JENNIFER L. GOOCH, PH.D.

Contact information

Department of Medicine / Renal Division
Emory University
1639 Pierce Drive, WMB RM 338
Atlanta, Georgia, 30322

phone:
fax:
e-mail: _____

Citizenship

United States

Current Titles and Affiliations

DATE APPOINTED	POSITION	INSTITUTION/LOCATION
Oct 1, 2004	Assistant Professor, tenure track	Dept of Medicine, Renal Division, Emory University, Atlanta, GA
Oct 1, 2004	Secondary academic appointment	Dept of Physiology, Emory University, Atlanta, GA
Oct 1, 2004	Staff Research Scientist	Atlanta Veterans Affairs Medical Center, Decatur, GA

Previous titles and Affiliations

Sept 1, 2003	Assistant Professor (Research)	Dept of Medicine, Renal Division, UT Hlth Science Center, San Antonio, San Antonio, TX
Oct 1, 2002	Staff Research Scientist	Audie L. Murphy Memorial Veterans Hospital, South Texas Veterans Health Care System, San Antonio, TX
July 1, 2002	Instructor (Research)	Dept of Medicine, Renal Division, UT Hlth Science Center, San Antonio, San Antonio, TX

Education

YEAR	DEGREE	MAJOR	INSTITUTION/LOCATION
1994	B.S.	Biology	Southwest Texas State University, San Marcos, TX
1999	Ph.D.	Molecular Medicine, Douglas Yee, MD, Advisor	University of Texas Health Science Center, San Antonio, TX

Postgraduate Training

YEAR	DEGREE	MAJOR	INSTITUTION/LOCATION
1999 – 2002	Post-Doctoral Fellow	Nephrology, Hanna E. Abboud, MD, Mentor	Dept of Medicine, Renal Division, UT Hlth Sci Center, San Antonio, San Anonio, TX.
2000 – 2002	Associate Investigator	Nephrology (Research)	Audie L. Murphy Memorial Veterans Hospital, South Texas Veterans Health Care System, San Antonio, TX.

Leadership Training

YEAR	PROGRAM	INSTITUTION/LOCATION
2007	Higher Education Resource Services (HERS) Management Institute	Wellesley College, Wellsley MA
2007	Professional Development Series (travel grant awardee)	American Society of Nephrology 2007 Meeting, Women in Nephrology

Committee Memberships

YEAR(S)	ORGANIZATION
2000 – 2004	Member, Research Enhancement Award Program (REAP) committee, Audie Murphy Memorial Veterans Hospital, San Antonio; San Antonio, TX
2003 – 2004	Member, REAP education and professional development committee, Audie Murphy Memorial Veterans Hospital, San Antonio, TX
2005	Member, Memorandum of Understanding (MOU) Policy Committee, Emory University
2005 – present	Member, Center for Cell and Molecular Signaling, Emory Univerisity
2005 – present	Member, Atlanta Veterans Administration Medical Center Institutional Animal Care and Use Committee (IACUC)
2006 – present	Member, Early Career Professional Development Advisory Committee, Emory University
2006 – present	Member, Women in Leadership Sub-committee of the President's Committee on the Status of Women, Emory University

Editorships and Editorial Board Service

YEAR(S)	ORGANIZATION
2005 – 2008	Member, Editorial Board of the Americal Journal of Physiology, Renal Physiology

Manuscript Review

YEAR(S)	ORGANIZATION
2002 – 2004	American Journal of Physiology, Cell; Blood; Journal of Pharmacological Experimental Therapeutics; Neoplasia; Experimental Medicine;
2003 – 2007	Americal Journal of Physiology, Renal Physiology;
2006 – 2007	Journal of Biological Chemistry; Americal Journal of Nephrology

Ad-hoc Grant Review

YEAR(S)	ORGANIZATION
2007	American Heart Asociation, Southwest Study Section

Selected Honors and Awards

YEAR(S)	ORGANIZATION
1998	Department of Defense pre-doctoral fellowship
1998	Outstanding Research Award, Institute of Biotechnology, Department of Molecular Medicine Annual Symposium
2000	Research Enhancement Award Program (REAP) training fellowship, Audie Murphy Memorial Veterans Hospital, South Texas Health Care System, and the Department of Medicine, Division of Nephrology, UTHSCSA
2002	Howard Hughes Medical Institute Junior Faculty Award, Department of Medicine, University of Texas Health Science Center, San Antonio, TX
2003	Junior Faculty Research Day Winner, Department of Medicine , University of Texas Health Science Center, San Antonio
2005	Presidential Early Career Award in Science and Engineering (PECASE)
2007	Graduate of the Higher Education Resource Services (HERS) Management Institute, Wellesley College, Wellesley MA.
2007	Elected Fellow, World Innovation Foundation (WFI).

Memberships in Professional Societies

YEAR(S)	ORGANIZATION
1999 – 2004	Endocrine Society
2003 - present	American Diabetes Association
2005 - present	American Society of Nephrology
2007 - present	American Physciological Society

Research Focus

Dr. Gooch's research is focused on the role of calcineurin in normal and disease processes in the kidney. Calcineurin is a serine/threonine phosphatase and is the target of pharmacological agents known to cause nephrotoxicity. Dr. Gooch's goal is to determine how calcineurin acts in the kidney in order to prevent nephrotoxicity.

Active Grant Support

2004 – 2008 NIH/NIDDK, RO1 DK066422-01, *Specificity of calcineurin signaling in the kidney*. (Jennifer Gooch, P.I.).

2005 – 2008 Department of Veterans Affairs MERIT, *Regulation of AQP2 trafficking by calcineurin*. (Jennifer Gooch, P.I.).

2006 – 2008 NIH/NIDDK, R21 DK074854-01, *Isoform-specific inhibition of calcineurin to prevent nephrotoxicity.* (Jennifer Gooch, P.I.).

2006 – 2011 Department of Veterans Affairs PECASE (Jennifer Gooch, awardee).

2007 - 2010 NIH/NIDDK, RO1 DK (Co-investigator), *Muscle-specific nutritional adaptations to catabolic stress* (SR Price, PI).

Completed Grant Support (previous 5 years)

2001 – 2002 Southern Arizona Foundation Equipment Grant, *Role of calcineurin in renal hypertrophy,* (Jennifer Gooch, P.I.).

2001 – 2002 South Texas Health Research Center Equipment Grant, *Role of calcineurin in renal hypertrophy,* (Jennifer Gooch, P.I.).

2002 – 2003 Howard Hughes Medical Institute New Faculty Startup, *Role of Calcineurin in Diabetic Nephropathy,* (Jennifer Gooch P.I.).

2003 – 2004 NIH/NIDDK George M. O'Brien Kidney Research Center, (Hanna Abboud, PI), *Project 5: Role of calcineurin in renal hypertrophy,* (Jennifer Gooch, P.I.).

2003 – 2004 American Heart Association, Texas Affiliate, Beginning Grant-in-Aid, *Mechanisms of IGF-I-mediated calcineurin signaling,* (Jennifer Gooch, P.I.).

2002 – 2005 Department of Veterans Affairs Merit Review Entry Program, *Mechanisms of IGF-I-mediated calcineurin signaling.* (Jennifer Gooch, P.I.) $132,000/year direct costs.

2002 – 2005 American Diabetes Association, Junior Faculty Award, *Role of calcineurin in diabetic nephropathy.* (Jennifer Gooch, P.I.) $120,000/year direct costs ($132,000/year total).

Patents

"Assay to measure calcineurin activity" US Provisional Patent Application 60/962,884

Formal Teaching

YEAR(S)	COURSE	HOURS	STUDENTS
2005	Journal Club	1	Renal Fellows
2006	Renal Physiology small group	8	MS I
2006	Renal Physiology lecture	1	MS I
2006	Introduction to Renal Fellowship	.5	Renal Fellows
2006	Fellows Bench Research Day	8	Renal Fellows
2007	Renal Physiogy small group	18	MS I
2007	Renal Physiology lecture	1	MS I
2007 - Fall	Exerecise Physiology small group	2	MS I
2007 - Fall	Exercise Physiology Lecture	2	MS I
2007 - Fall	Fellows Research/Grant writing day	1	Renal Fellows

Supervisory Teaching

YEAR(S)	TRAINEE	ROLE	CURRENT POSITION
2002 - 2003	Juan J. Toro, MD	Advisor, Masters of Science in Clinical Investigation, UTHSCSA	Instructor, UTHSCSA and Research Coordinator at the Audie Murphy Veterans Hospital, San Antonio, TX.
2004 - 2007	Scott Cobbs, PhD	Mentor, Post-doctoral fellowship, Emory SOM	MBA graduate
2005 - present	Tiffany Roberts	PhD dissertation Committee member, Emory	Graduate student
2005 - present	Maria Chacon-Heszele	PhD dissertation Committee member, Emory	Graduate student
2006 - present	Ramesh Reddy, DVM, PhD	Mentor, Post-doctoral, fellowship, Emory SOM	Post-doctoral fellow
2007 - present	Osama El-Minshawe, MD	Mentor, Research Fellowship, Emory SOM	Visiting Scholar on Sabbatical from El Minia University, El Minia Egypt
2007 - present	Juan Pena, MD	Menron, Post-doctoral fellowship, Emory SOM	Post-doctoral fellow

Invited Lectures

1997 Gooch JL, Lee AV, Yee D. "Interleukin-4 induces growth inhibition and apoptosis in breast cancer cells" Workshop presentation. 7th Annual Symposium on Cancer Research in San Antonio, San Antonio, TX.

1998 Gooch JL, Lee AV, Yee D. "Ligand dependent degradation of insulin receptor substrate-1 (IRS-1) in human breast cancer" Oral Presentation. 8th Annual Symposium on Cancer Research in San Antonio, San Antonio, TX.

1998 Gooch JL, Lee AV, Yee D. "Ligand dependent degradation of insulin receptor substrate-1 (IRS-1) in human breast cancer" Oral Presentation. Endocrine Society 80th Annual Meeting, New Orleans, LA.

1999 Gooch, JL, Van Den Berg CL, Yee D. "Inhibition of chemotherapy-induced apoptosis by insulin-like growth factor (IGF) -I involves both proliferative and anti-apoptotic mechanisms" Oral Presentation. 81st Annual Endocrine Society Meeting, San Diego, CA.

2002 Gooch, JL. "The role of calcineurin in diabetic nephropathy" Department of Medicine Research Seminar, University of Texas Health Science Center, San Antonio, San Antonio, TX.

2002 Gooch, JL. "The role of calcineurin in diabetic nephropathy" Invited lecture, Internal Medicine / Nephrology, University of Texas Southwestern Medical School, Dallas, TX.

2003 Gooch, JL. "Calcineurin – A new pathway for TGFβ-mediated regulation of ECM" Invited Lecture, Department of Pediatrics, University of Texas Health Science Center, San Antonio.

2003 Gooch JL, Guler R. "Calcineurin A-alpha is required for normal kidney development and function" Invited Lecture, Astrazeneca Young Investigator's Seminar series: Kidney development and disease, Experimental Biology Meeting, San Diego, CA.

2003 Gooch JL. "Specificity of calcineurin action in the kidney". Department of Molecular and Cellular Physiology, Louisiana State University, Shreveport. Shreveport, LA.

2003 Gooch JL. "Specificity of calcineurin isoforms in the kidney" Department of Anatomy and Cell Biology Seminar presentation, University of Kanses Medical Center, Kansas City, KA.

2003 Gooch JL, Guler RL, Vanden-Heuvel G. "Defects in nephrogenic zone development and mesangial cell proliferation in calcineurin A alpha knockout mice". Free communication, the American Society of Nephrology 36th Annual Meeting, San Francisco CA.

2003 Gooch JL, Guler RL, Suniga V, and Toro JJ. "Calcineurin A alpha is required for normal trafficking and function of AQP2". Free communication, the American Society of Nephrology 36th Annual Meeting, San Francisco CA.

2003 Gooch JL. "Kidney development and function in calcineurin A-alpha knockout mice" NHLBI, Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health, Bethesda, MA.

2004 Gooch, JL. "New insights into calcineurin function in the kidney" Department of Medicine, Renal Division Grand Rounds, Emory University Medical School, Atlanta, GA.

2004 Gooch JL. "New insights into calcineurin function in the kidney" Physiology seminar series, Department of Physiology, Emory University, Atlanta, GA.

2005 Gooch JL. "Calcineurin in protein regulation and trafficking" Physiology seminar series, Department of Physiology, Emory University, Altanta, GA.

2005 Gooch JL. "Inducible knockout of calcineurin isoforms" Dinner lecture series, Department of Physiology, Emory University, Altanta, GA.

2005 Gooch JL. "Calcineurin: A new factor in the trafficking of AQP2 through the endoplasmic reticulum" Free communication, the American Society of Nephrology 37th Annual Meeting, Philadelphia, PA.

2005 Gooch JL. "Calcineurin: A new factor in the trafficking of AQP2 through the endoplasmic reticulum" Oral presentation, Annual Southern Sand and Salt Water Club Meeting, Sarasota, FL.

2006 Gooch JL. "Calcineurin as a target for improved immune suppression" PECASE award presentation, Department of Veterans Affairs, Washington D.C.

2007 Gooch JL. "Calcineurin -let's start over at the beginning". Department of Medicine, Renal Division Grand Rounds, Emory University Medical School, Atlanta, GA.

2007 Willis B, Usher J, Gooch J, and Wong D. "PCSW Panel on the HERS Management Institute", Emory University, Atlanta, GA.

2007 Gooch JL. "Calcineurin - lets start at the beginning" Grand Rounds, Department of Medicine / Renal Division, Emory Unviersity, Atlanta, GA.

2007 Gooch JL. "Calcineurin - lets start at the beginning" Grand Rounds, Department of Medicine / Renal Division,Medical College of Georgia, Augusta, GA.

Bibliography

Published and Accepted Papers

1. Gooch JL, Lee AV, Yee D. Interleukin-4 (IL-4) induces growth inhibition and apoptosis in human breast cancer cells. *Cancer Research*. 58:4199-4205, 1998.

2. Lee AV, Jackson JG, Gooch JL, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Enhancement of the insulin-like growth factor signaling in human breast cancer: Estrogen regulation of insulin receptor substrate-1 (IRS-1) in vitro and in vivo. *Molecular Endocrinology*, 13(5): 787-796, 1999.

3. Gooch JL, Van Den Berg CL, Yee D. Insulin-like growth factor (IGF) -I rescues breast cancer cells from chemotherapy-induced cell death: proliferative and anti-apoptotic effects. *Breast Cancer Research and Treatment*, 56:1-10, 1999.

4. Gooch JL, Yee D. Strain-specific differences in the formation of apoptotic DNA ladders in MCF-7 breast cancer cells. *Cancer Letters*, 144:31-37, 1999.

5. Lee AV, Gooch JL, Osterreich S, Guler RL, Yee D. IGF-I-induced degradation of IRS-1 is mediated by the 26S proteosome and requires PI-3 kinase. *Molecular Cell Biology*, 5:1489-1496, 2000.

6. Gooch JL, Herrera R, Yee D. The role of p21 in IFN-gamma-mediated growth inhibition in human breast cancer cells. *Cell Growth and Differentiation*, 6:335-342, 2000.

7. Gooch JL, Tang Y, Gilroy-Ricono JM, Abboud HE. Insulin-like growth factor-I induces renal hypertrophy via a calcineurin-dependent mechanism. *Journal of Bioogical Chemistry* 276:42492-42500, 2001.

8. Gooch JL, Christy B, Yee D. Stat6 is required for IL-4-induced growth inhibition and apoptosis in breast cancer cells. *Neoplasia*, 4:100-104, 2002.

9. Gooch JL, Barnes JL, Garcia S, Abboud HE. Calcineurin is activated in diabetes and is required for glomerular hypertrophy and extracellular matrix regulation. *American Journal of Physiology, Renal Physiology*, 284: F144-F154, 2003.

10. Gooch JL, Gorin Y, Zhang B-X, Abboud HE. Involvement of calcineurin phosphatase in TGFβ-mediated regulation of extracellular matrix accumulation. *Journal of Biological Chemistry*, 279:15561-15570, 2004.

11. Gooch JL, Guler RL, Pergola PE, Barnes JL. Regulation of calcineurin A isoform expression in the diabetic kidney. *Journal of the American Society of Nephrology*, 15(6):1421-1429, 2004.

12. Gooch JL, Toro JJ, Guler RL, Barnes, JL. Calcineurin A-alpha but not A-beta is required for normal kidney development and function. *American Journal of Pathology* 165: 1755-1765, 2004.

13. Gooch JL, Guler RL, Barnes JL, Toro JJ. Loss of calcineurin A-alpha results in altered trafficking of AQP2 and is a new model of nephrogenic diabetes insipidus. *Journal of Cell Science*, 119:2468-76, 2006.

14. Alcalay NI, Brantley JG, Sharma M, Gooch JL, Vanden Heuvel GB. Ectopic expression of the homeobox gene Cux-1 restored metanephric growth inhibition by cyclosporin A. *Developmental Dynamics*, 236(1):184-91, 2007.

15. Gooch JL, Roberts B, Cobbs SL, and Tumlin JT. Calcineurin A-alpha mediates nephrotoxicity and alters expression of TGFbeta. *Transplantation*, 83(4):439-47, 2007.

16. Cobbs SL and Gooch JL. NFATc is required for TGFβ-mediated transcriptional regulation of fibronectin. *Biochem and Biphysl Res Comm*, 2007 Oct 19;362(2):288-94. 2007.

17. Roberts BR, Pohl J, Gooch JL. A fluorimetric method for determination of calcineurin activity. *Cell Calcium*, In Press. 2007.

Submitted Manuscripts

1. Gooch JL, Roberts BR, Kokko KE, El-Minshawe O, Tumlin JT. Racial differences in calcineurin activity of control and post-transplant patients, 2007.

2. Kelly FM, and Gooch JL. Development of lymphoproliferative disorder in mice heterozygous for calcineurin A-alpha. (In Preparation), 2007.

Reviews, editorials

1. Gooch JL. An emerging role for calcineurin A-alpha in the development and function of the kidney. (Invited Review). *American Journal of Physiology, Renal Physiology*, 290: F769-F776, 2006.

2. Gooch JL. Calcineurin inhibition and development *Clinical Enzyme Inhibition*. In Press, 2007.

Book Chapters

1. Yee D, Jackson JG, Weng C-N, Gooch JL, Lee AV. The IGF system in breast cancer. In: K. Takano, Hizuka N, Takahashi S-I (ed.). Molecular Mechanisms to regulate the activities of insulin-like growth factors, pp. 319-325: Elsvier Science B.V., Amsterdam, 1998.

Abstracts

1. Yee D, Gooch JL, Jackson JG. IGF-I, insulin, and IL-4 activate IRS-1 in human breast cancer cells: differential IRS1 tyrosine phosphorylation by IGF-I is associated with increased MAPK and PI3K activation. American Association of Cancer Research, 1996.

2. Jackson J, Gooch J, Yenish L, White M, Lee A, Yee D. Expression and activation of insulin receptor substrate-1 and -2 (IRS-1and -2) in human breast cancer cells, 78th Annual Endocrine Society Meeting, San Francisco, CA, 1996.

3. Gooch JL, Yee D. Interleukin-4 induces growth inhibition and apoptosis in breast cancer cells. UTHSCSA Cancer Research Day, June 1997.

4. Gooch JL, Lee AV, Yee D. Interleukin-4 induces growth inhibition and apoptosis in breast cancer cells. 7th Annual Symposium on Cancer Research in San Antonio. San Antonio, TX, 1997.

5. Gooch JL, Yee D. Interferon gamma signaling in breast cancer (Abstract). 20th Annual San Antonio Breast Cancer Symposium, San Antonio, TX. *Breast Cancer Research and Treatment* 46: 116, 1997.

6. Gooch JL, Van Den Berg CL, Yee D. Insulin-like growth factor (IGF) -I rescues breast cancer cells from chemotherapy-induced cell death: proliferative and anti-apoptotic effects. 21st Annual San Antonio Breast Cancer Symposium, December 1998.

7. Gooch JL, Yee D. Interferon-gamma mediated growth inhibition in human breast cancer cells: p21-dependent and p21=independent mechanisms. UTHSCSA Research Day, San Antonio, TX 1998.

8. Gooch JL, Yee D. Interferon-gamma mediated growth inhibition in human breast cancer cells: p21-dependent and p21=independent mechanisms. Department of Medicine Research Day, UTHSCSA Research Day, San Antonio, TX 1998.

9. Gooch JL, Jackson JG, Yee D. Interleukin-4 induced apoptosis is associated with STAT6 activation, IRS-1 phosphorylation, and activation of the SAPK pathway in human breast cancer cells. Keystone Symposia : Specificity in Signal Transduction, Lake Tahoe, NV, 1998.

10. Gooch JL, Lee AV, Yee D. Ligand dependent degradation of insulin-like growth factor-I (IRS-1) in human breast cancer. 8th Annual Symposium of Cancer Research in San Antonio, July 1998.

11. Lee AV, Jackson JG, Gooch JL, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Enhancement of the insulin-like growth factor signaling pathway by estrogen in human breast cancer cells. 80th Annual Endocrine Society Meeting, New Orleans, LA, June 1998.

12. Gooch JL, Lee AV, Yee D. Insulin receptor substrate-1 (IRS-1) is required for insulin-like growth factor-I (IGF-I) but not interleukin-4 (IL-4) mediated growth effects in human breast cancer cells. Department of Medicine Research Day, San Antonio, TX 1999.

13. Gooch JL, Jackson JG, Yee D. Interleukin-4 induced apoptosis is associated with STAT6 activation, IRS-1 phosphorylation, and activation of the SAPK pathway in human breast cancer cells. National Student Research Forum. Galveston, TX, 1999.

14. Gooch JL, Tang Y, Gilroy-Ricono JM, Abboud HE. Insulin-like growth factor-I (IGF-I) induces renal hypertrophy via a calcineurin-dependent mechanism. American Society of Nephrology 33rd Annual Meeting, Toronto, Canada, 2000.

15. Gooch JL, Tang Y, Garcia S, Abboud HE. Inhibition of calcineurin blocks diabetic renal hypertrophy. American Society of Nephrology 34th Annual Meeting, San Francisco, CA, 2001.

16. Gooch JL, Gorin Y, Tang Y, Abboud HE. TGFβ-mediated activation of Erk1/Erk2 and induction of ECM synthesis is associated with activation of calcineurin phosphatase and generation of reactive oxygen species (ROS). American Society of Nephrology, 34th Annual Meeting, San Francisco, CA, 2001.

17. Gooch JL, Gorin Y, Zhang B, Abboud HE. TGFβ mediates extracellular matrix accumulation in mesangial cells through a pathway that includes generation of reactive oxygen species, mobilization of calcium, and activation of calcineurin phosphatase. Department of Medicine Research Day, May 2002.

18. Gooch JL, Gorin Y, Zhang B, Abboud HE. TGFβ mediates extracellular matrix accumulation in mesangial cells through a pathway that includes generation of reactive oxygen species, mobilization of calcium, and activation of calcineurin phosphatase. American Society of Nephrology 35th Annual Meeting, Philadelphia, PA October 2002.

19. Gooch JL, Guler RL, Barnes JL. Regulation of calcineurin A isoforms in the diabetic kidney. Society for Experimental Biology and Medicine Annual Meeting, San Diego, CA, April 2003.

20. Gooch JL, Guler RL. Calcineurin A alpha is required for normal kidney development and function. Society for Experimental Biology and Medicine Annual Meeting, San Diego, CA, April 2003.

21. Gooch JL and Suniga V. Cross-talk between calcineurin and MAPK is involved in TGFbeta-mediated regulation of fibronectin. American Society of Nephrology, 37th Annual Meeting, St. Louis, IN, 2004.

22. Gooch JL. Calcineurin A-alpha knockout is a new model of nephrogenic diabetes insipidus. Nephrogenic Diabetes Insipidus Foundation 2004 Global Conference, Phoenix Arizona, 2004.

23. Gooch JL. Indications of nephrotoxicity with loss of calcineurin A-alpha but not A-beta. Society for Experimental Biology and Medicine Annual Meeting, San Diego, CA, April 2005.

24. Gooch JL. Cross-talk between calcineurin and MAPK is involved in TGFbeta-mediated regulation of fibronectin. American Society of Nephrology, 37th Annual Meeting, St. Louis, IN, 2004.

25. Gooch JL. Indications of nephrotoxicity with loss of calcineurin Aalpha but not Abeta isoform. American Society of Nephrology, 38th Annual Meeting, Philadelphia, PA, 2005.

26. Cobbs SC and Gooch JL. NFATc is required for TGFbeta-mediated regulation of fibronectin. American Society of Nephrology, 38th Annual Meeting, Philadelphia, PA, 2005.

27. Kelly FM, and Gooch JL. Development of lymphoproliferative disorder in mice heterozygous for calcineurin A-alpha. American Society of Nephrology, 39th Annual Meeting, San Diego, CA, 2006.

28. Gooch JL, Klein RL, and Roberts BR. Calcineurin regulates AQP2 trafficking in the ER. American Society of Nephrology, 39th Annual Meeting, San Diego, CA, 2006.

29. Roberts BR, Tumlim JA, and Gooch JL. Activity of pharmacological inhibitors of calcineurin is dependent upon relative expression of isoforms of the catalytic subunits. American Society of Nephrology, 39th Annual Meeting, San Diego, CA, 2006.

30. Cobbs SL and Gooch JL. NFATc is required for TGFβ-mediated transcriptional regulation of fibronectin. American Society of Nephrology, 39th Annual Meeting, San Diego, CA, 2006.

CURRENT AND FUTURE RESEARCH PLANS

INTRODUCTION:

Calcineurin is well known as the enzyme target of the immunosuppressant drugs cyclosporin A and FK506. Use of calcineurin inhibitors (CIs) increased one-year survival following organ transplantation from 5% to over 80% and remain the cornerstone of post-transplant therapies. Unfortunately, prolonged treatment with CIs frequently causes serious side-effects including skin disorders, cancer, and renal failure. CIs are known to suppress T cell function by blocking activation of nuclear factor of activated T cells (NFAT) transcription factors ([1]). However, actions of calcineurin in other tissues including the kidney have not been fully investigated.

Previously, I became interested in calcineurin as a mediator of growth factor-induced cell hypertrophy and extracellular matrix induction in glomerular mesangial cells. I found that calcineurin was required for IGF-I-and TGFβ-mediated matrix accumulation [2, 3] and IGF-I-mediated hypertrophy [2]. These pathways are rapidly activated in the diabetic kidney and regulate matrix expansion and compensatory hypertrophy. Consistent with in vitro data, inhibition of calcineurin with cyclosporin reduced glomerular matrix expansion and hypertrophy in diabetic rats [4]. While calcineurin inhibition reduced matrix expansion in glomeruli, cyclosporin alone was sufficient to induce matrix proteins in the renal tubulo-interstitium and produced an additive effect with diabetes [4]. This experiment proved to be an elegant demonstration of cell-specific calcineurin function and identified a signaling system where calcineurin inhibition in the kidney does not lead to fibrosis. As a result, the broad goal of my research became understanding mechanisms of calcineurin signaling specificity.

Isoforms may mediate cell-specific actions of calcineurin. One important mechanism for cell specific action that we investigated was the contribution of different isoforms of the catalytic subunit of calcineurin. Calcineurin is a multi-subunit enzyme. Full activity requires interaction of catalytic (A) and regulatory (B) subunits, calmodulin and calcium [5]. Our work has now made clear that isoforms of the (A) subunit have unique functions that need to be fully explored.

To investigate how isoforms contribute to cell-specific calcineurin action, we obtained mice that lack either the Aα or the Aβ isoform. Both models had been previous created [6, 7], but kidney development and function had never been studied. Data showed that the Aα isoform is more important to kidney development and function than the Aβ [8, 9]. Moreover, when the phenotypes of Aα-/- mice were compared with cyclosporin-treated wildtype mice, many features of nephro-toxicity were present with loss of the Aα isoform but absent in mice lacking Aβ [9]. We also found a number of other phenotypic changes in Aα-/- mice that are present in humans taking calcineurin inhibitors including hyperlipidemia, anorexia, and lymphoproliferation [8, 10]. Thus, we concluded that loss of Aα was most likely responsible for many clinical manifestations of CI toxicity. Importantly, previously published reports showed, and work in our lab confirmed, that loss of the Aα isoform *does not significantly impair T cell function*. Mice with Aα-/- immune systems can still be suppressed with cyclosporin [6] and aged α heterozygous mice with severe nephrotoxicity have normal levels of IL2 [10], a cytokine closely associated with calcineurin action in T cells.

Study of Aβ-/- mice shed further light on calcineurin action in the immune system. Bueno et al reported a severe loss of mature T cells in Aβ-/- mice that mirrored the effects of cyclosporin on wildtype mice [7]. Moreover, Aβ-/- mice were resistant to graft rejection, similiar to cyclosporin-treated wildtype mice. Taken together, we conclude that the Aα and Aβ isoforms function in very different ways - ***the Aα isoform is required for normal kidney function while the Aβ regulates T cell activity.*** This finding presents an important clinical opportunity to improve CI therapy: targeted inhibition of the Aβ isoform to produce immune suppression without nephrotoxicity.

OBJECTIVES:

I. INVESTIGATE THE SPECIFIC ROLES OF CALCINEURIN ISOFORMS. *Rationale:* Calcineurin Aα and Aβ are products of two different genes. While the isoforms are closely related,

there are small regions of divergence in the sequences [1]. Moreover, mice lacking Aα and Aβ have very different phenotypes, suggesting that the two isoforms have specific functions. We have generated cell lines from kidneys of wildtype, Aα-/-, and Aβ-/- mice [9] and we find difference in upstream signals as well as downstream processes with loss of each isoform. Thus, one important area of work in my laboratory is to investigate upstream signals and downstream processes of each isoform.

A. Calcineurin Aα: an integral part of endoplasmic reticulum function. Previously, we reported that Aα mice have a urine concentrating defect and that AQP2 does not translocate to the membrane [11]. Further investigation showed that AQP2 is not localized to intracellular vesicles and remains trapped inside the endoplasmic reticulum (ER). In a project funded by a Department of Veterans Affairs MERIT award, I proposed experiments to determine the role of calcineurin in the ER. We find that calcineurin can dephosphorylate the ER chaperone protein calnexin. In the absence of calcineurin activity, calnexin-mediated chaperone function is altered, leading to aberrant trafficking of target proteins. In addition to AQP2, we find that a number of proteins including TGFβ are dysregulated in the absence of Aα. While there are numerous reports in the literature that calcineurin inhibitors can result in upregulation of TGFβ, we were the first to identify a mechanism to explain this observation. We hypothesize that loss of Aα alters ER protein sorting and results in aberrant trafficking of a wide range of targets including AQP2 and TGFβ. Current and future work is therefore directed toward the following experiments: 1) Establish the upstream signals that activate Aα - eg cAMP, IP_3 PLCγ; 2) Investigate what processes are dependent upon Aα activity including ER-associated degradation, unfolded protein response; and 3)Determine the consequences of Aα inhibition on normal protein trafficking, ER stress, and hypertrophy.

B. Calcineurin Aβ: regulator of NFATc-mediated transcription. We have found that Aβ regulates distinct downstream targets from that of Aα. Specifically, fibroblasts lacking the Aβ isoform no longer regulate NFATc [9]. As a result, we obtained mice that constitutively express an NFATc-responsive luciferase promoter and crossed the mice with our Aα and Aβ lines. Resulting offspring allow us to measure NFATc transcriptional activity in wildtype, Aα-/- and Aβ-/- tissues. Consistent with our in vitro data, we find a dramatic loss of NFATc-luciferase activity in Aβ-/- but not Aα-/- kidneys. In 2004, I received an NIH RO1 award to determine why calcineurin inhibition blocks extracellular matrix accumulation in glomerular mesangial cells but induces it in epithelial cells. Recent work on this project revealed that NFATc is required for up-regulation of fibronectin [12]. Since other in vitro experiments and data from NFATc-luciferase mice indicate that NFATc is regulated specifically by the Aβ isoform, we speculated that matrix proteins would not be upregulat-ed in diabetic Aβ-/- glomeruli. Recent experiments confirm this scenario. We therefore hypothesize that Aβ specifically regulates NFATc transcription factors. One downstream target is upregulation of matrix proteins including fibronectin. Our current and future work is therefore directed at the following: 1) Establish upstream signals required for activation of Aβ; 2) Determine what processes are dependent upon Aβ activity in addition to NFATc including kidney-specific transcription co-factors; and 3) Investigate the result of Aβ inhibition on matrix accumulation, growth factor signaling.

II. GENERATE NOVEL EXPERIMENTAL AND CLINICAL TOOLS TO STUDY CALCINEURIN ISOFORMS. *Rationale:* The bulk of my research is directed toward novel areas of investigation. Therefore, one focus of my laboratory is designing, evaluating, and implementing new technologies to enhance our research capabilities. We have shown that the isoforms of calcineurin have unique roles. As such, we are pursuing the generation of inducible, isoform-specific null mice, further developing our new fluorimetric method to measure isoform-specific calcineurin activity, and identifying compounds that selectively target Aα and Aβ isoforms.

C. Evaluate inducible, isoform-specific knockout mice to validate our model that the Aβ isoform regulates immune activity while the Aα isoform is vital in the kidney. Aα-/- mice have developmental defects that severely affect renal function [8]. Therefore, one goal in my laboratory is to create inducible, isoform-specific knockout mice. I am currently funded by an NIH R21 award to create

inducible Aα and Aβ knockout mice. Briefly, we have generated constructs with exons from the catalytic domains of the Aα and Aβ genes flanked by LoxP sites. Mice will be made with the "floxed" genes replacing the endogenous sites and then crossed to mice carrying a Cre recombinase protein that is fused to a modified estrogen receptor. Tamoxifen injection stimulates nuclear localization of the Cre enzyme and facilitates excision of the targeted exons throughout the mouse. This method will allow us to create isoform-specific null mice at any age. Once the mice are obtained, we will evaluate a number of important questions under controlled isoform selectivity.

The initial set of experiments we will evaluate transplant tolerance and nephrotoxicity. In collaboration with Drs. Ken Kokko and Christian Larsen (Emory SOM), we will perform skin transplants on adult Aα- and Aβ-/- mice. We will then follow both rejection and kidney function to determine if selective loss of either isoform produces a different result. We anticipate that rejection will be delayed in Aβ-/- mice and kidney function will be normal. In contrast Aα-/- mice will have no improved graft tolerance compared to wildtype mice and will develop nephrotoxicity.

D. Expand of our newly developed calcineurin assay to detect specific isoforms. Common methods to measure calcineurin activity have technical difficulties/obstacles. To facilitate our research objectives, we created a novel assay to measure calcineurin activity. We validated the assay [13] and now routinely use this method. Furthermore, with the Emory Office of Technology Transfer, I have submitted an application to pursue patenting and commercialization of this method. Our assay uses an amino acid sequence from the RII subunit of protein kinase A as a substrate, which both Aα and Aβ can dephosphorylate. In order to modify the assay to detect isoform-specific activity, we must either identify other peptides with isoform specificity or find a selective inhibitor to "subtract" the activity of one isoform (discussed further in *part E*).

E. Pursue mechanisms of isoform-specific modulation/inhibition in vitro and in vivo. The majority of my research is now focused on the possibility that selectively targeting calcineurin isoforms will produce new, safer therapeutic options. Our in vitro experiments will identify differences in upstream and downstream actions of the isoforms and characterize specific effects due to loss of each isoform. Using inducible, isoform-specific knockout mice we will perform proof-of-principle experiments that immune suppression and nephrotoxicity can be separated. These experiments will be aided by our novel assay methods and unique experimental models. Finally, we will pursue means to selectively inhibit the activity of each isoform.

In collaboration with Dr. Dennis Liotta (Emory University), we propose to screen peptide and chemical libraries for agents that interact with unique regions in the Aα and Aβ proteins. Using our high throughput assay, we will then screen candidate compounds for isoform selectivity. While Aβ selective agents would seem to have the most obvious therapeutic potential, agents that selectively inhibit the Aα isoform or that preferentially activate either isoform could also have research and clinical significance.

SIGNIFICANCE

I have developed a novel area of research into the specific roles of calcineurin isoforms. My data suggest that Aα and Aβ are activated by different upstream signals and act on separate downstream targets. Inhibition of each isoform produces different effects in vitro and in vivo. Objective I is directed toward understanding basic cellular actions of calcineurin isoforms. Objective II addresses our need for novel tools and reagents including inducible, isoform-specific knockout mice, techniques to study isoform-specific activity, and means to selectively modify isoform activity. Future work will be directed toward applying these new data and techniques to improve clinical calcineurin inhibitor use. My work and that from other laboratories form a compelling case that pathways associated with the Aβ isoform will lead to better immune suppression with fewer side-effects. The experiments described in this proposal are an overview of my current and future goals toward this objective.

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Racial differences in the inhibition of calcineurin in stimulated T cells

Jennifer L Gooch[1,2], Brian R Roberts[1], Kenneth E Kokko[1], Osama El Minshawy[1,3], James A Tumlin[1]. [1]Medicine / Nephrology, Emory University, Atlanta, GA, [2]Research, Atlanta Veterans Affairs Medical Center, Decatur, GA, [3]On sabbatical leave, El minia University, El Minia, Egypt

The introduction of calcineurin inhibitors (CIs) greatly improved short-term graft survival but long-term outcomes have not significantly improved. While multiple factors can effect organ survival, as a group, African American (AA) transplant recipients suffer higher rates of rejection than other racial groups. Despite intense effort, no explanation for this difference has been identified. The aim of this study was to determine if differences in sensitivity to CIs contributes to racial disparities. T cells were isolated from control volunteers and renal transplant recipients and calcineurin stimulation and response to CIs was determined. T cell CaN activity was significantly increased in response to CD3/CD28 co-stimulation in control but not transplant subjects. Despite comparable blood levels of FK506, T cell activation was markedly higher in AA patients compared to Caucasians (CC). Similarly, both FK506 and cyclosporin A failed to inhibit calcineurin activity in T cells of AA control subjects. Finally, increased stimulation of CaN activity was associated with elevated production of cytokines including IL-3, IL-4, and TGFâ. Consistent with this, AA transplant patients had significantly higher serum levels of TGFâ compared to CC. These data suggest that reduced efficacy of CIs at blocking T cell calcineurin activity may contribute to worse long-term outcomes of AA patients.

Current and Pending Grant Support
Jennifer L. Gooch, PhD

ACTIVE:

Source:	Department of Veterans Affairs - PECASE
Title:	Regulation of AQP2 trafficking by calcineurin
Period:	10/01/06 – 09/31/011
Total funding:	$125,000 total
% effort:	n/w
Role:	PI
Goals:	This award is additional support of another VA-funded project to identify mechanisms of calcineurin-mediated regulation of AQP2 trafficking.

Source:	National Institutes of Health, NIDDK, R21 DK074854-01
Title:	Isoform-specific inhibition of calcineurin to prevent nephrotoxicity
Period:	04/01/05 – 03/31/07
Total funding:	$273,000 total
% effort:	10%
Role:	Principle Investigator
Goals:	The goals of this project are to create inducible isoform-specific knockout mice and investigate transplant rejection and nephrotoxicity.

Source:	Department of Veterans Affairs - MERIT
Title:	Regulation of AQP2 trafficking by calcineurin
Period:	10/01/05 – 09/31/07
Total funding:	$494,900 total
% effort:	50%
Role:	PI
Goals:	The goal of this project is to identify mechanisms of calcineurin-mediated regulation of AQP2 trafficking.

Source:	National Institutes of Health, NIDDK, R01 DK066422-01
Title:	Specificity of calcineurin signaling in the kidney
Period:	07/01/04 – 06/31/08
Total funding:	$797,370 total
% effort:	30%
Role:	PI
Goals:	The goals of this project are to identify mechanisms of calcineurin specificity in the kidney by examining transcriptional regulation of fibronectin by NFATc, differential targets of calcineurin dephosphorylation, and the role of alpha and beta isoforms in calcineurin action.

Source:	National Institutes of Health (PR Price, PI) , NIDDK, 2RO1 DK050740
Title:	Muscle-specific nutritional adaptations to catabolic stress

Period:	07/01/07 – 06/31/10
Total funding:	$1,950,000 total
% effort:	5%
Role:	Co-investigator
Goals:	The goals of this project are to investigate the role of FOXO transcription factors in protein degradation in response to catabolic stress.

PENDING:

Source:	Department of Veterans Affairs - MERIT (renewal)
Title:	The role of calcineurin in calcium-activated protein sorting
Period:	10/01/08 – 09/30/12
Total funding:	$800,400
% effort:	50%
Role:	PI
Goals:	The goals of this project are to investigate a novel role for calcineurin Aα in calcium activated protein trafficking of target proteins including AQP2.

Source:	National Institutes of Health, NIDDK, R01 DK066422 (renewal)
Title:	Specificity of calcineurin signaling in the kidney
Period:	07/01/08 – 06/31/013
Total funding:	$1,912,499 total
% effort:	30%
Role:	PI
Goals:	The goals of this project are to identify mechanisms of calcineurin specificity in the kidney by identifying unique upstream and downstream signaling properties of calcineurin isoforms and by targeting the unique action of the beta isoform on NFATc.