

Development and Analysis of Mouse Models of Human Epithelial Ovarian Cancer

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The focus of my research program is the development and analysis of genetically engineered mouse (GEM) models of epithelial ovarian cancer (EOC). The primary goals of GEM model development are to create models that will enable us to study early disease development and define cellular origins of ovarian carcinoma, identify additional genetic alterations that occur during the course of ovarian carcinoma progression, and establish useful preclinical models of EOC for future translational research. Until recently, suitable gene promoters to target transgene expression to the mouse ovarian epithelium were unavailable. We developed the first transgenic mouse model of EOC in which the potently oncogenic early region of the Simian Virus 40 (SV40) genome is expressed under the transcriptional control of a portion of the 5' upstream regulatory region of the murine Müllerian inhibiting substance type II receptor (MISIIR) gene. We have established a stable line of TgMISIIR-TAg transgenic mice that develop spontaneous progressive ovarian carcinoma with complete penetrance. Disease development in mice is similar to humans in that mice with ovarian tumors present with few or no symptoms until animals have large bilateral ovarian tumors accompanied by the presence of malignant ascites and invasion of omentum and other intraperitoneal organs. Histopathological evaluation of tumors confirms the presence of poorly differentiated adenocarcinomas that closely resemble human serous ovarian cancers. Several projects in the laboratory employ the TgMISIIR-TAg model of ovarian cancer to investigate early disease development and identification of precursor lesions of EOC, the potential utility of these mice as a preclinical model for evaluation of therapeutic and prevention strategies, and the role of hormones and reproduction in the development of EOC.



A major goal of our laboratory is to develop additional GEM models of EOC by modulating genes known to be involved on the development of hereditary and sporadic EOC. In addition, as Co-Director of the Ovarian Cancer SPORE Core D: Mouse Engineering Core, we work with other SPORE investigators at Fox Chase and at other SPORE funded institutions to develop genetically relevant GEM models of human ovarian cancer.

Analysis of early disease in EOC prone TgMISIIR-TAg transgenic mice. Connolly, in collaboration with Hedrick-Ellenson^a

Although ovarian cancer can arise from any cell type found in the ovary (including oocytes, granulosa cells and theca-interstitial cells), almost 90% of cancers arise from epithelial components of the ovary such as the ovarian surface epithelium (OSE), ovarian inclusion cysts or components of the secondary Müllerian system (including the epithelial cells of the rete

ovarii, paraovarian/paratubal cysts, endosalpingiosis, endometriosis or endomucinoses). The origin of EOC remains somewhat controversial, therefore, the ovarian cancer prone TgMISIIR-TAg mice is a useful model system in which to investigate these possibilities.

We have begun histopathological evaluation of the process of early ovarian cancer development in TgMISIIR-TAg mice by obtaining serial sections through the entire ovaries removed from two, three and four week-old female mice.

In young mice, the ovaries appear nearly normal with abundant primary follicles and a small percentage of cells that stained positively for TAG. Among the cells expressing the TAG protein in the ovary, we found occasional TAG staining on the ovarian surface epithelium, small clusters of cells just below the surface and in glandular structures in or adjacent to the pedicle of the ovary. As noted above, it is widely held that ovarian cancer arises from the ovarian surface epithelium, often after it invades the ovarian cortex. Indeed, studies from T. Hamilton's laboratory at Fox Chase and recent studies by other investigators have supported this view. However, in looking at the TAG positive cells in the context of the whole ovary, we see a pattern that does not exclude the possibility that many of the TAG antigen positive tumor cells may have originated in the hilus of the ovary, perhaps from the rete ovarii. Thorough examination of numerous ovaries from mice in addition to the evaluation of markers of epithelial cell differentiation such as cytokeratins 8 and 19, as well as proliferation markers such as proliferating cellular nuclear antigen (PCNA), is ongoing in order to unravel the disease process at the histological level. Preliminary observations reinforce the need to carefully and systematically evaluate the whole ovaries in order to better understand the initiation and origin of EOC.

TgMISIIR-TAg mice as a preclinical model of human EOC. Williams, Quinn, Connolly, in collaboration with Hensley,[§] Wolf,[¶] Williams,[§] Hamilton[§]

In collaboration with the Small Animal Imaging Facility at Fox Chase, we developed strategies to accurately and noninvasively measure tumor burden and growth rate *in vivo* using Magnetic Resonance Imaging (MRI). The ability to monitor and calculate tumor growth rates and volume *in vivo* over the course of the treatment period will allow the use of fewer subjects and more precise determinations of treatment agent efficacy. Preliminary MRI and volumetric analyses have proven to be a safe and reliable method for accurate *in vivo* determination of tumor burden and tumor growth rate. To establish protocols for the use of TgMISIIR-TAg mice as preclinical models for the evaluation of novel therapeutic agents, we initiated studies to investigate the effects of treatment of TgMISIIR-TAg mice with cisplatin and paclitaxel based on

our prediction that they would result in a measurable increase in the survival of the ovarian cancer prone mice. Preliminary results suggested a measurable initial decrease in ovarian tumor size and increase in survival for the treated mice as compared to untreated mice. Future studies are aimed at increasing study group sizes and the determination of statistical requirements to accurately assess therapeutic effects. The long-term goal of these preliminary studies is to establish methods and criteria for the rational evaluation of novel therapeutic and prevention agents using this preclinical model of EOC.

The role of reproductive hormones in the development of EOC. Brake, Connolly, in collaboration with Hua,[§] Hamilton,[§] Ariazi,[§] Jordan[§]

Gonadotropic and steroid hormones of the hypothalamic-pituitary-ovarian axis have been implicated in ovarian tumorigenesis, including gonadotropin-releasing hormone (GnRH), FSH, LH/human chorionic gonadotropin (hCG), estrogen, progesterone and androgen. Gonadotropins and/or steroid hormones may increase the risk of ovarian cancer by stimulating the growth of ovarian epithelial cells. Previous studies have shown that normal human OSE cells and ovarian tumors express receptors for both gonadotropins and steroids and that both classes of hormones have mitogenic potential.

We are interested in understanding the role of estrogen in ovarian tumor initiation and progression in TgMISIIR-TAg mice. Our results indicate that tumors from TgMISIIR-TAg mice express estrogen receptors, suggesting that they may respond to estrogen. The availability of our TgMISIIR-TAg mice allows us to revisit the question of what role estrogen plays in ovarian carcinoma in a controlled animal model system. Specifically, we are investigating the growth effects of estrogen *in vitro* in mouse ovarian carcinoma (MOVCAR) cell lines and *in vivo* in MOVCAR tumor xenografts and MISIIR-TAg mice in order to determine if estrogen is sufficient to accelerate the growth and/or progression of ovarian cancer. Conversely, we are testing the efficacy of two anti-estrogenic compounds, tamoxifen and Faslodex™ (fulvestrant), to determine if inhibition of estrogen activity can slow the growth of ovarian cancer cells either in cell culture or *in vivo* in tumor xenografts and TgMISIIR-TAg mice. Our initial

results suggest that fulvestrant does have a growth inhibitory effect on cells MOVCAR cells in culture. Results of these experiments may have significant clinical implications.

Modeling inherited ovarian cancer in mice.

Brake, Williams, Baxter-Jones, Quinn, Connolly, in collaboration with Hua,[§] Nikitin,^c Lozano^d

About one out of every ten cases of epithelial ovarian cancer is inherited. Unlike non-hereditary (sporadic) ovarian cancer, the underlying genetic causes of hereditary ovarian cancer are well understood. The majority of cases are the result of inherited mutations in the breast cancer associated gene 1 (*BRCA1*). In addition to mutations of *BRCA1*, mutations of *p53* are often found in patients with breast and ovarian cancer syndrome. Based on the importance of both of these genes in the development of inherited ovarian cancer, we hypothesize that inactivation of *BRCA1* and *p53* in the ovaries of mice will result in epithelial ovarian cancer in the animals.

We have an ongoing project to: 1) develop models of inherited human EOC by inactivation of *BRCA1* and *p53* singly or at the same time in the mouse ovarian surface epithelial cells; 2) investigate whether there is a difference between the complete absence of *p53* or the presence of a dominantly acting *p53* mutant in ovarian tumorigenesis in mice; and 3) identify genes and cellular pathways, downstream of *BRCA1* and *p53* inactivation/mutation, that contribute to ovarian carcinogenesis.

To this end, we obtained *BRCA1^{loxP/loxP}* and *p53^{loxP/loxP}* mice from the National Cancer Institutes (NCI) Mouse Models of Human Cancer Consortium (MMHCC) Mouse Repository. Additionally, *p53^{R172H}* mice expressing a dominant negative mutant form of *p53* that corresponds to the human arginine→histidine hotspot mutation at codon 172 were obtained from G. Lozano's laboratory. These strains have been crossed to establish colonies of *BRCA1^{loxP/loxP}*, *p53^{loxP/loxP}*, *BRCA1^{loxP/loxP}/p53^{loxP/loxP}*, *BRCA1^{loxP/loxP}/p53^{R172H/WT}* and *BRCA1^{loxP/loxP}/p53^{R172H/oxP}* mice. One approach for restricted excision of floxed sequences in the ovarian surface epithelium is intrabursal injection of Adenovirus-Cre (Ad-Cre). As this has been shown by others to be an effective approach, we have performed intrabursal injections of 35–40 mice from each group (*BRCA1^{loxP/loxP}*,

p53^{loxP/loxP}, *BRCA1^{loxP/loxP}/p53^{loxP/loxP}*, *BRCA1^{loxP/loxP}/p53^{R172H/WT}* and *BRCA1^{loxP/loxP}/p53^{R172H/oxP}*) with Ad-Cre. We anticipate the development of ovarian tumors in mice with inactivated *BRCA1* and *p53*. *In vivo* monitoring by MRI has shown ovarian enlargement in a *BRCA1^{loxP/loxP}/p53^{loxP/loxP}* mouse six months after administration of Ad-Cre.

The role of *STAT3* and *p53* in EOC. Williams, Baxter-Jones, Quinn, Brake, Connolly, in collaboration with Jove,^e Nikitin^c

Recent studies have demonstrated that the *STAT3* gene is frequently activated in ovarian cancer cell lines and EOC tumor specimens. Additionally, the most common genetic alteration identified in EOC is mutation or loss of the *p53* gene. Interestingly, in addition to activation of *STAT3*, *p53* mutation is also frequently identified in many human cancer cell lines. Based on these observations, we are investigating whether *STAT3* activation alone or in combination with *p53* gene inactivation plays a direct role in the development of EOC in mice. Moreover, recent studies investigating the role of *STAT* signaling in *Drosophila* ovary indicate that activation of the *JAK/STAT* pathway results in a significant increase in the number of ovarian epithelial cells that become migratory and invasive. These findings are intriguing in light of the hypothesis that incessant ovulation and the resultant postovulatory wound repair of the ovarian surface epithelium (OSE) are key risk factors for human EOC.

To determine if activation of *STAT3* is involved in ovarian cancer development, we developed transgenic mice that express a constitutively activated *STAT3* gene under transcriptional control of the 5' regulatory region of the *MISIIR* gene promoter. Offspring of *TgMISIIR-STAT3C* mice and *TgMISIIR-STAT3C;p53^{+/-}* mice are currently under evaluation for alterations of ovarian structure and histology. Preliminary results indicate regions of hyperplasia in the Fallopian tube and irregular glandular morphology in the uterus of female *TgMISIIR-STAT3C;p53^{+/-}* mice. However, the more common tumors that occur in *p53^{+/-}* mice appear to outpace a reproductive tract phenotype. In order to achieve tissue-specific inactivation of *p53* in the ovary, *TgMISIIR-STAT3C* mice are being crossed with conditional *p53^{loxP/loxP}* mice. Inactivation of the *p53* gene

will be accomplished by localized administration and infection with Ad-Cre. We hypothesize that expression of activated *STAT3* and inactivation of *p53* in the mouse ovary will be sufficient to induce tumorigenesis and, based on previous studies in the fly, that these tumors will have an invasive phenotype. Future studies are aimed at

phenotypic analyses, identification of specific downstream mediators of the *STAT3* activation that are involved in the tumorigenic phenotype, identification of additional genetic and gene expression alterations that contribute to the malignant phenotype, and evaluation of *STAT3* or pathway-targeted therapeutic strategies.

Publications

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Sandy's Dream, the country's first known mural designed to promote cancer awareness, was painted on the wall of Propper Brothers Furniture in Manayunk by local artist Ann Northrup. The mural was funded by the Sandy Rollman Ovarian Cancer Foundation.