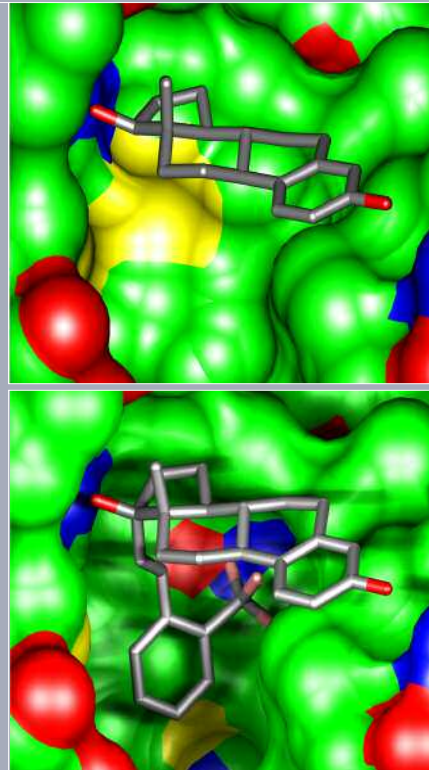
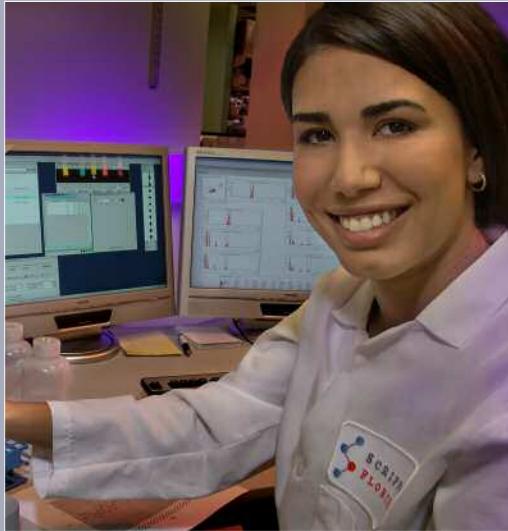


Cancer Biology

A novel binding pocket in the estrogen receptor. X-ray crystallography reveals a novel pharmacologic class of synthetic estrogens, which induce a unique restructuring of the ligand-binding pocket. Top panel, Part of the estrogen receptor ligand-binding pocket bound to estradiol. Bottom panel, The novel, extended region of the binding pocket when it is occupied by trifluoromethyl phenylvinyl estradiol, a 50-pM-affinity ligand. The 30%–40% increase in the size of the binding pocket indicates a new site for targeted chemistry, which can be used to develop improved therapeutic agents for breast cancer and other estrogen-responsive disorders. Reprinted with permission from Nettles, K.W., Bruning, J.B., Gil, G., et al. Structural plasticity in the oestrogen receptor ligand-binding domain. *EMBO Rep.* 8:563, 2007.





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Chairman's Overview

The Department of Cancer Biology was launched last year at the Florida campus, with close ties to cancer researchers at the California campus.

Broadly, the goals of our research programs are to understand the molecular pathogenesis of cancer. We focus on pathways directed by oncogenes and tumor suppressors that control cell division, growth, survival, and differentiation and that modify the response to therapeutic agents. The 7 faculty members currently in the department use a battery of state-of-the-art technologies, from biochemistry and cell biology to preclinical models to x-ray crystallography, for target discovery and validation. We have developed unique models to evaluate the efficacy of new leads in cancer prevention and therapeutics.

Our goal is to understand the molecular underpinnings of all of the major human cancers, but we are also interested in cancer in infants and children, the interplay between cancer and the immune system, and the relationships between aging and cancer. One of the many strengths at the Florida campus is the access to high-throughput technologies that enable investigators to explore potential leads quickly by using both genetic and small-molecule screens. Collaborations with the major cancer centers in Florida and with cancer researchers at the California campus will enable us to convert leads into translational and clinical studies.

INVESTIGATORS' REPORTS

Myc-Mediated Pathways in Cancer and Development

J.L. Cleveland, M.A. Hall, F.C. Dorsey, R. Rounbehler, D. Sofia, J. Doherty, M. Steeves, C. Yang, T. Bratton, W. Li

Myc oncogenes (*c-Myc*, *N-Myc*, and *L-Myc*) are activated in approximately 70% of all human cancers. This activation occurs directly via gene amplification or chromosomal translocations or indirectly via alterations in signal transduction pathways or loss of tumor suppressors that normally regulate and/or harness expression of the oncoprotein Myc. Myc oncoproteins function as transcription factors, and, in part, their activation in cancer reflects their essential roles as regulators of cell growth and division; they regulate up to 10% of the genome. In addition, Myc overexpression triggers accelerated rates of cell proliferation, a situation that puts malignant cells at a considerable advantage. Myc is also essential for the tumor angiogenic response and plays key roles in tumor cell metastasis. However, in normal cells, the hyperproliferative response provoked by Myc also triggers apoptosis, and mutations in regulators of this cell death response are a hallmark of most malignant neoplasms.

We have used mouse models to dissect the contribution of key targets downstream of Myc that control tumor development. These studies have shown that Myc triggers apoptosis through the Arf-p53 tumor suppressor pathway that is inactivated in most malignant tumors and by selectively affecting the expression of members of the Bcl-2 family of proteins that directly control the intrinsic apoptotic pathway. We have shown that mutations in these pathways are a hallmark of Myc-driven cancers in mice and humans. Genetic studies have established the key roles of these pathways in controlling tumor development and metastasis and in disabling differentiation programs. In addition, we have shown that Myc itself also often acquires somatic mutations during tumor progression that selectively disable its ability to activate some apoptotic pathways.

Although apoptotic regulators clearly act as guardians against Myc-induced tumorigenesis, we have found that the ability of Myc to provoke accelerated cell growth is also critical for tumorigenesis. First, Myc coordinately regulates the expression of cytokines that direct tumor

angiogenesis, and this regulation occurs through a circuitous route: Myc regulates the transcription of regulatory factors that in turn control the half-life of mRNAs that encode angiogenic cytokines. Second, Myc suppresses the expression of the universal cyclin-dependent kinase inhibitor p27^{Kip1} by inducing the expression of the Cks1 component of the SCF^{Skp2} E3 ubiquitin ligase complex that targets p27^{Kip1} for destruction by the 26S proteasome. Accordingly, loss of *Cks1*, the gene for Cks1, disables the ability of Myc to suppress p27^{Kip1} and markedly impairs Myc-induced proliferation and tumorigenesis, whereas loss of the gene for p27^{Kip1} accelerates Myc-induced tumorigenesis. Remarkably Cks1 overexpression is a hallmark of all lymphomas with Myc involvement, and this pathway is apparently not bypassed during lymphoma development (high levels of nuclear, and thus presumably functional, p27^{Kip1} are expressed in Myc-induced, Cks1-deficient lymphomas). Finally, our recent studies indicate that Myc also induces the expression of components of other SCF complexes, suggesting this pathway is a general route by which Myc coordinates cell growth and division.

Because Myc regulates such a large number of genes and is essential for cell growth and division, agents that directly target the transcription functions of Myc are likely to also effect the growth of normal cells. Therefore, we have focused on key transcription targets that might be suitable therapeutic targets. Indeed, we have shown that inhibiting ornithine decarboxylase, a direct transcription target of Myc and the rate-limiting enzyme of polyamine biosynthesis, impairs Myc-induced proliferation and transformation. These results were underscored by our findings that heterozygosity in the gene for ornithine decarboxylase, a condition that only reduces the enzyme activity of ornithine decarboxylase and the generation of its product by half, triples the life span of tumor-prone mice. Thus, agents that target the polyamine pathway have promise in both the prevention and treatment of cancer. Currently, we are defining the mechanism by which targeting ornithine decarboxylase disables the proliferative response of Myc.

We recently discovered that additional Myc transcription targets that can be exploited in cancer therapy include components of the autophagy pathway, an ancient survival pathway that directs the digestion of bulk cytoplasmic material and organelles when cells are faced with nutrient- or oxygen-deprived conditions, a scenario that certainly applies to the tumor microenvironment. We have shown that agents that disable

autophagy have tremendous potential in cancer prevention and treatment. Currently, we are defining the mechanism by which Myc regulates the expression of genes associated with the autophagy pathway and the potential of these genes as targets for cancer therapeutic agents.

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Steady-State T-Cell Lymphopoiesis

H.T. Petrie, A. Griffith, H. Nakase, M. Chang, J. Shi, M. Fallahi, K. McKevitt, B. Torres

Our main area of research is identification of intrathymic processes that induce bone marrow stem and/or progenitor cells to differentiate into cells of the T-lymphocyte lineage. Like most hematopoietic cells, T cells must be made not only during embryonic development but also throughout life; they are lost because of a variety of causes, including bleeding, cellular senescence, and immune activation. Deficiencies in the production of new T lymphocytes lead to a marked increase in susceptibility to infectious diseases, particularly viral diseases. The causes of such deficiencies may be congenital or acquired (as a consequence of exposure to retroviruses, chemotherapeutic drugs, or environmental agents) or may occur naturally as a consequence of aging. By understanding the production of T lymphocytes in normal circumstances, we hope to define the

underlying causes of diseases and autoimmune disorders due to T-cell deficiencies and to develop strategies that can be used to correct these abnormalities.

Both in the embryo and after birth, the main site of T-cell production is the thymus. One of the unique features of T-cell production by the thymus after birth is that the stem cells that perpetuate this process do not reside within the organ. Instead, new T lymphocytes are derived from bone marrow-derived stem cells circulating in the bloodstream. Consequently, the ability of the thymus to attract circulating stem cells out of the bloodstream is a crucial and obligatory first step in T-cell production. Remarkably, nearly nothing is known about the signals that induce stem cells to leave the blood and enter the thymus. We think that defining such signals is critical for developing new strategies to correct deficiencies in T-cell production. To this end, we first sought to define the anatomic sites where bone marrow-derived stem cells enter the postnatal thymus under normal conditions, because these sites are where such signals must be found.

We found that blood-borne cells enter the thymus through a particular type of blood vessels, postcapillary venules, that are found only in the perimedullary cortex, a very restricted tissue region. Using laser-based microdissection, we have developed a method to isolate these specific blood vessels, and we are using microarray screening to reveal the homing mechanism, including chemoattractive stimuli, ligands for adhesion of progenitor cells, and various other related factors required for the recruitment process. In parallel, we are using microarray technology to characterize cells that home to the thymus to evaluate expression of the appropriate receptors, counterreceptors, or adhesion molecules and to determine the lineage potential and proliferative capacity of the cells. This double-sided approach of homing sites and homing cells permits both greater scrutiny and built-in validation, and it has allowed us to rapidly move forward in an area that has long remained enigmatic.

Once blood-borne stem cells enter the thymus, T-lymphocyte production begins. Each stem cell is induced to proliferate and give rise to approximately 1 million immature thymocytes. Like the stem cells from which they are derived, the earliest intrathymic cells have the potential to give rise to numerous other types of blood cells. However, the environment provided by the thymus causes these multipotential precursors to undergo a series of developmental changes

that result in commitment to the T-lymphocyte lineage. Again, the signals that the thymus provides to induce this commitment and the subsequent proliferation of T cells are poorly understood. To date, only 3 critical interactions between the thymus and developing lymphoid cells have been defined (Notch, c-kit, and IL-7R), and even in these cases, the precise function of these signals is not clear.

To identify novel signals, we first determined the positional context of each stage of development of early progenitor cells. We found that the different developmental stages could be mapped to identifiable subregions of the cortex or the medulla of the thymus. By correlating these locations with the known functions at each developmental stage, we were able to generate a functional map of stromal signaling microenvironments in the thymus. Using this functional map, we are determining how each stromal region differs from the other regions and what unique signals stromal cells in each region deliver to developing lymphocytes. For these studies, we are using microdissection of each mapped region and then *in silico* subtraction of those genes expressed by the lymphoid cells. This method has allowed us to define, for the first time, global transcription signatures for stromal cells from the thymic cortex and medulla and from functionally defined subregions of each compartment. We are now correlating molecules secreted by stromal cells or found on the surface of stromal cells with the corresponding receptors on developing lymphocytes to identify new, region-specific signals for T-cell development. Ultimately, we wish to evaluate the roles of these signals in various immunodeficient states, including age-related immune disorders, and to develop strategies for correcting these immunodeficiencies through transplantation and/or gene therapy. Further, this unique stromal database has allowed us to understand the biology of thymic stromal cells at an unprecedented level and to define region-specific stromal promoters that can be used for region-specific overexpression or conditional gene deletion. We expect that these topics will provide a strong base for studies for many years to come.

The same methods that enabled us to characterize stromal signaling regions in the thymus have also provided tools uniquely suited for studies of the enigmatic process of thymic involution with age. Progressive deterioration of the thymus, beginning around the time of puberty, is primarily a consequence of aging thymic stroma. During the past few years, it has become increasingly clear that age-related decreases in the production

of naive T cells leads to the selective accumulation of memory T cells and, ultimately, to accumulation of "holes" in the immune repertoire. These immune insufficiencies result in increased susceptibility to infectious disease and in a decreased ability to resolve infections. Further, deterioration of thymic stroma, and the decreased efficiency in negative selection that results, probably plays a role in the advent of age-related autoimmune diseases, including diabetes, arthritis, and atherosclerosis. Of note, the thymus can be induced to completely regrow, although the best means for doing so—castration—is somewhat undesirable.

Using microdissection, microarrays, and *in silico* subtraction techniques, we can now analyze the differences between thymic stromal cells from young or old mice and during the initiation, log growth, and termination phases of thymic regeneration after induction of regrowth by castration. These methods provide us a unique opportunity to clearly characterize the molecular biology of aging in thymic stromal cells and the requirements for induction, sustenance, and termination of thymic regrowth. In a collaborative effort with scientists at StromaCyte Corporation, Jupiter, Florida, we will use the data generated by these studies to identify therapeutic targets that can be used to induce regrowth of the thymus without the need for castration.

Finally, we are interested in the use of T-cell reconstitution after bone marrow transplantation to treat hematopoietic or lymphoid malignant neoplasms. Particularly in adults, reconstitution of the pool of the naive T cells is poor, and, consequently, transplant recipients may succumb to otherwise trivial infections such as those caused by cytomegalovirus or Epstein-Barr virus. In most cases of transplantation in adults, the T cells that do eventually reconstitute the host are donor T cells that are neither tolerant nor naive. Rather, they are homeostatically expanded, donor-derived T cells with a memory phenotype.

Using a novel mouse model for thymic reconstitution, we found that the almost complete inability of the thymus in adults to make new T cells after bone marrow transplantation is due to the exquisite sensitivity of thymic stromal cells to chemotherapeutic or myeloablative drugs and radiation. We are now identifying the changes induced by such treatments in thymic stromal cells. Using these data and a better understanding of normal stromal cell biology and metabolism, we hope to define strategies that can be used either prophylactically to protect thymic tissue during chemotherapy or afterward to help regenerate it.

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Novel Regulators of the Anaphase-Promoting Complex

N. Ayad, D. Harmey, S. Simanski, L. Owens

The anaphase-promoting complex (APC) is an essential regulator of the eukaryotic cell cycle. New findings suggest that the APC integrates signals from multiple pathways to induce particular cell-cycle transitions. However, no unbiased genome-wide approach has been used to determine the signaling pathways that regulate APC activity. We used a cell-based screening method to identify APC regulators that are potential mediators of signaling pathways. We cotransfected 14,000 cDNAs with the N terminus of the APC substrate cyclin B fused with luciferase and identified several novel regulators of the APC (Fig. 1).

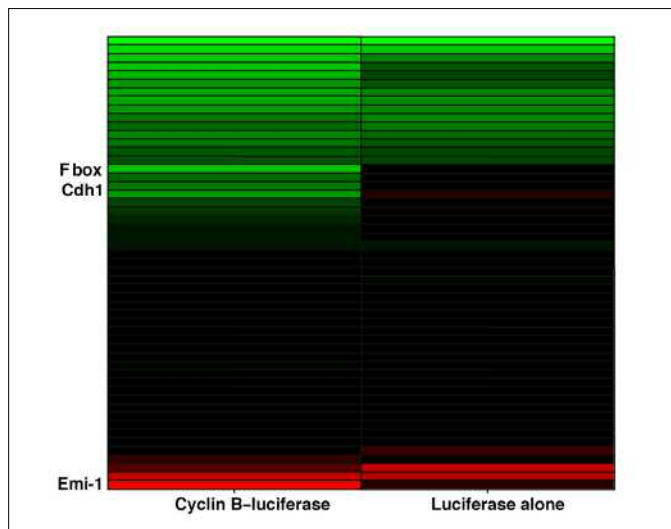


Fig. 1. The N terminus of the APC substrate cyclin B was fused to luciferase and used as a measure of APC activity. Cotransfection of 14,000 cDNAs with the fusion protein or luciferase alone allowed us to identify APC regulators. The known APC regulator Cdh1 was identified as a protein that reduced the steady-state levels of the fusion protein relative to luciferase alone. An uncharacterized F box protein also reduced levels of the fusion protein specifically. Heat map key: green is 2-fold lower than control plasmid transfection (Sport6); red is 2-fold or higher than control plasmid. The known APC inhibitor Emi-1 was used as a control on each plate.

One protein we have extensively characterized is an F box protein that stimulates APC activity. Expression of this protein is highest during exit from mitosis, suggesting that the protein may play a role in regulating APC activity at that time. Furthermore, depletion of this protein by transfection of short interfering RNA induced a delay in mitotic exit. Currently, we are analyzing the mechanism by which this protein regulates APC activity in vitro and in vivo. These studies suggest that cell-based screening is an effective tool for determining the signaling networks that regulate ubiquitin ligases and cell-cycle transitions.

PUBLICATIONS

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Molecular Mechanisms of cAMP-Mediated Transcription

M.D. Conkright, B.A. Mercer, A.L. Amelio

A variety of biological functions depend on the cAMP signaling cascade, including long-term memory, survival of beta cells, glucose metabolism, cardiomyopathy, and chondrocyte proliferation. We study the molecular mechanisms involved in the conversion of cAMP signals into transcriptional events. Increases in cellular levels of cAMP stimulate the expression of numerous genes by liberating the catalytic subunits of protein kinase A, which phosphorylates the transcription factor cAMP-responsive element binding protein (CREB). Phosphorylation of CREB promotes the recruitment of the coactivators CREB-binding protein/p300 and the initiation of transcription.

The diversity of biological functions associated with CREB and the cAMP pathway will be impossible to fully understand until all of the components involved in the pathway have been identified and characterized. Currently, we are using high-throughput cell-based screening technologies available at the Scripps Florida campus, including cDNA expression libraries, short interfering RNA libraries, and small-molecule libraries, to identify all of the proteins that make up and regulate the cAMP pathway. Using this technology, we have identified proteins called transducers of regulated CREB activity, a novel family of CREB coactivators. Ascertaining the factors involved in the cAMP signaling pathway will be paramount in delineating why the biological function of CREB differs so drastically between tissues.

Signaling Specificity Through the Nuclear Receptor Ligand-Binding Domain

K.W. Nettles, J. Bruning, G. Gil, J. Nowak

Our overall goal is to understand how small-molecule ligands control specific physiologic outcomes through the chemical-structural interface of the ligand with specific nuclear receptors.

We have developed a technique that speeds our ability to obtain x-ray crystal structures of the nuclear receptor ligand-binding domain by several orders of magnitude, allowing us to obtain structural information on entire classes of receptor-ligand complexes in a short time. We are currently using this approach to define the chemical and structural features that determine NF- κ B-selective signaling through the estrogen receptor, the mechanisms of partial agonist activity through peroxisome proliferator-activated receptor γ , and allosteric signaling across the retinoid X receptor heterodimer interface.

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