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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
Mouse homologs of the *Drosophila melanogaster* tumor suppressor gene, discs large 1, have been examined for their presence in the ovarian surface epithelium (OSE), the origin of the most virulent and common form of ovarian cancer. The presence of ten such mouse genes was verified in the ovary by RT-PCR analysis of whole ovarian homogenates. Antibodies specific to each of the homologs were utilized to assess the presence of each protein in the surface epithelial layer. CASK and ZO-2 were both expressed in the surface epithelium as well as in other ovarian cell types. ZO-1 and SAP102 were not found in the surface epithelium although they were determined to be present in the granulosa cells. ZO-3 was not found in any of the ovarian tissues. Immunofluorescence could not positively localize five additional proteins, Dlgh1, Dlgh2, Dlgh3, p55, and PSD95 to any ovarian tissue; however, Dlgh2 and p55 proteins were found in whole ovarian lysates by western blot analysis. Primary cell cultures from mouse ovary surface epithelium have been established. The challenge, as yet unrealized, has been to target specifically the OSE expression of a protein. We propose a change in research objectives during the third year of funding to accomplish this objective.

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INTRODUCTION

Cancer growth of the germinal epithelium of the human ovary gives rise to the most common and virulent form of human ovarian cancer. Since conventional approaches to investigating the biology of the ovarian surface epithelium have yielded frustratingly slow progress toward understanding the development and progression of ovarian cancer, we have implemented an approach involving "species hopping". The fundamentals of cellular biology and pathology are highly conserved through much of the evolutionary tree. Therefore, we have made use of strong genotype-phenotype correlations in the fruit fly, *Drosophila melanogaster,* to identify a gene whose mouse homologs could serve as candidates for study into the etiology of mammalian ovarian cancer. Homologs of the fruit fly tumor suppressor gene *discs large 1* (*dlg 1*) were identified as such candidates. The proposed study was designed to determine the ovarian expression of ten mouse dlg 1 homologs, to identify one or more homologs whose products were localized to the cells of the ovarian surface epithelial layer, to generate mice bearing a systemic deletion of a dlg 1 homolog normally expressed in the germinal epithelium, and to characterize the ovarian phenotype resulting from such systemic deletion for the select homolog.

BODY

The 10 mouse genes previously identified for investigation in this study included those encoding three discs large homologs (Dlgh1, Dlgh2, Dlgh3), a calcium/calmodulin-dependent serine kinase (CASK), an erthrocyte membrane protein (p55), post-synaptic density protein 95 (PSD95), a synapse-associated protein 102 (SAP102), and three zona occludens proteins (ZO-1, ZO-2, ZO-3). The expression of these genes in mouse whole ovary homogenate preparations was verified by using RT-PCR analysis.

Task 1. *To determine which of the known mouse dlg1 homologs are expressed in the germinal epithelium of the mouse ovary*

Progress Report #1 of this investigation described results obtained through immunofluorescent localization of proteins of interest in sections of intact whole mouse ovary. Briefly, these results indicated that CASK and Z0-2 were expressed in the surface epithelium covering the ovary, but were additionally identified in other cell types within the ovary. Both ZO-1 and SAP102 proteins localized exclusively to granulosa cells within the ovary, and ZO-3 was not expressed in the ovary at all. Multiple attempts at immunofluorescent identification of the proteins Dlgh1, Dlgh2, Dlgh3, p55, and PSD95 in ovary sections have yielded inconclusive results.

The antibodies raised against Dlgh2 and p55 have since detected proteins in whole ovary and kidney lysates in Western blot analyses, but since their cell specificity within the ovary is unknown, it has not been determined if these proteins may or may not be of further interest. The antibody raised against Dlgh3 has not recognized any proteins through either immunofluorescent tissue preparation or Western blot analysis.

In an attempt to create sample material enriched with surface epithelial cells, short-term primary cultures of mouse surface epithelial cells were created by using a method adapted from that of Kido and Shibuya (1998). Briefly, intact ovaries were

incubated in a weak trypsin solution to allow for the detachment of OSE (ovarian surface epithelium) cells from the ovary surface, the ovaries were discarded, and detached cells were cultured under 10% CO_2 in a complete growth medium consisting of Dulbecco's Modified Eagle Medium, fetal calf serum, penicillin and streptomycin, and murine EGF.

Although cultures were not exclusively OSE cells, the presence of EGF in the medium helped to create cultures enriched with OSE cells that eventually developed into intact epithelial monolayers over the plastic substrate (**Appendix, Figure 1**). Resulting cultures could be fixed and analyzed by immunofluorescent detection of ZO-2 and the epithelial junction proteins occludin and E-cadherin. The cellular localization of each of these proteins corresponded to that observed in similarly processed OSE cells in intact whole ovary sections.

Task 2. *To generate mice bearing a systemic deletion of a dlg 1 homolog expressed in the germinal epithelium of the mouse ovary.*

No knockout mice have been generated as yet through this project.

Task 3. *To characterize the phenotype of the ovarian germinal epithelium of mice nearing a systemic deletion of a dlg 1 homolog.*

No knockout mice have been generated as yet through this project.

Key research accomplishments

1. Dlgh2 and p55 proteins were identified in whole ovary lysates by Western blot, but their localization within the ovary remains undetermined.
2. Primary cultures of mouse ovarian surface epithelium were created and determined to maintain their phenotype *in vitro* based upon immunofluorescent localization of Z0-2, occludin and E-cadherin.

Reportable outcomes

No additional reportable outcomes at the time of this report.

Conclusions

Immunodetection results suggest that ZO-2 (**Appendix, Figure 2**) may be an excellent candidate for gene deletion based upon its discreet localization pattern in the ovarian surface epithelial cells and its colocalization with occludin, an important epithelial tight junction protein. However, as ZO-2 is also expressed in other cell types in the ovary as well as in other organs throughout the animal, systemic deletion will most likely result in widespread phenotypes that are unrelated to alterations in the biology of the surface epithelium. To date there are no known OSE-specific gene promoters to direct tissue-specific knockout for the investigation of ovarian cancer.

During the course of this investigation, the lack of an ovarian surface epithelium specific promoter has emerged as the most significant obstacle inhibiting fruitful investigation of ovarian cancer biology. We suggest that although our original proposal is still sound in theory, the absence of such a tool makes development of any knockout, as originally proposed, a nonproductive exercise at this time. Therefore, we propose to

utilize the third year of this research project to shift our focus from the original proposal to that of the identification of a gene(s) and thus a promoter whose expression is specific to the ovarian surface epithelium. A formal request to change the focus of the third year of grant will be submitted forthwith to the U.S. Army Medical Research Acquisition Activity office. Our laboratory is well positioned for this endeavor in that we have successfully identified through subtraction suppression hybridization over 80 genes which are expressed exclusively or primarily in the ovary (Hennebold et al. 2001). This was completed by subtraction of total ovary mouse cDNA collected at time points throughout a stimulated estrus cycle with cDNAs from 6 other organs (brain, heart, kidney, liver, spleen, and lung). The approach for the identification of an OSE-specific gene would be identical. Within the year remaining in this funding period, the isolation of sufficient OSE mRNA, the subtraction of OSE mRNA with cDNA from other major tissues, the confirmation of tissue specificity of the resulting library by Northern blot analysis, and the characterization of those cDNAs specific to the OSE after sequencing and analysis for homology to known genes with the Basic Local Alignment Tool (BLAST) could be accomplished. A useful gene will by necessity be a novel sequence since no OSE specific genes are known, but clues to function quite possibly may be forthcoming by sequence comparison with a wide range of databases including those for the zebra fish, worm, fly, and human.

The characterization of the promoter, although beyond the time frame of this proposal, will be accomplished by traditional promoter analyses which utilizes fusion of green fluorescent protein (gfp) to varying lengths of the 5' UTR and subsequent transfection into primary OSE cells. Expression of the fluorescent protein will be directed by those sequences required for tissue specific expression. Additional characterization of the promoter will result from analysis by RT-PCR of its temporal and hormonal–regulated expression profile throughout a stimulated estrus cycle.

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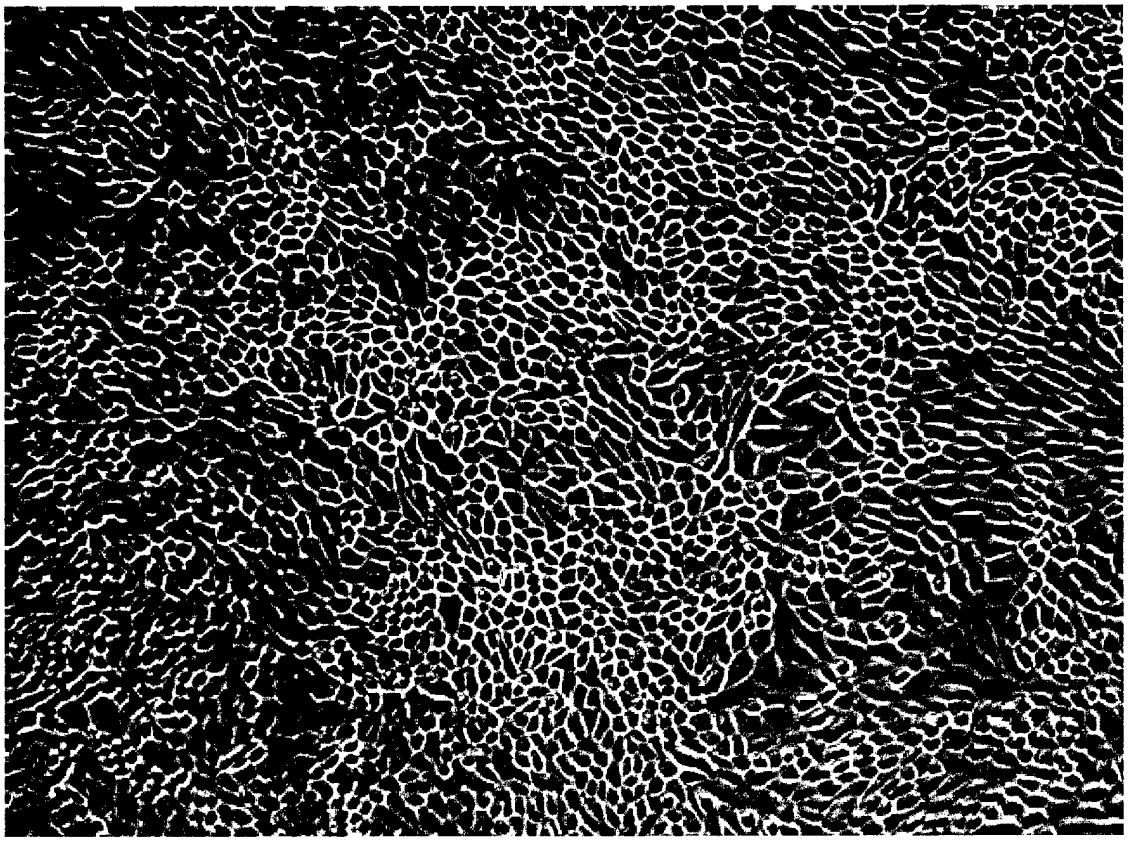

Figure 1. Primary ovary surface epithelium monolayer in culture.

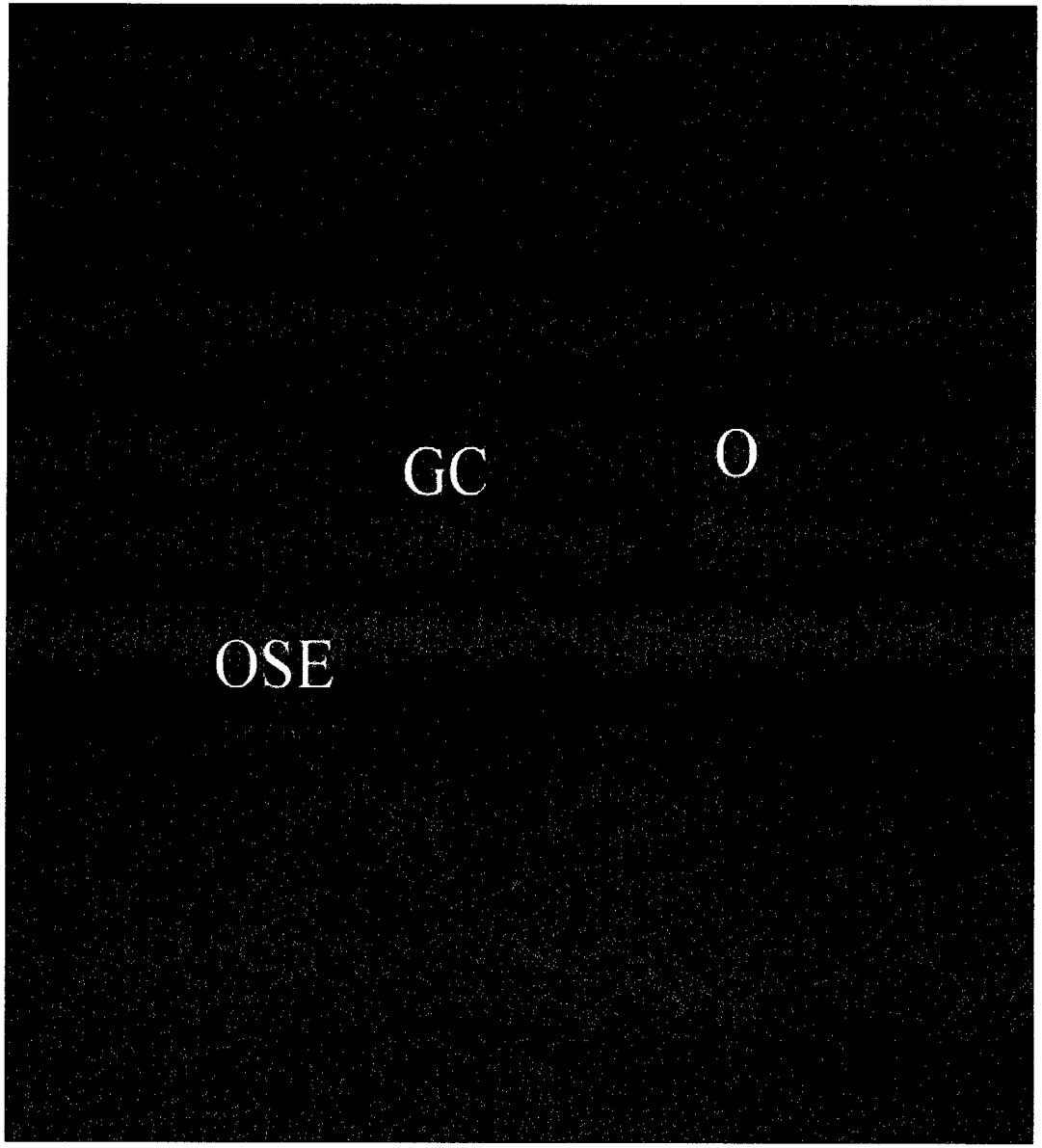

Figure 2. Immunodetection of mouse ZO-2 in mouse ovary sections (28X magnification). OSE=Ovarian Surfact Epithelium, GC=Granulosa Cell, O=Oocyte