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These experiments investigate a mouse model of human adrenal androgen biosynthesis and the role of these steroids in human breast cancer growth. An androgen-dependent human breast cancer model was established in the *scid* mouse. To provide zona reticularis function, essential for adrenal androgen biosynthesis, in human adrenal organoids in the mouse, two approaches are being taken; first, to form an organoid with a capillary bed adequate for proper zonation to be re-established, and second, the genetic engineering of clonal adrenal cells to suppress 3β-hydroxysteroid dehydrogenase (3β-HSD), the key enzyme regulating DHEA biosynthesis. Clonal bovine adrenal cells formed a functional organoid in *scid* mice that replaced the animals' adrenal function. Southwestern blotting using a probe in the regulatory region of the 3β-HSD gene has shown zonal differences in protein binding. The characterization of these transcription factors may provide future information on the molecular basis of zonation and thus indicate methods for obtaining zona reticularis function in the organoids.

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FOREWORD

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Peter Hung 9/30/97

PI - Signature Date

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Introduction

We reported last year that there had been both progress and problems in the development of a mouse model for human adrenal androgen biosynthesis. We reported that, when human adrenocortical cells are transplanted into immunodeficient mice, animals do not maintain high levels of DHEA (dehydroepiandrosterone) and DHEAS (dehydroepiandrosterone sulfate) and that this may indicate fundamental differences in the mouse in the handling of these steroids versus in humans and higher primates. Our assumption is that the key element that is required for the production of human adrenal tissue in the mouse that is able to make substantial amounts of DHEA is the ability of the tissue to form a zona reticularis. This will require an adequate development of a vascular capillary bed within the transplanted tissue.

Body

Task 1

Further develop the human adrenal organoid/SCID (severe combined immunodeficiency) mouse model for investigation of the regulation and effects of human adrenal androgens

As previously reported, we found that the production of the adrenal androgens, DHEA and DHEAS, by the adult human adrenal gland is exclusively the function of the innermost zone of the adrenal cortex, the zona reticularis. The essential feature of a model in which normal human adrenal androgen production is maintained in a mouse via an implanted human adrenal organoid is the maintenance of zona reticularis function.

In order to do this, we have developed, in addition to the previously reported model in which the cells are transplanted beneath the kidney capsule of the mouse, a model in which these cells are transplanted subcutaneously. Because the cells are subcutaneous, they are not subject to the limitations of size as when transplanted under the kidney capsule. We have shown that this will enable us to make much larger tissue constructs. A longer vascular bed may support proper zonation. In another development, we have developed a polymer, based on polylactic acid, that is capable of binding proteins and can be used to aid the tissue engineering of this structure. We have shown that beads can be made from this polymer and that the beads can encapsulate proteins. We are testing whether the encapsulation of angiogenic factors such as FGF or VEGF can provide a directed angiogenesis to the subcutaneous tissue transplants. In this way, it may be possible to guide vascularization in such a way as to create a long capillary bed which is likely to be required for re-zonation to occur. So far, we have shown that the subcutaneous method of transplantation yields normal adrenocortical tissue, that the cells are functional, and that the addition of tissue-engineered polymer beads is compatible with cell survival and growth. In future experiments, we will determine if directional vascularization occurs and if this is sufficient to form a zone secreting DHEA and DHEAS.

Task 2

Assess the influence of circulating adrenal androgens on human estrogen response in human breast cancer cell growth.

We want to assess the influence of adrenal androgens produced by implanted human adrenal organoids in the SCID mouse on the growth of co-implanted tumor cell cells (MCF-7). MCF-7 cells were used which have been transfected with aromatase. During the past year, we have set up a successful model of androgen-dependent growth of this tumor and we present the data from these experiments in this report in the Appendix. The success of this model now will allow the testing of adrenal androgens in human breast cancer growth in the mouse model.

Task 3

Investigate the molecular biology of adrenal androgen regulation focusing on the key enzyme 3β-hydroxysteroid dehydrogenase

An alternate approach to the question of setting up a mouse model with high adrenal androgen secretion is to form an organoid from cells genetically engineered to produce high levels of DHEA. We showed last year that the development of a genetically engineered cell to oversecrete DHEA would be possible if a clonal cell were engineered with an antisense 3β-HSD

construct. We have shown and published that clonal bovine adrenocortical cells can form functional tissue in SCID mice (Thomas et al., 1997). The key finding that clonal cells can form transplanted tissue indicates that genetically engineered clonal cells are likely to be able to form functional tissue. We have constructed the antisense 3β-HSD plasmid and have transfected it into bovine adrenocortical cells. Clones are being grown and characterized. We hope during the following year to show whether clones, when transplanted and made into tissue, secrete large amounts of DHEA.

Task 4

The physiological influences on adrenal androgen production in the human adrenal organoid SCID mouse model

This task awaits the development of adrenal organoids producing high amounts of DHEA as described in Tasks 1-3. We intend to commence this portion of the work when such organoids have been developed.

Task 5

Identify the transcription factors which regulate the human type II 3β-HSD gene and test their effects on adrenal androgen synthesis in the human adrenal organoid SCID mouse model

To identify the region of the type II gene that is likely to be targeted by transcription factors, we took advantage of a previous observation that a 40-base pair region in the first intron of the type I 3β-HSD gene appears to be essential for regulation of this gene in tissues other than the adrenal cortex. We also noted that this region differs significantly in the type II gene. Because the type II gene is expressed in the adrenal cortex, but not in other tissues, and the type I gene is expressed in other tissues, but not in the adrenal cortex, the presence of substantial nucleotide differences in this region makes it a likely region for the binding of transcription factors which are differentially regulated between the two genes and between the different tissues. We reported last year that gel shift assays using this probe strongly suggested differences in the proteins between the zones of the human adrenal cortex. We have now shown using Southwestern blotting a protein of molecular weight 35,000 to be present in the fasciculata, but not in the reticularis, and also present at much higher concentrations in the bovine adrenal cortex (see Appendix). The protein is present in proportion to the level of expression of the 3β-HSD gene in the three tissues. Our present goal is to try to identify this protein. One technique would be to use Southwestern screening using an expression library from the zona fasciculata, but we also are approaching this task by taking advantage of the fact that, because of the human genome project, many cDNAs of human genes are now available on gridded filters. By using labeled cDNAs from fasciculata and reticularis and probing such filters, differences may become apparent which may allow rapid identification of this protein. Because this approach, if successful, would be much more rapid than library screening, we are now beginning these experiments.

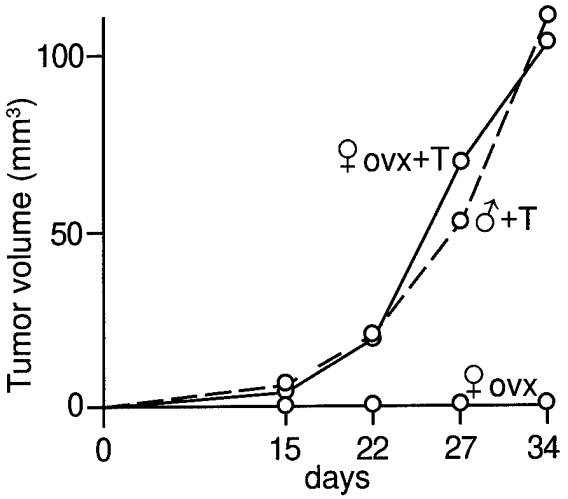
Conclusions

Despite problems in the mouse model due to differences between mice and humans in the metabolic handling of the adrenal androgens, the advances of the creation of clonal organoid tissues and progress in the molecular biology of the 3β-HSD genes allow confidence that the major aims of this grant are achievable.

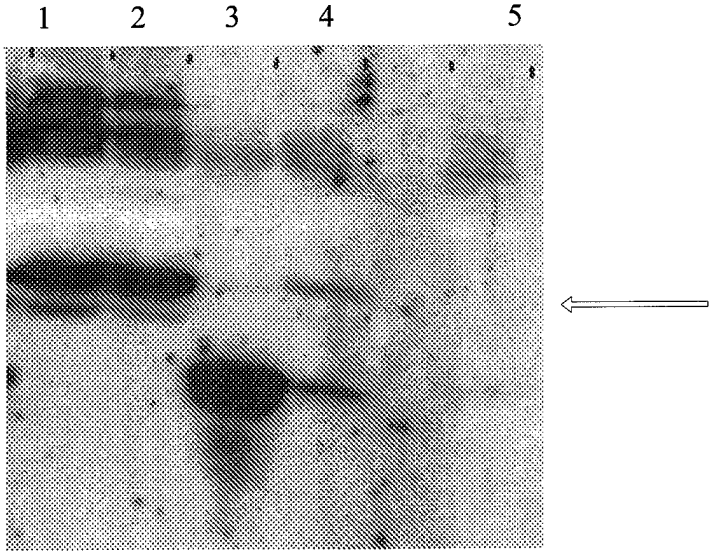
Reference

Thomas, M., Northrup, S.R., and Hornsby, P.J. (1997) Adrenocortical tissue formed by transplantation of normal clones of bovine adrenocortical cells in *scid* mice replaces the essential functions of the animals' adrenal glands. *Nature Med.* 3: 978-983

Appendix



Growth of aromatase-transfected MCF-7 tumors in *scid* mice (ovariectomized females, and males; controls or injected wth testosterone 3x at 0, 7, 14 days, 1 mg/g) (averages of 8 mice each group)



Southwestern blot of nuclear proteins from various tissues, probed with a regulatory region of the human 3β-hydroxysteroid dehydrogenase gene. The protein indicated by the arrow is not expressed in HeLa cells (5) or in zona reticularis (3) but is present in the zona fasciculata (4) and at high concentrations in the bovine adrenal cortex (1 and 2)