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2001 Program
New Investigator (3-year project)

Project Title: Structural and mechanistic studies of the proto-oncogene PDK1

Project Summary: The serine-threonine protein kinase PDK1 contributes to tumor progression and is therefore a target for anti-cancer drug discovery. Methods were developed for high-level production of the native active and inactive phospho-specific isoforms of human PDK1. In addition, methods were developed for high-level expression, purification, and intein-mediated ligation of the ¹⁵N-isotopic labeled regulatory PH domain to a site-directed paramagnetic spin-labeled catalytic kinase domain of PDK1. Homogeneous preparations of the active and inactive forms of both native and "segmentally-labeled" PDK1 were confirmed by analytical SDS-PAGE, ion exchange HPLC, and phosphopeptide mapping studies. The catalytic kinase activities of the active and inactive forms of both native and segmental-labeled PDK1 towards model substrates were evaluated. The establishment of conditions for high-level production of the active and inactive forms of both native and segmentally-labeled PDK1 will greatly facilitate high-throughput screening and NMR structure- and fragment-based discovery of novel anti-cancer drugs against phospho-specific isoforms of human PDK1.

Project Successes: Protein structure-based approaches represent the newest and most promising areas of focus for discovery of drugs for treatment of tobacco-related diseases such as cancer and heart failure. Since numerous drug targets, such as the PDK1 tumor growth and survival promoting enzyme, behave as flexible multi-domain proteins, X-ray three-dimensional structures can be solved for only the individual functional domains. This hinders development of more potent and selective drugs that could simultaneously interact with either (i) known binding pockets of proximal domains or (ii) unknown potential binding pockets that may exist within clefts formed between two interacting domains. In this regard, a novel structure-based strategy was developed for PDK1, which utilizes segmental isotopic labeling of its regulatory domain in combination with site-directed spin labeling of its catalytic domain. NMR studies are used to determine long-range distance restraints between the regulatory and catalytic domains, which can be used to determine the overall structure and dynamics of the full-length flexible multi-domain protein. Clefts and crevices detected around the dynamical domain-domain interface will provide new targeting sites for fragment-based extension of current small molecule lead compounds to produce more potent and selective anti-cancer agents.

Publications from BRP funded research in Peer Reviewed Journals:

Harris TK. PDK1 and PKB/Akt: Ideal targets for development of new strategies to structure-based drug design. *IUBMB Life*. 2003;55:117–126.

Gao X, Yo P, Keith A, Ragan TJ, **Harris TK.** Thermodynamically balanced inside-out (TBIO) PCR based gene synthesis: A novel method of primer design for high fidelity assembly of longer gene sequences. *Nuc Acids Res*. 2003;31(22):e143.

Articles listed below are currently being prepared for submission for publication

Gao, X., Yo, P., and **Harris, TK**. High-Level Production and Characterization of Phospho-Specific Isozymes of Human PDK1 and PKB2: Prospective for High-Throughput Screening of Phospho-Specific Inhibitors. *Protein Expr. and Purif.* 2004 (in preparation).

Ragan, T. J., Keith, A., and **Harris, TK**. Expression, Purification, and Intein-Mediated Ligation of the Regulatory PH and Catalytic Kinase Domains of Phospho-Specific Isozymes of Human PDK1 and PKB2: Prospective for NMR Structure- and Fragment-Based Discovery of Phosphospecific Inhibitors. *Protein Expr. and Purif.* 2005 (in preparation).

Gao, X., and **Harris, TK**. Contribution of the Pleckstrin Homology Domain in Regulation of Phosphoinositide-Dependent Protein Kinase-1 (PDK1) Catalyzed "Trans"-Autophosphorylation and Activation. *Biochemistry* 2005 (in preparation).

Gao, X., and **Harris, TK**. Contribution of the Pleckstrin Homology Domain of Protein Kinase B-2 (PKB2) in Regulating its Catalytic Activation by PDK1-Catalyzed Transphosphorylation. *Biochemistry* 2005 (in preparation).

Keith, A., Ragan, T. J., and **Harris, TK**. Solution Structure and Backbone Dynamics of the Pleckstrin Homology Domain of the Human Phosphoinositide Protein Kinase-1 (PDK1) *J. Biomol. NMR* 2005 (in preparation).

Presentations from BRP funded research:

"Discovering New Drug Targeting Sites on Flexible Multi-Domain Protein Kinases", June 13, 2004 American Society of Biochemistry and Molecular Biology (ASBMB) Annual Meeting, Boston, MA

"Combining Segmental Isotopic and Site-Directed Spin Labeling for NMR Detection of New Drug Targeting Sites on Flexible Multi-Domain Protein Kinases", October 2, 2004, Southwest Regional Annual Meeting of American Chemical Society, Fort Worth, Texas

New grants based in part on BRP-funded work:

NIH

Title: Second-messenger induced activation of PDK1 and PKB/Akt

Project period: 01/01/04 to 12/31/08

Award amount: \$1,136,275

* Grant closed early.