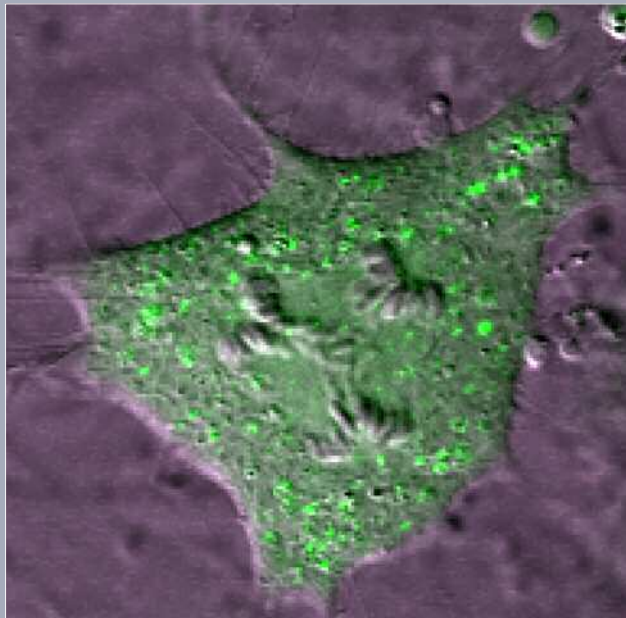
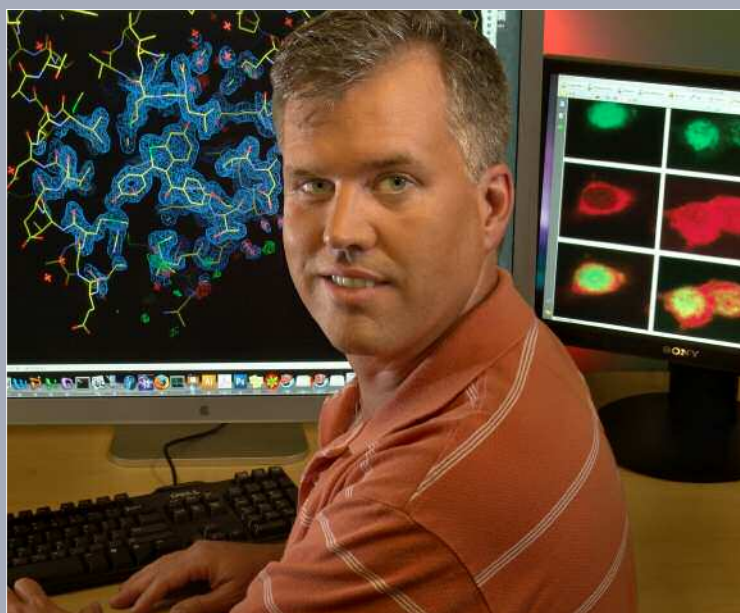


Cancer Biology



Aberrant cell division in a precancerous cell: Shown is a differential interference contrast image of an early-passage p53-null mouse embryo fibroblast. Note that the chromosomes in the cell are being pulled in 3 directions. Daughter cells that arise are aneuploid and/or polyploid. Work done by Frank C. Dorsey, Ph.D., research associate, in the laboratory of John L. Cleveland, Ph.D., professor.



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Chairman's Overview

The Department of Cancer Biology on the Florida campus was established in November 2006. The department has rapidly grown to now include



John L. Cleveland, Ph.D.

8 faculty members. The broad goals of the research programs of the department are to fully define the molecular events that underlie human cancer and then apply this knowledge to the development of novel therapeutic strategies and new agents for cancer prevention and therapeutics.

The programs include those that examine the roles of signal transduction pathways, oncogenes, and tumor suppressors that are altered in cancer and how these alterations control cell division, growth, survival, differentiation, cell migration and metastasis, tumor angiogenesis, transcriptional circuits, and genomic stability and how they modify the response to therapeutic agents. In addition, the interplay between tumors and the immune system in cancer is a new major thrust of research. Faculty members in the department use a

battery of state-of-the-art technologies for target discovery and validation, ranging from biochemistry and cell biology to preclinical models to x-ray crystallography. In addition, unique models have been developed to evaluate the efficacy of new leads in cancer prevention and therapeutics. Investigators in the department have interests in understanding the molecular underpinnings of all of the major human malignant neoplasms, including lung, breast, prostate, colon, and brain cancer, and several hematologic malignant neoplasms. Other interests include pediatric oncology, the interplay between malignant neoplasms and metabolism, and the relationships between aging and cancer.

One of the many strengths at the Florida campus is high-throughput technologies that enable investigators to rapidly move forward potential leads by using both genetic and small-molecule screens. Strong collaborations with the major cancer centers in the State of Florida and with cancer researchers at the California campus of Scripps Research will allow leads that are identified to rapidly advance to translational and clinical studies.

INVESTIGATORS' REPORTS

Myc-Mediated Pathways in Cancer and Development

J.L. Cleveland, M.A. Hall, F.C. Dorsey, R. Rounbehler, K. Yekkala, J. Doherty, M. Steeves, C. Yang, T. Bratton, S. Prater, W. Li

Myc oncoproteins function as master regulators of transcription and regulate up to 10%–15% of the genome. Three *Myc* oncogenes (*c-Myc*, *N-Myc*, *L-Myc*) are activated in about 70% of human cancers. Their activation can occur directly via gene amplification, chromosomal translocations, or somatic missense mutations or indirectly via alterations in signal transduction pathways or the loss of tumor suppressors that normally regulate and/or harness *Myc* expression. The pervasive selection for *Myc* activation in cancer in part reflects the essential roles of *Myc* as a regulator of cell growth and division, but overexpression of *Myc* also triggers accelerated rates of cell proliferation, tumor angiogenesis, and metastasis. Further, *Myc* regulates stem cell fate and supercompetition, a scenario in which cells that overexpress *Myc* kill their neighboring, normal cells.

We have used mouse models to dissect the contribution of key targets downstream of *Myc* that control tumorigenesis. In normal cells, *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 these pathways hold *Myc*-induced tumorigenesis in check and that mutations in these apoptotic regulators are a hallmark of most malignant tumors.

Although apoptotic regulators clearly serve as guardians against *Myc*-induced cancer, 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 cell growth and tumor angiogenesis. Second, *Myc* suppresses expression of the universal cyclin-dependent kinase (Cdk) inhibitor p27^{Kip1} that normally inhibits the activity of cyclin E–Cdk2 and cyclin A–Cdk2 complexes that are necessary for entry and progression through the DNA synthesis (S) phase of the cell cycle. Notably, we found that *Myc* suppresses p27^{Kip1} protein levels by inducing transcription 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* disables the ability of *Myc* to suppress p27^{Kip1} and markedly impairs *Myc*-induced proliferation and tumorigenesis, whereas loss of p27^{Kip1} accelerates *Myc*-induced tumorigenesis. Remarkably, *Cks1* overexpression is a hallmark of *all* lymphomas with *Myc* involvement, suggesting this pathway is a general route by which *Myc* coordinates cell growth and division and that the pathway can be targeted by directed therapeutic agents.

Because *Myc* regulates such a large number of genes and is essential for cell growth and division, the adverse effects of agents that directly target the transcription functions of *Myc* might be greater than the agents' beneficial effects. We therefore have focused our efforts on key transcription targets of *Myc* that might be suitable therapeutic targets. We found that inhibiting ornithine decarboxylase, a direct transcription target of *Myc* and the rate-limiting enzyme of polyamine biosynthesis, impairs *Myc*-induced proliferation and tumorigenesis. These results were underscored by our findings that heterozygosity in the gene that encodes 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 the treatment of cancer. Currently, we are defining the mechanism by which targeting ornithine decarboxylase disables the proliferative response of *Myc*. Our results indicate, quite remarkably, that targeting ornithine decarboxylase disables the ability of *Myc* to suppress p27^{Kip1} by short-circuiting of the *Myc*-to-*Cks1* pathway.

Finally, 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 manifests in the tumor microenvironment. We have shown that agents that disable autophagy have tremendous potential in cancer prevention and treatment. Currently, we are defining the mechanisms by which *Myc* regulates the expression of genes that control the autophagy pathway and their potential as targets for agents to prevent and treat cancer.

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Structural Dynamics in Adhesion Complexes

T. Izard, P.R. Bois, J.H. Lee, G.T.V. Nhieu,* H. Park, E.S. Rangarajan, S.D. Yogesha

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Cell migration and morphogenesis are essential for the development, growth, and survival of metazoans, and these processes are also involved in pathophysiologic conditions such as cancer metastasis and myopathies. Migration and morphogenesis rely on the ability of a cell to dynamically form and break specific contacts, called adhesion junctions, with neighboring cells (adherens junctions) or the extracellular matrix (focal adhesions). Vinculin is an essential regulator of both cell-cell (cadherin-catenin mediated) and cell-matrix (integrin-talin mediated) junctions, where it provides links to the actin cytoskeleton by binding to talin in integrin complexes or to α -catenin and α -actinin in cadherin junctions. Previously, little was known about the structure and activation of vinculin, although the accepted belief was that activation required severing intramolecular interactions of the vinculin head and tail domains.

VINCULIN STRUCTURE AND REGULATION

Our crystal structures, biochemical studies, and biological experiments have redefined vinculin structure and regulation. First, in its resting, inactive conformation, vinculin is held in a closed-clamp conformation through interactions of a 7-helical bundle domain present in its head domain (Vh1) with a 5-helical bundle in the tail domain (Vt); 3 additional helical bundle domains that were identified likely also serve as docking sites for interactions with partners. Second, contrary to dogma, we found that, talin itself is a direct activator of vinculin; α -helical vinculin-binding sites (VBSs) in the central rod domain of talin trigger vinculin activation by displacing Vt from a distance. More importantly, our structures revealed that this activation of vinculin and displacement of Vt occurred via a heretofore unknown change in protein structure, by a process we termed helical bundle conversion (Fig. 1). Third, our studies

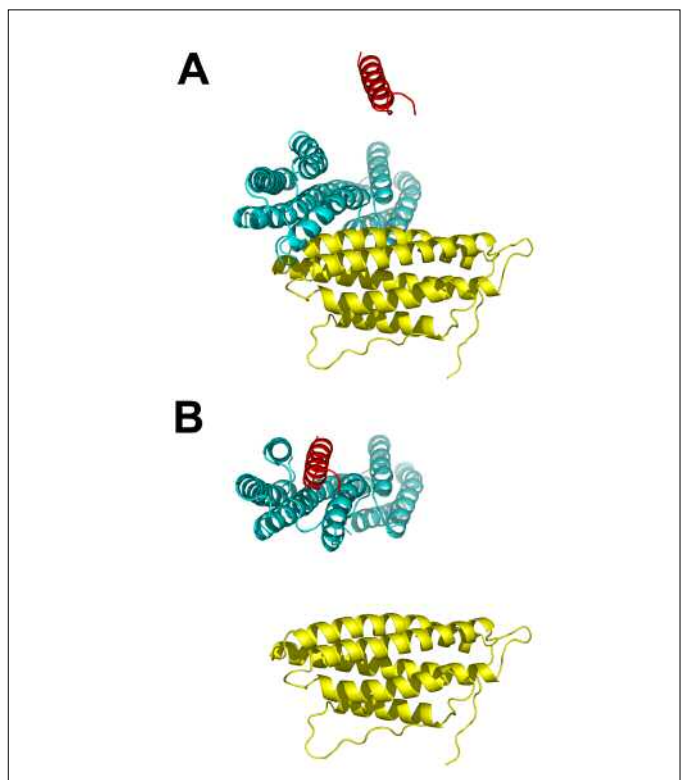


Fig. 1. Vinculin activation by talin through helical bundle conversion. Ribbon drawing of the vinculin head (Vh1; cyan) and tail (Vt; yellow) domains activated by talin's VBS (red). A, The crystal structure of the Vh1-Vt complex revealed that vinculin is held in a closed conformation through many hydrophobic interactions between the Vh1 and Vt domains. B, The crystal structure of talin-VBS3 bound to Vh1 shows that talin binds to vinculin at an accessible site distal from the Vh1-Vt interface and that talin-VBS3 binding displaces Vt from a distance. Wholesale structural changes occur upon talin binding, whereby the 4-helical bundle of Vh1 incorporates the amphipathic VBS helix of talin to form an entirely new 5-helical bundle via helical bundle conversion.

of the complex composed of α -actinin and vinculin revealed that α -actinin activates vinculin and alters its structure in unique ways. These findings supported our model in which the vinculin Vh1 domain functions as a “molecular switch” that undergoes rapid and unique changes in its structure after binding to different activators, which then endow vinculin with the ability to bind to unique partners in adherens junctions vs focal adhesions. Finally, our studies indicated that adhesion signaling involves a chain reaction of structural alterations in which, after their activation, the VBSs of talin or α -actinin first unravel from their buried locations and then bind to and activate vinculin, which then undergoes wholesale changes in its structure (Fig. 2).

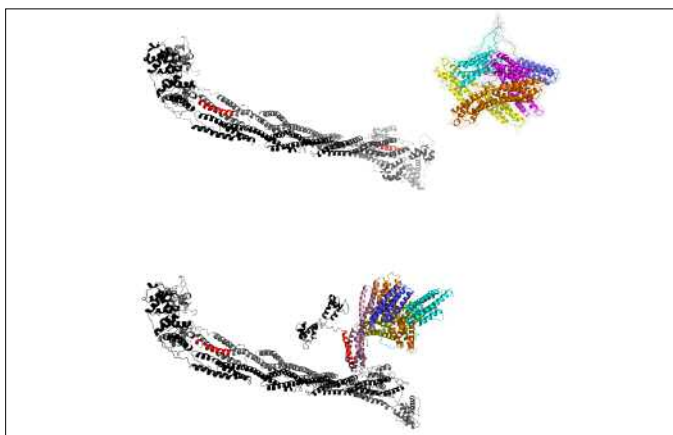


Fig. 2. Relays in adherens junctions. Ribbon drawing of α -actinin (gray and black) and vinculin. Top, In their resting state, both α -actinin and vinculin are in a closed conformation. The VBS is shown in red. Bottom, When activated, α -actinin unfurls to expose its VBS, which then binds to and activates vinculin, resulting in helical bundle conversion of the vinculin Vh1 domain and severing of vinculin’s head-tail interaction. The α -actinin antiparallel homodimer has 2 VBS sites. For clarity, only 1 vinculin molecule is shown bound to the α -actinin homodimer.

TARGETING VINCULIN IN PATHOGEN-HOST INTERACTIONS

We have also made significant inroads in understanding how vinculin is co-opted by pathogens. Initially, we have focused on *Shigella flexneri*, the principal pathogen of bacillary dysentery. *Shigella* organisms inject invasin proteins (IpaA-IpaD) that create pores in intestinal epithelial cells and that trigger the formation of filopodial and lamellopodial extensions that surround the bacteria. IpaA, a protein of approximately 70 kD essential for the pathogenesis of *Shigella* in vivo, facilitates entry of the bacteria into host cells by binding to vinculin. We established that IpaA has 2 high-affinity VBSs that bind to the Vh1 domain of vinculin and induce unique alterations in the domain’s structure. Strikingly, our

studies showed that IpaA acts as a talin mimic that disrupts vinculin’s contacts with talin and α -actinin, and our results suggest that this mechanism is a general one that is exploited by other pathogens. Importantly, our biological studies have shown that this interaction is necessary for efficient entry of *Shigella* into host cells.

Our recent studies have revealed additional layers of functional complexity of IpaA. First, we found that the second, somewhat lower affinity, VBS of IpaA can bind to a second motif of the vinculin Vh1 domain, a situation that would stabilize IpaA-vinculin interactions (Fig. 3). Second, our biochemical and genetic screens

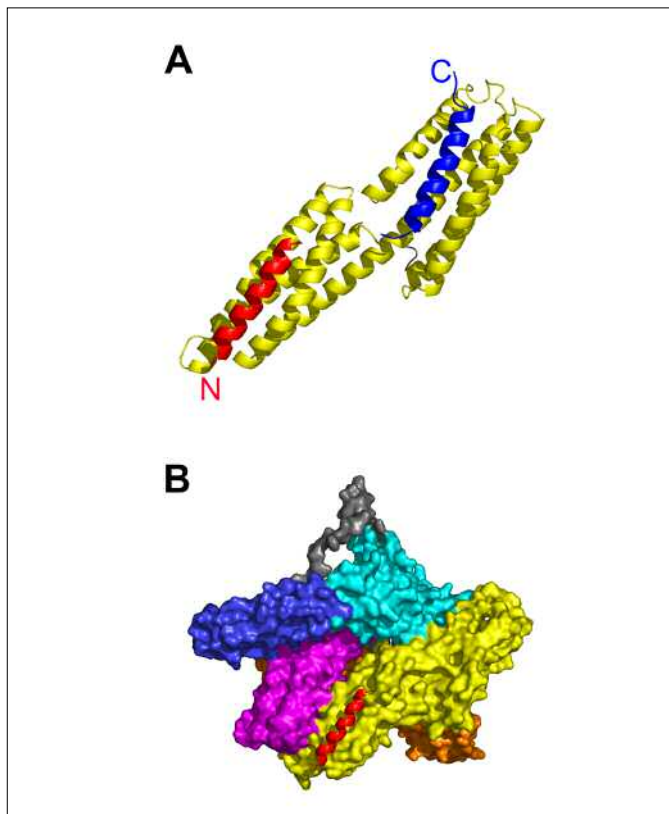


Fig. 3. A novel second binding site on the vinculin Vh1 domain allows IpaA to bind vinculin in its closed conformation. A, Ribbon drawing of the vinculin head domain (Vh1; yellow) bound by the 2 VBSs of *S flexneri* IpaA. The first IpaA-VBS (blue) binds to Vh1 with femtomolar affinity by molecular mimicry of the Vh1-talin interaction, via helical bundle conversion. In contrast, the second IpaA-VBS (red) binds vinculin by a helix addition mechanism, a scenario that allows the *S flexneri* invasin to also recruit pools of inactive vinculin and that would also facilitate the bridging of 2 molecules of vinculin by IpaA. B, Surface drawing of full-length human vinculin (yellow, orange, magenta, blue, gray, and cyan) bound to IpaA-VBS (ribbon drawing, red). The weaker binding IpaA-VBS binds vinculin by a helix addition mechanism, which has no allosteric effects on vinculin, allowing IpaA to bind to vinculin in its closed conformation. Reprinted from Nhieu, G.T., Izard, T. Vinculin binding in its closed conformation by a helix addition mechanism. EMBO J. 26:4588, 2007.

have revealed new cytoskeletal binding partners for IpaA. The physiologic roles of these interactions will be tested, and along with our structural analyses, these studies may point to new therapeutic avenues.

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Regulation of Mitotic Entry and Exit by Ubiquitin-Mediated Proteolysis

N. Ayad, S. Simanski, N. Nagarsheth

Ubiquitin-mediated proteolysis is one of the main ways cells eliminate intracellular proteins. This elimination is important for cell homeostasis, development, and growth. Recent studies have also indicated that one or more components of this system are overexpressed in cancer cells, making the components attractive targets for pharmacologic inhibition. Although a fair amount is known about the pathways leading to ubiquitin-mediated degradation, many essential components have not been identified.

We devised a means of identifying regulators of the anaphase-promoting complex (APC), an essential ubiquitin ligase required for the metaphase-to-anaphase transition and exit from mitosis. We fused cyclin B1, a known APC substrate, to luciferase and cotransfected this fusion construct with 14,000 cDNAs. This genome-wide screen led to the identification of multiple regulators of the APC, including some that are overexpressed in breast cancer. Currently, we are identifying the mechanism by which these proteins regulate the APC during exit from mitosis and are developing high-throughput screens. Our eventual goal is to eliminate the activity of the proteins selectively in cancer cells.

APC activity is inhibited before mitosis because its premature activation would lead to genomic instability. One way the APC is inhibited is by inhibiting the cdk1/cyclin B complex required for entry into mitosis. This complex is kept inactive before mitosis because it is phosphorylated by the tyrosine kinase Wee1. Wee1 is degraded during the G₂ phase and mitosis to tip the balance to active cdk1 and allow mitotic entry to proceed. We analyzed Wee1 degradation in somatic cells and found that 2 separate ubiquitin ligases containing

β-transducin repeat-containing protein and trigger of mitotic entry 1 are required for Wee1 destruction and mitotic entry. These findings indicated that eukaryotic cells have multiple means of regulating Wee1 degradation, because inactivation of a single critical component would lead to premature mitosis and disastrous consequences for the organism. We are determining the respective roles of these ligases in cancer progression.

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Anatomy and Regulation of Mouse Recombination Hot Spots

P.R. Bois, M. Fallahi-Sichani, I. Getun, Z.K. Wu

Crossover events are necessary for meiosis progression and for genome reshuffling and diversity before the generation of gametes. A peculiarity of meiosis is the programmed nature of double-strand breaks, which are induced by the conserved endonuclease Spo11. Studies in a variety of model systems have shown that recombination occurs in distinct regions termed hot spots. An estimated 5%–10% of the genomes of higher eukaryotes are recombinogenic; the remainder reside in the “cold.” However, little is known about the nature and mechanisms that control recombination hot spots in mammalian genomes.

We have focused on identifying novel hot spots in the mouse chromosome 19 by directly detecting crossover events. Characterization of a highly polymorphic hot spot (HS23.7) revealed quite unexpectedly that repair does not have to be complete for meiosis to proceed. The persistence of these unrepaired heteroduplex regions at crossover sites in mature spermatozoa promotes genome instability that was revealed by the high diversity and rearrangements observed in laboratory strains and wild mouse populations. We are identifying and characterizing more of these recombinogenic regions where chromosomes are reshuffled between generations and provide the driving mechanism at the origin of diversity.

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Molecular Mechanisms of cAMP-Mediated Transcription

M.D. Conkright, A.L. Amelio, N.E. Bruno

Glucose homeostasis is maintained by coordinating glucose metabolism in skeletal muscle, lipid storage in adipose tissue, and glucose production in the liver. Insulin and glucagon are central hormone regulators of glucose homeostasis. Glucagon initiates the gluconeogenic program in hepatocytes by activating the cAMP signaling pathway, whereas insulin inhibits hepatic glucose output. Our recent detection of a new component of the cAMP pathway, transducers of regulated CREB (TORCs), established that cAMP signaling is more sophisticated than previously recognized and provided new insights into glucose homeostasis. Collectively, recent studies have indicated that insulin, glucagon, and energy signals converge on TORC2 phosphorylation to modulate glucose output via CREB-mediated expression of hepatic genes. However, the specific nuclear actions of TORC2 are unknown. Thus, the mechanisms involved in differentiating TORC2-transmitted signals are of biological and clinical interest.

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Structural and Molecular Mechanisms of Nuclear Receptor Signaling

K.W. Nettles, G. Gil, J. Nowak, M. Zhou

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 new chemical probes for estrogen receptors that selectively suppress inflammatory gene expression programs. We characterized a series of these probes by using x-ray crystallography, revealing the structural features of signaling specificity. The

novel compounds are also being used to explore the biological effects of inhibiting inflammatory programs in cancer cell proliferation and invasion and the role of estrogen signaling in immune function.

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