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Award Number: W81XWH-08-1-0181

TITLE: Development of a Conditional Transgenic Mouse Model to Test the Fallopian Tube Origin of Serous Ovarian Cancer

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REPORT DATE: March 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 March 2009	Annual	1 Mar 2008 – 28 Feb 2009

4. TITLE AND SUBTITLE

Development of a Conditional Transgenic Mouse Model to Test the Fallopian Tube Origin of Serous Ovarian Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0181

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Western Ontario
Ontario, Canada, N6A 5B8

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of this research study is to generate transgenic mice to specifically address fallopian tube tumorigenesis and test the potential for tubal origin of high-grade serous ovarian cancer observed in patient samples. To this end, we have generated two independent lines of transgenic reporter gene mice using the mouse oviduct-specific glycoprotein-1 (Ovgp1) promoter driving expression of the bacterial beta-galactosidase (lacZ) gene. Transgene expression is readily observed in the oviduct of female mice and is concomitant with the onset of puberty. Expression is specifically localized to the oviductal epithelium lining the lumen. In the highest expressing Ovgp1-lacZ transgenic line, however, expression is also significant in the stroma of the uterus. Ongoing studies are focused on understanding the regulation of transgene expression to develop a better method to restrict expression to the oviduct epithelium specifically. We believe our results will lay the foundation to generate an accurate mouse model to study the tubal origin of serous ovarian cancer in humans.

15. SUBJECT TERMS
serous ovarian cancer, transgenic mice, oviduct-specific glycoprotein-1, lacZ reporter gene, oviduct epithelium, uterus

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

Serous ovarian cancer represents the most common form of ovarian cancer diagnosed in women[1]. Serous ovarian cancers can be further subdivided into low-grade, borderline, and high-grade serous ovarian cancers. However, current evidence from gynecologic pathologists is that high-grade serous ovarian carcinoma may be a distinct disease[2]. Contrary to the pervasive theory that epithelial ovarian cancer originates from the ovarian surface epithelium (OSE)[3], a more recent hypothesis states that the high-grade serous ovarian cancer may originate in the distal fallopian tube[4]. Precursor lesions in the fallopian tube called tubal intraepithelial neoplasia possess upregulated tumour suppressor p53 expression and mutations in p53 protein[5]. Salpingo-oophorectomy samples from high risk BRCA1+ carriers have increased tubal intraepithelial neoplastic lesions even before ovarian pathology emerges[6, 7]. Thus, perhaps high-grade serous ovarian cancer follows a different pathogenetic program as compared to other ovarian cancer histotypes: the origin of high-grade serous adenocarcinoma is in the distal fallopian tube followed by secondary metastasis to the ovarian surface. Thus, the purpose of this research study is to generate transgenic mice to specifically address fallopian tube tumorigenesis and test the potential for tubal origin of high-grade serous ovarian cancer observed in patient samples. Most efforts to produce mouse models of ovarian tumorigenesis have focused on targeting the mouse OSE with conditional genetic alterations[8-13]. The objective of this research study is to derive an alternative animal model for high-grade serous ovarian carcinoma by targeting the epithelium of the mouse oviduct (homologous to the fallopian tube in women). To achieve this end, we have been testing the facility of the mouse oviduct-specific glycoprotein-1 (*Ovgp1*) gene promoter[14].

BODY:

Generation of *Ovgp1-lacZ* transgenic mice

The 2.2 kilobase region of the proximal promoter of the mouse *Ovgp1* gene was isolated by polymerase chain reaction using primers specific for *Ovgp1* and the bacterial phagemid containing this region of the mouse genome. The PCR amplified fragment was cloned into the pSCA vector (Stratagene) by standard TA cloning and subsequently DNA sequenced to confirm that is was *Ovgp1*. The *lacZ* cDNA as well as polyadenylation sequences from SV40 present at the 3' end, were cloned directly into the pSCA-*Ovgp1* plasmid. The resultant pSCA/ *Ovgp1-lacZ* /pA plasmid was prepared for linearized DNA microinjection into fertilized one-cell mouse embryos (C57Bl/6 x CBA mice). Microinjections and implantation into recipient female foster mice was performed by the in-house Transgenic and Gene Targeting Facility at the London Regional Cancer Program. Thirty-four offspring were produced after microinjection, and genotyping was performed by Southern blotting using the SV40 polyA cassette as a radiolabelled probe and reconfirmed by PCR analysis using primers specific for the *Ovgp1* promoter (forward primer) and *lacZ* (reverse primer) sequences. For all subsequent genotyping, PCR analysis was performed using these same primers. Genotype analysis revealed that 5 of the 34 offspring carried the *Ovgp1-lacZ* transgene; the positive lines were denoted as 238, 242, 243, 252 & 253. These initial founders were individually mated with wildtype mice to test for germline transmission and to initiate stable lines of *Ovgp1-lacZ* mice. Four of the 5 lines (238, 242, 243 & 252) produced offspring that also harboured the transgene, whereas the 253 line produced very few litters and none had pups with the transgene present in the genome. These four independent *Ovgp1-lacZ* transgenic lines were quickly expanded up to perform preliminary assays to determine whether the transgene is expressed in the reproductive tract.

Ovgp1-lacZ transgene expression in the female reproductive tract

Since the female reproductive system can already harbour significant levels of endogenous beta-galactosidase activity, several small experiments were performed to determine the optimum conditions to observe bacterial *lacZ*-derived beta-galactosidase activity versus endogenous background activity. Staining was performed at room temperature (~22°C) or 37°C for several hours or over night, and using pH buffers ranging from 6.5 to 8.5. We were able to reduce the endogenous beta-galactosidase activity to undetectable levels in the female reproductive tract of wildtype mice when compared to *Ovgp1-lacZ* expression in transgenics. Analysis of *Ovgp1-lacZ* transgene expression by X-gal staining of wholemounts of female reproductive tracts demonstrated that two of the four lines (238 and 242 lines) possessed detectable transgene expression. Very low levels of positive X-gal staining was observed in the 238 line of female *Ovgp1-lacZ* mice by 8 weeks of age (nearing the end of puberty) and this was restricted to the oviduct (Fig. 1). Strikingly, the 242 line of *Ovgp1-lacZ* mice exhibited very strong transgene expression, where positive X-gal staining was generated within hours after starting the assay. *Ovgp1-lacZ* expression in line 242 female mice is first observed as early as 5 weeks of age (the early stages of puberty in female mice), and is tremendously upregulated by 8 and 12 weeks of age (Fig. 1). To further investigate the cells that are expressing the *Ovgp1-lacZ* transgene, frozen sections were prepared from 12-week-old female 242 line transgenic mice. The sections were subsequently processed for X-gal staining and counterstained with eosin. Positive transgene expression was readily observed in the epithelium lining the lumen of the oviduct (Fig. 1). Interestingly, we also determined that the transgene is expressed in cells of the stroma underlying the uterine myometrium (data not shown). Thus, we have determined that the mouse *Ovgp1* promoter may be a useful tool to target genetic manipulation to the female mouse oviduct; however, significant transgene expression can also occur in other cells of the female reproductive tract, namely the uterine stroma compartment. We have since generated an *Ovgp1*-Cre transgene plasmid to follow up on this next strategy.

KEY RESEARCH ACCOMPLISHMENTS:

- molecular cloning of *Ovgp1-lacZ* reporter gene plasmid
- generation of five independent *Ovgp1-lacZ* transgenic founder lines: lines 238, 242, 243, 252, and 253
- detectable transgene expression in the female reproductive tract of two lines of mice, with one line having low expression restricted to oviduct (line 238) and another with very high levels of expression from oviduct and throughout uterus (line 242)
- preliminary histological analysis to localize transgene expression in the oviduct and uterus of high-expressing 242 line
- molecular cloning of *Ovgp1*-Cre transgene plasmid

REPORTABLE OUTCOMES:

- generation of two *Ovgp1-lacZ* transgenic lines of reporter gene mice
- applied for CDMRP OCRP Career Development Award application (Title: “Development of novel transgenic mouse models of ovarian cancer”) which was based in part on the work supported from this current CDMRP OCRP Concept Award; the proposal was reviewed as excellent but was not recommended for funding

• the results generated thus far will be presented at the University of Western Ontario Dept. of Obstetrics & Gynaecology Resident Research Day in May 2009 and the Dept. of Oncology Annual Research & Education Day in June 2009; both are local meetings in London, Ontario, Canada

CONCLUSION:

The mouse *Ovgp1* promoter may prove useful in targeting specific transgene expression to the oviductal epithelium of female mice in order to generate a much-needed accurate model of a fallopian tube origin of serous ovarian cancer. Since significant transgene expression can also occur in other cells of the female reproductive tract, particularly in the uterine stroma, we are further characterizing the regulation of transgene expression in *Ovgp1-lacZ* transgenic mice which have been generated before proceeding with the production of several additional transgenic mice as detailed in the original Statement of Work.

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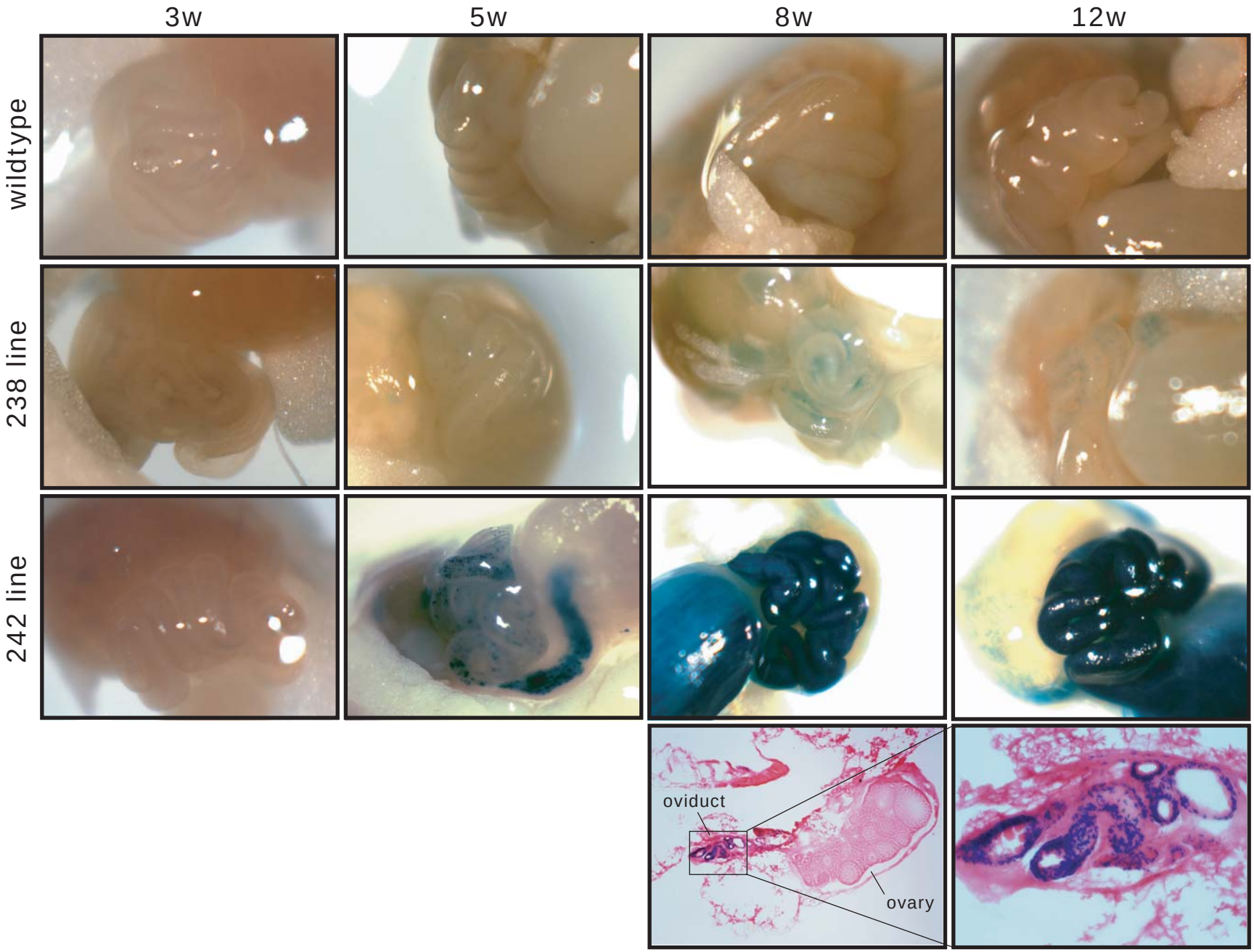

Figure 1. Wholemount X-gal staining to visulaize *Ovgp1-lacZ* transgene expression in the female mouse reproductive tract. Female mice of two different *Ovgp1-lacZ* lines (238, 242) and representative wildtype mice were sacrificed at 3, 5, 8 & 12 weeks of age, as indicated and the dissected intact reproductive tracts were subjected to X-gal staining overnight. Weak staining is seen in the 238 line and very high levels of expression in the 242 line as evidenced by the blue staining of tissue. No staining was observed in the wildtype mice. To localize transgene expression to specific cells within the tissue, cryosections of the female reproductive tract were prepared and subjected to Xgal staining on glass slides. Photos were taken with a stereomicroscope at 40x magnification for wholemounts and inverted microscope at 40x and 200x for sections using ImagePro imaging software.