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Role of p53 and Rb in Ovarian Cancer

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Abstract

Ovarian cancer remains a major health concern worldwide, primarily in postmenopausal women. Among the most common genetic alterations in human sporadic epithelial ovarian cancer (EOC) are *p53* mutations, defective retinoblastoma (RB) pathway ($p16^{INK4a}$/RB) and activation of oncogenes such as c-*myc*, *K-ras* and *Akt*. Although these alterations are frequently associated with poor clinical prognosis, their specific contributions to EOC formation remain unclear. In order to gain a better understanding of the roles of these proteins *in vivo*, a number of mouse models have been generated, largely based upon inducing specific genetic lesions in the ovarian surface epithelium from which the majority of carcinomas are thought to arise in humans. Here, we review the role of tumor suppressor p53 and the Rb pathway in EOC with particular attention to association of p53 to high grade serous carcinomas as opposed to low grade and benign tumors. We also provide an overview of the utility and application of genetically engineered mouse models, in particular towards rational drug design and development of improved imaging techniques in ovarian cancer.

1 Introduction

Ovarian cancer is the second most common gynecological neoplasm with over 20,000 new cases and 15,000 deaths predicted in 2006 (Jemal et al., 2006). While significant decreases in mortality have been observed in cancers of the breast and cervix, mortality rates for cancer of the ovary has remained essentially constant over the past thirty years. The majority of cases present at advanced stages, at which point the disease is rarely curable by existing treatment schemes. Accordingly, the 5-year survival rate for advanced ovarian cancer is twenty-nine percent. In addition to asymptomatic development, a scarcity of accurate animal models has resulted in a marked lack of knowledge of how the disease progresses, which in turn has precluded the development of desperately needed treatment regimens and screening programs.

Ovarian cancer is a wide-ranging term that groups together a diverse set of neoplasms originating from the ovary, with carcinomas comprising ninety percent of ovarian cancers. Based upon morphological criteria, epithelial ovarian cancers (EOCs) are classified as serous, mucinous, endometrioid, clear cell, transitional cell, squamous cell, and mixed epithelial neoplasms (Scully, 1999). The ovarian surface epithelium (OSE) is a single layer of flat-to-cuboidal cells covering the ovary and is the presumed cell of origin for EOCs (Auersperg et al., 2001; Nikitin et al., 2004; Scully, 1977; Vanderhyden et al., 2003). Recent studies indicate that this layer may possess stem cell properties and both tumors and cell lines of transformed mouse OSE cells contain a side population (Szotek et al., 2006) which is considered by many investigators as an indicator of cancer stem cells in other tissues (Chiba et al., 2006; Haraguchi et al., 2006; Hirschmann-Jax et al., 2004; Kruger et al., 2006).

2 Disease Etiology

The etiology of EOC is poorly understood and although several risk factors have been identified, their direct involvement remains largely unaddressed. Of all proposed risk factors, ovulation has received the widest attention. The theory that persistent ovulation increases ovarian cancer incidence was first proposed by Fathalla in 1971 (Fathalla, 1971; Fathalla,

1972) and has been supported by numerous studies demonstrating that a reduction in ovulatory events by pregnancy and/or oral contraceptive decreases EOC risk (Riman et al., 2002; Risch et al., 1994; Risch et al., 1983; Titus-Ernstoff et al., 2001; Whittemore et al., 1992). Advocates of this so-called incessant ovulation hypothesis argue that repeated rupture of the ovarian surface during ovulation and subsequent repair by OSE proliferation may increase the frequency at which mutations arise. However, some have deemed this model too simplistic, since neither the effects of reproductive hormones nor acute inflammation is taken into account, both of which may be mutagenic (Bose, 2005; Bukulmez and Arici, 2000; Cramer and Welch, 1983; Fleming et al., 2006; Konishi, 2006; Mohle et al., 1985; Ness and Cottreau, 1999; Nikitin, 2005).

In a recent study using a serial transvaginal ultrasonography approach, approximately fifty percent of ovarian carcinomas were shown to develop from pre-existing benign-appearing cysts or endometriotic cysts, while no pre-existing lesions had been evident in the remaining cases 12 months prior to diagnosis (Horiuchi et al., 2003). Strikingly, upon histopathological analysis, the majority of tumors that arose from pre-existing lesions were mucinous, endometrioid or clear cell carcinomas with adjacent benign- or borderline-like lesions in the vicinity of the carcinoma. In stark contrast, tumors with no evidence of pre-existing lesions were mostly of a serous pathological nature. While a minority of serous carcinomas were of low grade and located adjacent to borderline-like lesions, the majority were high grade with no evidence of precursor lesions in the vicinity of the carcinoma. These observations give significant weight to the hypothesis that low grade serous carcinomas arise in a stepwise manner from benign lesions, while high grade serous carcinomas are distinct and arise *de novo* from the OSE (Shih Ie and Kurman, 2004).

3 Genetics of Ovarian Cancer

While germline mutations in *BRCA1* and *BRCA2* are the most common genetic aberrations in hereditary ovarian carcinomas, by far the most frequent alterations in sporadic EOC are in the p53 and RB pathways. Defects in these two tumor suppressor pathways are present in over eighty percent of human cancers (Hahn and Weinberg, 2002; Sherr and McCormick, 2002) and have been associated with poor prognosis in ovarian carcinomas (Bali et al., 2004; Fujita et al., 1997; Hashiguchi et al., 2001; Katsaros et al., 2004; Kusume et al., 1999; Sui et al., 2000; Tachibana et al., 2003).

3.1 Mutations in the p53 Pathway

Mutation of the *p53* gene at the locus 17p13.1 is the most common single genetic alteration in sporadic human EOC. The p53 protein contains four functional domains – a transcriptional activation domain, a tetramerization domain and two DNA binding domains. In addition to possessing transcriptional activating properties, transcriptional repression has been described, although binding sites are less well characterized (Curtin and Spinella, 2005; D'Souza et al., 2001; Hammond and Giaccia, 2005; Hoffman et al., 2002; Imbriano et al., 2005).

Either loss of wild type p53 function, gain of oncogenic function or the ability to activate p53 inappropriately severely compromises the capacity for controlled cellular proliferation and growth. Numerous stimuli have been demonstrated to activate p53, including UV irradiation-induced DNA damage, inappropriate proto-oncogene activation, mitogenic signaling and hypoxia. Depending upon the cellular context one of several responses is implemented, such as cell cycle arrest, senescence, differentiation or induction of the apoptotic cascade. Through its activity as a transcription factor, p53 executes each response by directly binding p53-binding sites in regulatory regions of target genes. Using bioinformatic approaches, over 4,000 putative target genes were identified (Wang et al., 2001). Validated target genes include the Cdk inhibitor p21, members of the pro-apoptotic family Bcl-2, the death receptor Fas and p53

repressor Hdm2 (mdm2 in mice) (el-Deiry et al., 1993; Miyashita and Reed, 1995; Oda et al., 2000; Owen-Schaub et al., 1995).

The majority of *p53* mutations are missense mutations that cause single residue changes, largely occurring in the DNA binding domain (Sigal and Rotter, 2000). Mutant p53 protein has the ability to form a tetramer with wild type p53, acting as a dominant negative to repress normal physiological processes of p53, possibly by inducing an inactive conformation of the DNA binding domain and reducing the ability to transactivate/repress target genes (Chene, 1998; Kern et al., 1992; Shaulian et al., 1992; Unger et al., 1993). Normally, p53 exists in a negative feedback loop with Hdm2 which tightly controls both p53 and Hdm2 levels in the cell. Loss of transcriptional activity, however, may result in decreased Hdm2, with the consequence of mutant p53 stabilization and therefore increased amount of non-functional/gain-of-function mutant p53 protein (Blagosklonny, 2000).

Although *p53* mutations have been detected in all histological types of EOC, a number of studies have demonstrated higher frequencies of such mutations in serous carcinomas (Table 1).

Furthermore, a number of studies that have paid particular attention to histological criteria of malignancy of serous tumors have found that *p53* mutations are strongly associated with high grade serous carcinomas, but are rare in low grade or borderline serous carcinomas (Kupryjanczyk et al., 1995; Kupryjanczyk et al., 1993; Skomedal et al., 1997; Zheng et al., 1995). In contrast, borderline/low grade tumors frequently harbor mutations in *K-ras*, which are very rare events in high grade serous adenocarcinomas (Cuatrecasas et al., 1997; Diebold et al., 2003; Singer et al., 2002; Singer et al., 2003a; Singer et al., 2003b; Zheng et al., 1995). These observations have given strong support to the hypothesis that high grade and low grade serous carcinomas arise via discrete pathways (Shih Ie and Kurman, 2004). Lending further support to this hypothesis is the observation that *p53* is mutated in early stage high grade carcinomas as well as adjacent dysplastic epithelium in prophylactically removed ovaries from *BRCA1* heterozygotes (Pothuir, 2001; Werness et al., 2000). This supports a model in which *p53* mutation is not only required for carcinogenesis, but also is an early event in the pathogenesis of high grade serous carcinoma.

Of interest are the interactions between p53 and BRCA1 in ovarian carcinogenesis. *Brca1*$^{-/-}$ mouse embryos are embryonic lethal at embryonic (e) day 6.5 yet if embryos are compound null mutants for both *Brca1* and *p53*, lethality is delayed, leading to a “death by checkpoint” hypothesis (Scully and Livingston, 2000). This stipulates that in order for accelerated tumor development, p53 function must be lost so that genome instability is tolerated. In one epidemiological study (Villeneuve et al., 1999), no instance of *p53* loss was observed without simultaneous loss of *BRCA1*. To test this model in a more defined setting, Xing and Orsulic (Xing and Orsulic, 2006) generated a mouse model in which to study p53 and Brca1 interaction further. They observed that inactivation of *Brca1* and *p53* in mouse OSE cells of ovary explants did not lead to transformation unless the *Myc* oncogene is over expressed virally, while Clark-Knowles *et al.* reported increased proliferation in mouse OSE cells deficient for *Brca1* and *p53* but no increase if *Brca1* or *p53* was inactivated independently (Clark-Knowles et al., 2006). Both of these studies are in good agreement with the observation that transformation of *p53* deficient mouse OSE cells requires multiple hits for transformation to occur (Orsulic et al., 2002).

3.2 Mutations in the RB Pathway

The *Retinoblastoma 1* (*RB*) gene was originally identified as a tumor suppressor gene in hereditary and sporadic retinoblastoma in children (Friend et al., 1986; Fung et al., 1987;

Knudson, 1971; Lee et al., 1987; Weissman et al., 1987). Mutations in either RB or its pathways are also common in neoplasms of adults (Sherr and McCormick, 2002).

RB is the founding member of a three-member family of tumor suppressors which also contains p107 and p130. All three interact with a large number of proteins yet their direct binding to the E2F family of transcription factors is fundamental to their roles as tumor suppressors (Sherr and McCormick, 2002). RB is only able to interact with E2F when hypophosphorylated. When RB is phosphorylated by cyclin D-dependant kinases, E2Fs are no longer bound and are free to bind regulatory regions of E2F-responsive genes leading to progression into S phase of the cell cycle. In addition to cell cycle effects through E2F, RB also has wide-ranging and frequently poorly understood functions in several cellular processes, including control of cell death and differentiation and histone modification. For example, RB plays a role in the transition of proliferating myoblasts to differentiating myocytes (Huh et al., 2004) and differentiation of fetal liver macrophages by opposing inhibitory functions of Id2 on transcription factor PU.1 (Iavarone et al., 2004). Furthermore, inactivation of *Rb* results in p53-independent apoptotic death in the developing nervous system of the mouse (Macleod et al., 1996). Rb is involved in epigenetic modifications (Brehm et al., 1998; Luo et al., 1998; Magnaghi-Jaulin et al., 1998) and most recently *Caenorhabditis elegans* homologs of the RB pathway have been implicated in repressing the RNA interference pathway (Wang et al., 2005).

Although loss of heterozygosity (LOH) of *RB* is well demonstrated in many somatic cancers, a specific role of RB in ovarian cancer has been difficult to determine given conflicting data. Liu et al. observed inactivation of *RB* in sixty percent of ovarian cancer samples (Liu et al., 1994), while a study by Gras et al. reported LOH of the *RB* locus in seventeen percent of EOC samples and thirty percent of tumors with serous differentiation (Gras et al., 2001). However, due to a limited number of samples, statistical significant was not attained in the later study. In contrast, independent studies by Dodson et al. and Kim et al. show RB immunohistochemistry staining in over ninety percent of clinical EOC samples that showed LOH at the *RB* locus, suggesting the presence of a second tumor suppressor at this locus (Dodson et al., 1994; Kim et al., 1994). Unfortunately, no corroborating experiments such as Western blots or RT-PCR assays were performed to confirm immunohistochemical results at that time.

While the frequency of *RB* mutation in EOC is of debate, more concrete evidence exists demonstrating that the RB pathway is frequently altered. Mutations in either INK4 protein $p16^{INK4a}$ (*p16*), *RB* or *cyclin D1*/*Cdk4* are observed in almost fifty percent of EOC clinical samples in a very thorough piece of work (Hashiguchi et al., 2001; Kusume et al., 1999). In order to control Cdk-mediated inhibitory phosphorylation of RB, tumor suppressor p16 specifically antagonizes cyclin D dependent kinases leading to continued RB-E2F binding and repressing activation of the E2F transcriptional program. Specifically analyzing *p16* expression and alteration, numerous studies reported that alteration in *p16* via either mutation, LOH or promoter methylation occur in between thirty and sixty five percent of EOCs, although a far lower percentage has also been reported (Table 2).

Of great interest is the observation that over fifty percent of EOC patients have mutations in both the p53 and RB pathways, including forty percent of serous carcinomas (Hashiguchi et al., 2001). It is well known that extensive interaction exists between these two pathways (Sherr and McCormick, 2002). The *INK4a* locus encodes two proteins through use of an alternative reading frame: $p16^{INK4a}$ and a second tumor suppressor involved in activating p53, $p14^{ARF}$ ($p19^{Arf}$ in mice). p14 represses Hdm2, modulating the p53-Hdm2 negative feedback pathway. In *p14*-null cell lines, E2F over expression enforces S phase entry (Qin et al., 1994), while deregulated E2F induces p14 expression. Together, these data provide several possibilities for

p53-RB pathway interaction and indicate the significance of concomitant deregulation in both pathways.

3.3 Mouse Models to Analyze p53 and RB Function in EOC

Given the aforementioned data from clinical samples, several groups have attempted to model the roles of p53 and RB using the mouse as a model system. The first approach taken was to direct expression of the transforming region of SV40 large T antigen (SV40 Tag) in the mouse OSE by using the *Mullerian inhibitory substance type II receptor* (*MISIIR*) promoter. SV40 Tag binds and inactivates both p53 and Rb proteins. Necropsy of *MISIIR-SV40-Tag* transgenic mice revealed bilateral ovarian masses in 50% of cases and bloody ascites were frequently present in the abdominal cavity (Connolly et al., 2003). Pathological analysis classified the tumors as poorly differentiated carcinomas.

However, while clearly an important breakthrough in EOC modeling, this approach has several shortcomings. Firstly, while the *MISIIR* promoter directs expression to the OSE, neoplastic lesions were also observed at other sites demonstrating a degree of promote leakiness. Secondly, expression of MISIIR is also evident during early embryonic development; tumors therefore arise during early adult life, which is unlike that observed in humans. Thirdly, and more importantly, through alternative splicing, *SV40* early region encodes several viral proteins including small t and 17kT antigens in addition to large T. All three proteins directly bind Hsc70 through a J domain at the N terminus, while large T and 19kT share a LXCXE binding motif allowing inactivation of all known members of the RB family. RB family members *p107* and *p130* are rarely mutated in human neoplasms (Weinberg, 1991). Furthermore, small t antigen has been implicated in cell transformation (Hahn et al., 2002).

In order to test that p53 and Rb are directly involved in epithelial ovarian carcinogenesis, we established a more defined and controlled approach to inactivate *p53* and/or *Rb* in the mouse OSE through Cre-*loxP* technology (Flesken-Nikitin et al., 2003). By taking advantage of the enclosed anatomical location of the mouse ovary within the ovarian bursa, selective exposure of OSE to any agent can be achieved. In order to inactivate *p53* and/or *Rb*, adenovirus expressing Cre recombinase under control of the *immediate early cytomegalovirus* promoter (Ad*Cre*) is injected through the oviductal infundibulum into the bursa of transgenic mice carrying conditional alleles of each gene. While $Rb^{loxP/loxP}$ mice do not have any ovarian tumors and only six percent of $p53^{loxP/loxP}$ mice develop neoplasia, ninety seven percent of $p53^{loxP/loxP}Rb^{loxP/loxP}$ mice develop ovarian tumor after single exposure to Ad*Cre*. Following a similar clinical course to that seen in humans, tumors spread intraperitoneally (27%), form hemorrhagic or serous ascites (24%) and frequently metastasize to the contralateral ovary (15%), lung (18%) and liver (6%). Pathological evaluation of the early stages of carcinogenesis combined with cytokeratin 8 (CK8) immunostaining demonstrated an epithelial origin of induced neoplasms in eighty four percent of cases. Consistent with a proposed role of p53 in the initiation of high grade serous adenocarcinomas, induced tumors were most comparable to this subset of human EOC tumors.

This approach has several advantages over other methods to model EOC in the mouse. Firstly, intrabursal administration of Ad*Cre* removes the requirement for an OSE-specific promoter, of which none are currently known. While OSE-specific infection was performed previously (Orsulic et al., 2002), our approach involves no cell culture stage and all tumor development is accomplished in adult immunocompetent mice. The approach also allows conditional and temporal control of the initiating events, which is particularly useful for modeling the early stages of EOC initiation. As such, an identical approach was recently used to demonstrate the role of *K-ras* and *Pten* in the initiation of endometrioid ovarian cancer (Dinulescu et al., 2005), and *Brca1* in preneoplastic changes (Clark-Knowles et al., 2006). Taken together, these

results clearly demonstrate that different genetic alterations lead to distinct subsets and stages of EOC.

4 Applications of Genetically Defined Models

Although the primary goal of generating genetically engineered mouse models is to attain a better understanding of the molecular pathways behind EOC carcinogenesis, other significant goals are to allow rational drug design and testing in a defined and reproducible environment and to allow development of improved imaging techniques. In this section, we describe recent novel applications of mouse models of EOC.

4.1 Rational Drug Design

Treatment options for patients with advanced stages of ovarian cancer are almost non-existent and severely limited in efficacy. Due to the high percentage of patients succumbing to the disease, ovarian cancer is a good candidate for chemoprevention.

A large body of work, largely in colorectal cancer studies, has indicated that non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin or sulindac, reduce the number and size of colonic polyps in patients with familial adenomatous polyposis (FAP) (Giardiello et al., 1993; Labayle et al., 1991; Nugent et al., 1993). Chronic administration of aspirin over a ten to fifteen year period has been reported to reduce risk of developing colon cancer by up to 50% (Thun et al., 1991), indicating a protective effect of NSAIDs. Nobel Prize winner John Vane proposed that the effects of NSAIDs is mediated by inhibiting the enzymatic activity of cyclooxygenase (COX) (Vane, 1971). COX is responsible for catalyzing arachadonic acid into PGG_2. PGG_2 is then converted into PGH_2, which is subsequently converted into one of many prostaglandins: hormone-like, lipid soluble molecules involved in a wide range of physiological processes, including platelet aggregation, muscular contraction/relaxation and immunity. Two isoforms of COX protein exist – COX-1 and COX-2, the later having received the most attention since COX-1 appears to be constitutively expressed while COX-2 is not normally expressed unless induced by pro-inflammatory cytokines.

Work in $Apc^{\Delta 716}$ mice, which spontaneously develop numerous polyps in the intestinal tract similar to FAP in humans, confirmed a link between NSAIDs and COX-2. $Apc^{\Delta 716}$ mice on a *Cox-2*-null background develop significantly fewer polyps compared to a *Cox-2*-wild type background. Treatment of $Apc^{\Delta 716}$ mice on a *Cox-2*-wild type background with either sulindac or a novel Cox-2 inhibitor MF-tricyclic similarly reduced polyp number (Oshima et al., 1996). Since this initial report, there has been much interest in developing COX-2 isoform-specific inhibitors over NSAIDs due to fewer adverse effects. In contrast, COX-1 has received little attention, despite having been purified and cloned prior to COX-2.

While NSAIDs appear to reduce risk of cancers at sites such as esophagus and stomach (Farrow et al., 1998), their effect in cancers of the ovary remain inconclusive. Although some groups have reported high levels of COX-2 in ovarian cancer (Klimp et al., 2001; Matsumoto et al., 2001), others have reported elevated COX-1, but not COX-2, in ovarian cancer tissue samples (Dore et al., 1998; Gupta et al., 2003) or cell lines (Kino et al., 2005; Yang et al., 2005), suggesting tissue-specific roles for each isoform. Furthermore, Cox-1 over expression was previously demonstrated in tumors arising from *p53*-null mouse OSE cells also over expressing either *c-myc* and *K-ras* or *c-myc* and *Akt* (Daikoku et al., 2005), while Cox-2 was either not expressed or expressed at very low levels. Therefore, in a large collaborative effort, Daikoku and co-workers investigated Cox-1/2 expression status in a defined and controlled manner using three genetically engineered mouse models to gain a better understanding of the roles of this class of protein in EOC and whether Cox over expression is unique to specific genetic alterations or is widespread (Daikoku et al., 2006). The previously characterized models used

were based upon intrabursal Ad*Cre* administration to inactivate *p53* and *Rb* (Flesken-Nikitin et al., 2003), or inactivate *Pten* and activate *K-ras* (Dinulescu et al., 2005) or based upon *MISIIR*-directed expression of *SV40 Tag* (Connolly et al., 2003), as outlined above. In all three models Cox-1, but not Cox-2, was over expressed in the mouse EOCs as judged by RT-PCR, *in situ* hybridization, Western blotting and immunohistochemistry with Cox-1/2 isoform-specific primers, probes and antibodies. The observation that Cox-1 is over expressed in an identical pattern in four different mouse models based upon different genetic lesions suggests that Cox-1 over expression may be widespread and a conserved aspect of EOC.

The investigation by Daikoku and colleagues has opened a new avenue for the rational design of preventive and therapeutic agents against ovarian cancer and may lead to a fundamental shift in approach towards COX inhibitors. Perhaps most significantly, in a microarray study comparing global gene expression between $p53^{loxP/loxP}Rb^{loxP/loxP}$ OSE cells treated with either Ad*Cre* or control virus in culture, Cox-1 over expression was detected at the earliest passages (Daikoku et al., 2006), indicating the potential usefulness of Cox-1 as a screening marker.

4.2 Development of New Imaging Techniques

While identification of screening markers associated with EOC are undoubtedly of critical importance to allow early and accurate diagnosis, it is extremely difficult to find markers that are flawless, since both a high degree of specificity and sensitivity is essential. The most widely used biomarker for ovarian tumors is the serum tumor marker CA125 (Verheijen et al., 1999). Unfortunately, while 80% of patients with advanced EOC have high CA125 serum levels, only half of them are positive at the early stage of disease (Nagele et al., 1995; Zurawski et al., 1988), whereas conversely, CA125 concentration may be elevated in individuals free of disease, resulting in false positive tests. For this reason, CA125 has limited diagnostic value and positive results must be substantiated by exploratory surgery or laparoscopy, which, like all surgical procedures, carries a certain degree of risk. Consequently, adequate monitoring of patients, especially those at elevated risk of developing EOC, such as women carrying germline mutations in *BRCA* genes, is difficult and prophylactic oophorectomy is recommended, which is not a viable option for nulliparous women who wish to raise a family. For this reason, minimally invasive imaging techniques need to be developed to allow improved patient monitoring.

Multiphoton microscopy (MPM, (Denk et al., 1990)) offers one possible means to improve diagnostic imaging. Two-photon MPM is based upon the theory that two low-energy infrared photons may arrive simultaneously at a fluorophore and result in electronic transition normally observed upon absorption of a single photon. Several endogenous molecules, such as NAD(P)H and flavins, emit photons upon two-photon excitation, while fluorescent proteins such as green fluorescent protein is also detectable via MPM. In addition, second harmonic generation (SHG) allows direct imaging of anisotropic biological molecules such as collagen (Williams et al., 2001) with no requirement for exogenously added fluorophores and may be imaged at the same time as two-photon microscopy. MPM has several advantages over traditional fluorescence imaging due to its low phototoxicity and lack of out-of-focal plane excitation (Williams et al., 2001). Together with our collaborators Drs Warren Zipfel, Rebecca Williams and Watt Webb, we have demonstrated the utility of two-photon microscopy to image deep into the mouse ovary (Zipfel et al., 2003). In contrast to transvaginal ultrasonography and traditional laparoscopy which provide either low resolution images or images only of the ovary surface, respectively, MPM is able to image at high resolution (cellular level) deep (~200-300μm) into the ovary, allowing one to rapidly acquire images of quality comparable to that of traditional hematoxylin and eosin-stained histological sections.

MPM has been used to help answer diverse biological questions such as how gene expression correlates with metastasis, whether senile plaques change size in a mouse model of Alzheimer's

disease and the role of sensory deprivation in cortical plasticity (Brown et al., 2001; Christie et al., 2001; Lendvai et al., 2000; Wang et al., 2002). In addition to low phototoxicity, MPM can allow analysis of individual cell migration and motility in a time-lapse manner (Flesken-Nikitin et al., 2005), while long-term, repeated imaging procedures may be carried out by performing MPM during several rounds of survival surgery (Christie et al., 2001), allowing one to closely follow development of EOC from the very earliest stages of carcinogenesis. The construction of an endoscopic MPM device should facilitate translation of this imaging method into clinical practice. Such a device is currently under development.

5 Concluding Remarks

Due to asymptomatic development, the initiating events of ovarian cancer remain obscure and much of our current understanding is based upon circumstantial and correlative evidences. To this end, the development of accurate mouse models of ovarian cancer is of utmost importance in expanding our knowledge of ovarian carcinogenesis. Based on the observations that *p53* and *Rb* pathways are commonly altered in human EOC we have inactivated both tumor suppressors in the mouse OSE and demonstrated formation of neoplasms that are most comparable to human high grade serous carcinomas of the ovary. Importantly, the approach for conditional induction of OSE-specific genetic alterations described in our work is well applicable to other studies seeking to test roles of specific genetic alterations in the OSE in a time-, location- and lineage-dependant manner. This study, and others in the field, has given significant weight to the hypothesis that *p53* and *Rb* mutations play critical roles in ovarian carcinogenesis, in particular at the very earliest stages. We have gone on to demonstrate the usefulness of genetically engineered mouse models in identifying proteins for therapeutic targeting and development of improved imaging techniques and it is our hope that these approaches will lead to a more complete picture of ovarian carcinogenesis, as well as facilitate its detection, treatment and prevention.

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Table 1

Frequency of *p53* mutations in histological subtypes of epithelial ovarian carcinoma (EOC)

Type of EOC (average %)		Defective/Total cases (%)	Reference
Serous	Low grade (16%)	4/22 (18)	(O'Neill et al., 2005)
		1/12 (8)	(Singer et al., 2005)
		33/190 (17)	(Lassus et al., 2003)
		5/27 (19)	(Chan et al., 2000)
	High grade (66%)	30/47 (64)	(O'Neill et al., 2005)
		30/59 (51)	(Singer et al., 2005)
		167/180 (93)	(Lassus et al., 2003)
		25/46 (54)	(Chan et al., 2000)
	Grade not determined (64%)	33/46 (72)	(Gadducci et al., 2006)
		47/71 (66)	(Havrilesky et al., 2003)
		16/26 (62)	(Caduff et al., 1999)
		14/31 (45)	(Fujita et al., 1994)
		11/20 (55)	(Henriksen et al., 1994)
		18/23 (78)	(Renninson et al., 1994)
		31/42 (74)	(Dogan et al., 2005)
		73/126 (58)	(Eltabbakh et al., 1997)
Clear cell (8%)		6/38 (17)	(Ho et al., 2001)
		0/4 (0)	(Otis et al., 2000)
		1/12 (8)	(Caduff et al., 1999)
Endometrioid (45%)		5/15 (33)	(Dogan et al., 2005)
		7/13 (54)	(Henriksen et al., 1994)
		13/27 (48)	(Caduff et al., 1999)
Mucinous (19%)		1/12 (8)	(Dogan et al., 2005)
		3/12 (25)	(Renninson et al., 1994)
		3/11 (27)	(Henriksen et al., 1994)
		3/21 (14)	(Caduff et al., 1999)

Table 2

Defects of p16 and Rb in human ovarian carcinomas*

Gene	Defect (average %)	Defective/Total cases (%)	Reference
p16	Homozygous mutation (7%)	2/7 (29)	(Kamb et al., 1994)
		1/50 (2)	(Brown et al., 2001)
		2/27 (7)	(Wong et al., 1997)
		2/70 (3)	(Fujita et al., 1997)
		2/88 (2)	(Shih Ie and Kurman, 2004)
		5/30 (17)	(Kanuma et al., 1997)
		0/22 (0)	(Shigemasa et al., 1997)
		1/94 (1)	(Milde-Langosch et al., 1998)
		0/23 (0)	(Niederacher et al., 1999)
		0/49 (0)	(Havrilesky et al., 2001)
		1/35 (3)	(Saegusa et al., 2001)
		8/45 (18)	(Kudoh et al., 2002)
	Methylation (15%)	8/43 (17)	(Fujita et al., 1997)
		16/44 (36)	(Milde-Langosch et al., 1998)
		0/23 (0)	(Ryan et al., 1998)
		6/23 (26)	(Niederacher et al., 1999)
		2/49 (4)	(Wong et al., 1999)
		2/37 (5)	(McCluskey et al., 1999)
		0/35 (0)	(Saegusa et al., 2001)
		6/46 (13)	(Hashiguchi et al., 2001)
		100/249 (40)	(Katsaros et al., 2004)
		5/50 (10)	(Ibanez de Caceres et al., 2004)
	Loss of expression (37%)	22/60 (37)	(Fujita et al., 1997)
		19/94 (20)	(Milde-Langosch et al., 1998)
		6/22 (27)	(Niederacher et al., 1999)
		20/59 (34)	(Kusume et al., 1999)
		22/29 (76)	(McCluskey et al., 1999)
		28/47 (60)	(Sui et al., 2000)
		10/46 (22)	(Hashiguchi et al., 2001)
		70/117 (60)	(Saegusa et al., 2001)
		28/82 (34)	(Havrilesky et al., 2001)
		9/73 (12)	(Tachibana et al., 2003)
		23/107 (21)	(Hashiguchi et al., 2001)
		60/134 (45)	(Bali et al., 2004)
RB	Homozygous mutation (9%)	1/24 (4)	(Sasano et al., 1990)
		2/15 (13)	(Liu et al., 1994)
	Loss of or aberrant expression (19%)	2/25 (8)	(Dodson et al., 1994)
		2/26 (8)	(Kim et al., 1994)
		3/22 (14)	(Taylor et al., 1995)
		7/34 (21)	(Niemann et al., 1998)
		2/59 (3)	(Kusume et al., 1999)
		5/46 (11)	(Hashiguchi et al., 2001)
		7/9 (78)	(Gras et al., 2001)
		10/84 (12)	(Havrilesky et al., 2001)
		1/78 (1)	(Konstantinidou et al., 2003)
		28/134 (21)	(Bali et al., 2004)
		12/107 (37)	(Hashiguchi et al., 2001)

* Only experiments on freshly collected surgical material are included.