genes Dev 2000; 14:994-1004.

31. Jonkers J, Mewissen R, Gulden H van der, Petters H, Valk M van der, Berns A: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001; 29:418-25.

32. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001; 29:418-25.

33. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001; 29:418-25.

34. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001; 29:418-25.

35. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001; 29:418-25.
sion using the promoter of a retrovirus-like element. Cancer Res 2001, 61:1291-5.

57. Boerboom D, Paquet M, Hsieh M, Liu J, Jamin SP, Behringer RR, Siros J, Taketo MM, Richards JS: Misregulated Wnt/beta-catenin signaling leads to ovarian granulosa cell tumor development. Cancer Res 2005, 65:9206-15.

58. Lague MN, Paquet M, Fan HY, Kaatmen MJ, Chu S, Jamin SP, Behringer RR, Fuller Pj, Mitchell A, Dore M, Hunesault LM, Richards JS, Boerboom D: Synergistic effects of Pten loss and WNT/CTNNB1 signaling pathway activation in ovarian granulosa cell tumor development and progression. Carcinogenesis 2008, 29:2062-72.

59. Green JE, Shibata MA, Yoshidome K, Liu ML, Jorcyk C, Anver MR, Wigginton J, Wiltrout R, Shibata E, Kaczmarczyk S, Wang W, Liu ZY, Calvo A, Coulter C: The C3(1)/SV40 T-antigen transgenic mouse model of mammary cancer: ductal epithelial cell targeting with multistage progression to carcinoma. Oncogene 2000, 19:1020-7.

60. Bergsagel Dj, Finegold Mj, Butel Js, Kupsky Wj, Garcea Rl: DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N Engl J Med 1992, 326:988-93.

61. Carbone M, Pass Hl, Rizzo P, Marinetti M, Di Muzio M, Mew Dj, Levine As, Procopio A: Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994, 9:1781-90.

62. Hachana M, Trimeche M, Ziadi S, Amara K, Korbi S: Evidence for a role of the Simian Virus 40 in human breast carcinomas. Breast Cancer Res Treat 2009, 113:43-58.

63. Yamamoto H, Nakayama T, Murakami H, Hosaka T, Nakamata T, Tsuoyama T, Oka M, Nakamura T, Toguchida J: High incidence of SV40-like sequences detection in tumour and peripheral blood cells of Japanese osteosarcoma patients. Br J Cancer 2000, 82:1677-81.

64. Viletche R, Madden Cr, Kozinetz Ca, Halvorson Sj, White Zs, Jorgensen Jl, Finch Cj, Butel Js: Association between simian virus 40 and non-Hodgkin lymphoma. Lancet 2002, 359:817-23.

65. Wong Na, Rae F, Herriot Mm, Mayer Nj, Brewerst Dh, Harrison Dj: SV40 Tag DNA sequences, present in a small proportion of human hepatocellular carcinomas, are associated with reduced survival. J Clin Pathol 2003, 56:904-9.

66. Pacini F, Vivaldi A, Santoro M, Fedele M, Fusco A, Romei C, Basolo F, Pinchera A: Simian virus 40-like DNA sequences in human papillary thyroid carcinomas. Oncogene 1998, 16:665-9.

67. Kusakari T, Kariya M, Mandai M, Tsuruta Y, Hamid Aa, Fukuhara K, Nambu K, Takakura K, Fujii S: C-erbB-2 or mutant Ha-ras induced malignant transformation of immortalized human ovarian surface epithelial cells in vitro. Br J Cancer 2003, 89:2293-8.

68. Kido M, Shibuya M: Isolation and characterization of mouse ovarian surface epithelial cell lines. Pathol Res Pract 1998, 194:725-30.

69. Auersperg N, Maines-Bandiera S, Booth Jh, Lynch Ht, Godwin Ak, Hamilton Tc: Expression of two mucin antigens in cultured human ovarian surface epithelium: influence of a family history of ovarian cancer. Am J Obstet Gynecol 1995, 173:538-65.

70. Neelesh D: Ovarian cancer screening. Aust Fam Physician 2007, 36(3):126-128.