REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Annual (17 Sep 2002 - 16 Sep 2003)

4. TITLE AND SUBTITLE
Smad-Mediated Signaling During Prostate Growth and Development

5. FUNDING NUMBERS
DAMD17-01-1-0100

6. AUTHOR(S)
Charles F. Shuler, D.M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Southern California
Los Angeles, California 90033

E-Mail: shuler@hsc.usc.edu

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. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

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

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)
The regulation of prostate morphogenesis is regulated by signaling molecules from the transforming growth factor beta family. The binding of the TGF-beta ligand to the cell surface receptors leads to the activation of a kinase activity in the TGF-beta receptors. The Smad family of molecules mediates the propagation of the signal through the cytoplasm. In the absence of TGF beta no signal is initiated. The null mutation of TGF-β3 can be specifically rescued at the molecular level by over expression of Smad 2. The rescue is correlated both with the quantity of Smad 2 produced through the over expression and the quantity of Smad 2 that is phosphorylated. The mechanism for Smad 2 phosphorylation could be through activation of the receptor kinases by another growth factor or through an alternative phosphorylation pathway. The ability to rescue the growth factor deficient phenotype through induction of a downstream effector molecule provides new insight into this regulatory pathway. The potential exists to develop strategies to activate the signal transduction mechanism in the absence of the growth factor and circumvent the deficiency. These results on the basic understanding of the signaling pathway will lead to new strategies for therapeutic intervention to alter cell growth and differentiation that may lead to neoplastic events.

14. SUBJECT TERMS
Smad-mediated TGF-β signal transduction; prostate ductal branching morphogenesis; prostate growth & development; cell proliferation & etc.

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

AD_____

Award Number: DAMD17-01-1-0100

TITLE: Smad-Mediated Signaling During Prostate Growth and Development

PRINCIPAL INVESTIGATOR: Charles F. Shuler, D.M.D., Ph.D.

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, California 90033

REPORT DATE: October 2003

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

Table of Contents

Cover.....1

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....4

Key Research Accomplishments.....5

Reportable Outcomes.....6

Conclusions.....6

References.....

Appendices.....

Introduction:

The mechanisms of signal transduction by growth factors represents an important area of investigation to understand fetal development and tissue differentiation. Our studies are focused on the transduction of signal by transforming growth factor-β (TGF-β) during development. Molecules critical to the transduction of TGF-β are the from the Smad family. In particular Smad 2 and Smad 3 are activated following ligand binding to specific receptors. The phosphorylation of these two Smads results in an up-regulation of TGF-β dependent events. Previous studies and preliminary data have demonstrated that transforming growth factor-β (TGF-β)-mediated signaling regulates prostate growth and morphogenesis. The studies are focused on the molecular mechanism of TGF-β signal transduction and the role of these mechanisms in morphogenesis.

Body:

The current research has continued to focus on studies in Specific Aim 2 examining the relationship of TGF-β signaling and Smad 2 and Smad 3 transduction of that signal. These studies have been greatly facilitated by the introduction of transgenic mice that have over-expression of Smad 2, and deletion of the TGF-β Type II receptor. The Smad 2 over expression transgenic mice have been mated to TGF-β3 null mutant mice to examine the rescue of the null mutant phenotype by forced over expression of the intracellular mediator. The requirement for phosphorylation in the Smad 2 over expression has also been examined to determine the requirement for this post-translational modification to activate the signaling pathway. The studies have remained focused on fundamental biomedical research aimed at examinations of the mechanisms of signal transduction.

TGF-β binding to the specific cell surface receptors results in activation of their kinase activity. The receptor kinases specifically phosphorylate the Smad 2 and Smad 3 molecules and the phosphorylation is absolutely required to activate the signaling pathway. In the previous report we provided evidence that the TGF-β3 null mutant mouse did not activate the receptor kinases and the absence of phosphorylated Smad 2 specifically indicated that the intracellular signaling pathway was not activated. There remains the potential that alternative mechanisms could exist and be inhibited in the null mutant mice so that a molecular rescue approach was developed using animals transgenic for over expression of Smad 2. Since Smad 2 is down stream from TGF-β binding to the receptors, over expression may rescue the null mutation and phosphorylation of the Smad 2 may also be required for the rescue. This type of molecular rescue approach provides a unique opportunity to examine specific linkage between molecules in a signal transduction pathway. This approach was indicated as a plan for the present year in last year's progress report and the progress on this line of investigation follows.

The results have provided clear evidence that over expression of Smad 2 is capable of rescuing the TGF-β3 null mutation. Western blot analysis of the proteins produced provide clear evidence of the over expression of Smad 2. Analysis of the Western blots with antibodies specific for phosphorylated Smad 2 indicate that some of the Smad 2 is phosphorylated. In quantitative analyses only a minimal amount of the over expressed Smad 2 is actually phosphorylated while the majority of the Smad 2 remains non-phosphorylated. The amount of phosphorylated Smad 2 approaches the quantities observed in animals without the null mutation. The amounts of total Smad 2 expressed and the amounts of phosphorylated Smad 2 were correlated with the rescue of the TGF-β3 null mutation. The rescue was directly correlated with both the total quantity of Smad 2 present and the amount of Smad 2 that was phosphorylated.

There are several very important outcomes that occur through this research. The rescue of the null phenotype by Smad 2 over expression establishes an epistatic relationship in the signal transduction and demonstrates that the absence of TGF-β can be compensated by the intracellular messenger molecules. This means that approaches can be developed to circumvent the requirement for growth factor ligand binding to the receptor to initiate the signaling pathway. A second key observation is that Smad 2 phosphorylation is occurring in the absence of TGF-β3 which means that either different growth factors are activating the kinase activity or that the Smad 2 molecule is phosphorylated by a different mechanism. Both of these are important possibilities since there would exist the opportunity to initiate the signaling with the alternative growth factor or the alternative phosphorylation pathway. These findings are novel in they represent the first approach to rescue the TGF-β3 null mutation and that Smad 2 over expression is a specific mechanism required for the molecular rescue. Since TGF-β signaling is a short range mechanism the molecular rescue indicates that the potential exists to circumvent a specific deficiency by induction of downstream elements of the signaling pathway. Examination of the regionally specific pathways for gene expression can be correlated with the intracellular signaling mechanisms to evaluate spatially restricted events during glandular morphogenesis.

We are building on these findings by examining the mechanism for Smad 2 phosphorylation and whether this is linked to activated of the TGF-β receptor kinases. This approach is facilitated by the use of a TGF-β Type II receptor conditional knockout. The approach is to specifically remove the TGF-βRII from specific cells at precise stages of development through a cre-lox approach. Cells deficient in TGF-βRII are incapable of activating the TGF-β dependent kinase. If Smad 2 can be phosphoryated in these cells then there is definite evidence that an alternative pathway exists to activate this intracellular specific signaling mechanisms. If Smad 2 is not phosphorylated in these cells then there is definite evidence for the substitution of other growth factors binding to these receptors and activating the Smad 2. Both outcomes will provide important new information on the basic processes regulating this intracellular signal transduction pathway.

Future studies will continue to examine the role of Smad 2 expresssion and Smad 2 post-translation modification as important events in the regulation of TGF-β signaling during development. The on-going studies have been specifically focused on basic mechanisms of growth factor binding and subsequent transduction of the signal in the cell. Analysis of these basic mechanisms has identified the potential to circumvent a growth factor deficiency by rescuing a null mutation. In the future these types of studies will provide targets for potential therapeutic strategies to either augment or inhibit growth factor signaling associated with pathologic processes.

Key Research Accomplishment:

- Smad 2 over expression rescues the effects of the TGF-β3 null mutation
- Over expressed Smad 2 is phosphorylated in the cell and thus is potentially activated in the downstream signaling pathway.
- Smad 2 over expression rescue is correlated with both the total amount of Smad 2 present and the minority amount of Smad 2 that is phosphorylated.
- Rescue of the null mutation with down stream effector molecules represents a potential target for developing therapeutic interventions.

Reportable Outcomes:

Manuscripts are in preparation to present the effects on Smad2 phosphorylation of the TGF-β3 null mutant. The current studies will be publishable in three areas; 1) TGF-β ligand specific activation of subsets of pathway restricted Smads through differential activation of receptor kinase activity; 2) Kinase activity of the TβIR-TβIIR complex following binding of different members of the TGF-β family; 3) Genetic rescue of TGF-β null mutations using specific overexpression of pathway restricted Smads and the relationship to the downstream activation of TGF-β regulated gene expression; 4) Identification of the mechanism for activation of Smad 2 in the null mutation background. All of these studies are focused on the basic biomedical principles of fundamental growth factor signaling pathways. Dissection of the different elements of the pathways are required to initiate strategies focused on specific molecular mechanisms that will generate targeted therapies. These findings will have important implications for the basic understanding of prostate growth and development.

Conclusion:

These studies are focused on the specific signal transduction mechanism related to TGF-β binding to specific receptors, subsequent activation of kinase activity and activation of downstream mediators through phosphorylation. The identification of molecular rescue in the face of growth factor deficiency is an important outcome that indicates that downstream effectors can be targets for intervention strategies. Further investigation of the mechanisms responsible for activation of the Smad 2 molecules will provide additional insight into the regulation of this critical developmental pathway. The use of novel transgenic mice provides approaches that are focused on specific elements of the signaling pathway. Further investigation of the fundamental mechanisms will provide improved characterization of control of the critical developmental events.

Appendices:

None