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14. ABSTRACT
The purpose of this study is to determine the functional role of the putative oncogene BORIS in ovarian cancer. The proposed studies will use a newly developed mouse model system to assess the oncogenic potential of BORIS expression in the ovarian surface epithelium, alone and in combination with Rb and p53 knockout. In the past year we have accomplished a number of objectives: i) we have obtained IACUC, ACURO, and Biosafety approvals for the proposed studies, ii) we designed and constructed a new conditional overexpression construct (iZEG-CTCFL) to drive BORIS expression in mice, iii) we obtained double conditional knockout mice (Rb, p53) in FVB/N background and bred with FVB/N wild-type mice, obtained F1 mice and intercrossed to generate stocks of single Rb^{flox} or $p53^{flox}$ mice. iv) we obtained founder BORIS-Tg mice and are currently crossing into the FVB/N strain to fully characterize the transgenic gene configuration, v) we conducted intrabursal Ad-Cre injections to generate cohorts of conditionally deleted Rb and p53 in mice, measured ovary/tumor volume over 300 days by MRI, and confirmed that double mutant mice have significantly enhanced ovary/tumor growth, and vi) Ms. March has learned how to isolate and culture OSE cells from mice and has begun to isolate and utilize these cultures to meet the study objectives.

15. SUBJECT TERMS
BORIS, CTCFL, ovarian cancer, mouse models, transgenic mice

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INTRODUCTION

BORIS (CTCFL) is the antagonistic paralog of CTCF, a protein involved in global regulation of chromatin structure and genomic imprinting. In human epithelial ovarian cancer (EOC), BORIS is aberrantly expressed due to promoter DNA hypomethylation. An increased expression ratio of BORIS/CTCF in EOC is associated with global DNA hypomethylation, advanced stage, and reduced survival. These findings suggest BORIS activation may be oncogenic in EOC. Based on the known function of CTCF in chromatin insulation, BORIS activation may also remodel the epigenome, particularly via DNA methylation changes (both hypermethylation and hypomethylation). It is plausible that BORIS activation may act cooperatively with Rb and p53 loss to drive epigenetic changes and EOC progression, as BORIS activation in EOC is coincident with activation of E2F target genes, and wildtype p53 is a negative regulator of BORIS expression. To test these hypotheses, we will develop and utilize a murine transgenic model that allows for the specific expression of BORIS in the ovarian surface epithelium (OSE), following delivery of adenovirus expressing Cre recombinase into the ovarian bursa.

BODY

Task 1. The goal of the first task is to determine the impact of BORIS expression, alone and in combination with loss of p53 and/or Rb, on EOC development.

1a. IACUC, ACURO, and Biosafety Approvals

The existing IACUC protocol was amended to permit the studies described in the remainder of the proposal and approved for an additional three years in October of 2012. Moreover, ACURO approval for *USAMRMC Proposal Number OC110111, Award Number W81XWH-12-1-0456, entitled "Functional Assessment of the Role of BORIS in Ovarian Cancer Using a Novel in Vivo Model System"* was obtained on 01/31/14. Finally, the Institutional Biosafety Protocol for the use of adenoviruses was also resubmitted and approved by Roswell Park Cancer Institute.

1b. Generation of transgenic mouse model with conditional CTCFL (BORIS) transgene in an FVB/N background.

As outlined in the SOW, we decided to generate a new *CTCFL* (*BORIS*) transgenic mouse so that it would be in the FVB/N background. In addition, we decided to remake the transgene in the iZEG conditional expression construct instead of the originally used pCLEG vector (described in original proposal) because of it contains reporters for both pre-activation (LacZ) and post-activation (EGFP) states. To help prevent epigenetic gene silencing that can take place with randomly inserted transgenes, we attempted to use a newly-developed transcription activator-like effector nucleases (TALENs) designed to target the ROSA26 locus. This approach was unsuccessful so we returned to the more traditional strategy (i.e. pronuclear injection with random integration).

Of 25 pups born following pronuclear injection, 4 were found to carry the BORIS transgene. These mice were mated to FVB/N mice and their progeny genotyped by PCR. Two litters showed evidence of germline transmission after genotyping by PCR; however, only one male mouse was confirmed by Southern analysis. This founder mouse appears to carry multiple copies of the BORIS transgene and has been mated to several FVB/N females to determine whether the transgene is present at a single chromosome location or at more than one locus and to propagate the mouse line. In addition mouse embryonic fibroblasts are being made from these offspring so that expression of the transgene can be tested following infection with adenoviruses that express Cre-recombinase.

1c. Generation of control and experimental mice and intrabursal injection of adenovirus expressing Cre-recombinase

The initial double conditional knockout mouse (pRb$^{flox/flox}$;p53$^{flox/flox}$ in FVB/N background) was bred with FVB/N wild type mice, and F1 mice intercrossed to generate stocks of single p53$^{flox/flox}$ knock out mice. As controls, cohorts of 15 double mutant (pRb$^{flox/flox}$;p53$^{flox/flox}$) and 15 single mutant (p53$^{flox/flox}$) female mice were established by conventional breeding and were treated with adeno-Cre-IRES-eGFP by intrabursal injection. To control for surgery and/or response to viral infection, contralateral ovarian bursa were injected with adenovirus that expresses only eGFP (adeno-eGFP). Since the BORIS transgenic mouse has only recently been made, mouse cohorts containing the transgene (e.g. pRb$^{flox/flox}$;p53$^{flox/flox}$; Tg$^{iZEG\text{-}CTCFL\ /+}$) have not yet been produced. Once we have identified BORIS transgenic mice with single integration sites (above), transgene-carrying cohorts will be produced by *in vitro* fertilization techniques with the help of the Transgenics Core Facility at RPCI. This should expedite the generation of sufficient experimental animals.

Following surgery and intrabursal injection, control cohorts (i.e. pRb$^{flox/flox}$;p53$^{flox/flox}$ and p53$^{flox/flox}$) were monitored by MRI for enlargement of ovaries and/or tumor development. As anticipated, ovary enlargement/tumor development increased dramatically in the double mutant cohort compared to the single mutant cohort (Fig. 1).

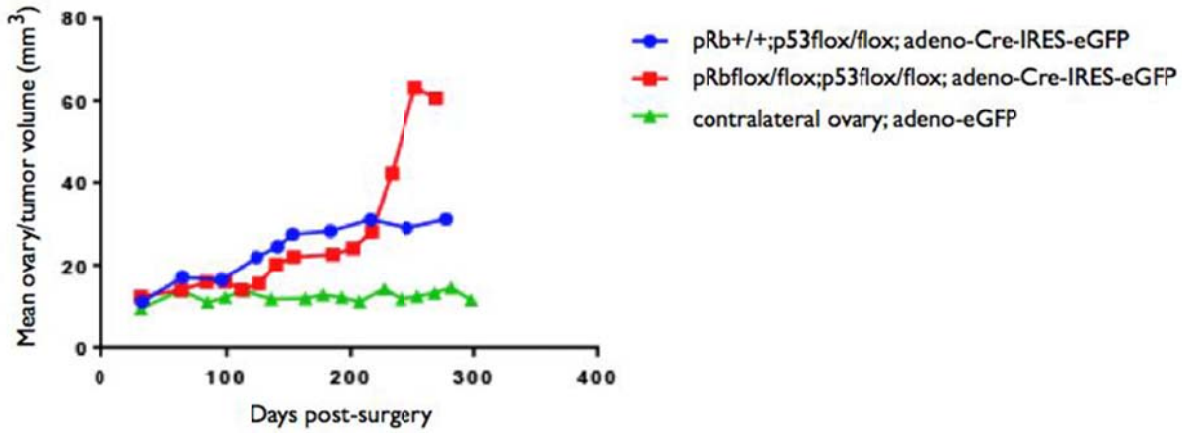

Figure 1. Tumor development in control cohorts. Single mutant (pRb+/+; p53$^{flox/flox}$) or double mutant (pRb$^{flox/flox}$;p53$^{flox/flox}$) female mice were subjected to intrabursal injection of adenoviruses expressing Cre-recombinase and eGFP or just eGFP (contralateral ovary), and monitored by MRI for 280-300 days. The graph shows the mean (n=15) ovary/tumor volume for the indicated cohort.

Alternative strategy to ectopically express BORIS in OSE:

Since it has taken so long to generate the BORIS-Tg mouse line we have been investigating an alternative approach to ectopically express BORIS in the mouse ovarian surface epithelium (OSE). With help from the Iowa University Vector Core Facility, we have constructed an adenovirus that expresses human BORIS. We are currently characterizing this adenovirus for infection efficacy and gene expression in HEK293 and OSE cells. The BORIS adenovirus can be co-injected with adeno-Cre-IRES-eGFP adenovirus into the ovarian bursa of mice circumventing the need to use the BORIS-Tg mouse line.

Task 2. The goal of the second task is to determine the impact of BORIS expression, alone and in combination with loss of Rb and/or p53, on epigenomic and genomic stability in EOC

Similar cohorts of "control" animals (i.e. $pRb^{flox/flox}$;$p53^{flox/flox}$ and $p53^{flox/flox}$) containing 15 female mice each have been established. These mice have all undergone ovarian intrabursal injections of adeno-Cre and are currently being aged. At various time points, mice will be sacrificed and tumor material collected for genomic (CGH) and epigenomic (DNA methylation) analysis. Similar cohorts of animals carrying the BORIS-Tg with or without floxed p53 and/or floxed pRb will be established by *in vitro* fertilization, injected with adeno-Cre, and used for tumor collection.

Task 3. The goal of the third task is to determine the impact of BORIS expression, alone and in combination with loss of p53 and/or Rb, on OSE transformation *in vitro*.

While visiting Dr. Flesken-Nikitin at Cornell, Ms. March learned to isolate and culture ovarian surface epithelial (OSE) cells. She has successfully established OSE cultures from double conditional knockout mice ($pRb^{flox/flox}$;$p53^{flox/flox}$) as well as from $p53^{flox/flox}$ knock-out mice. As described above, we are currently generating BORIS-Tg mice. Once mice are available from BORIS-Tg mice in the various genetic backgrounds ($pRB^{fl/fl}$, $p53^{fl/fl}$, $pRB^{fl/fl}$; $p53^{fl/fl}$), she will determine the oncogenic properties of OSE cells harvested from mice with all genetic configurations. These experiments will include testing for growth in soft agar and invasive growth through Matrigel.

KEY RESEARCH ACCOMPLISHMENTS

- Obtained IACUC, ACURO, and Biosafety approvals for all proposed studies.
- Designed and constructed a new conditional overexpression construct (iZEG-CTCFL) to drive BORIS expression in mice.
- Obtained double conditional knockout mice (Rb, p53) in FVB/N background and bred with FVB/N wild-type mice, obtained F1 mice and intercrossed to generate stocks of single Rbflox or p53flox mice.
- Obtained founder BORIS-Tg mice and are currently crossing into the FVB/N strain to fully characterize the transgenic gene configuration
- Conducted intrabursal Ad-Cre injections to generate cohorts of conditionally deleted Rb and p53 in mice, measured ovary/tumor volume over 300 days by MRI, and confirmed that double mutant mice have significantly enhanced ovary/tumor growth
- Ms. Joanna March (Teal Scholar) has learned how to isolate and culture OSE cells from mice and has begun to isolate and utilize these cultures to meet the study objectives

REPORTABLE OUTCOMES

N/A

CONCLUSION

We have made significant progress towards functionally assessing the role of BORIS in ovarian cancer. We have completed the necessary experimental design, have trained with the necessary experimental methods, and are currently producing mice to test the central hypotheses of the proposal. Successful completion of this proposal will reveal important new information about the molecular pathology of ovarian cancer, and the role of aberrant BORIS expression in the disease. This knowledge can be used to develop novel biomarkers and therapeutic approaches for ovarian cancer.

REFERENCES

N/A

APPENDICES

N/A

SUPPORTING DATA

N/A