Frederick National Laboratory for Cancer Research

Visiting Scholar Opportunity

Affinity Reagents

Cancer Challenge

It is estimated that 1,260 proteins are differentially expressed in human cancers (Polanski and Anderson, *Biomarker Insights* 2006:1, 1-48). These proteins are thought to represent candidate biomarkers that, if better understood, could accelerate the development of early-detection diagnostics, cancer-monitoring strategies, and cancer therapeutics. The availability of high-quality affinity reagents and the development of standardized methodologies for their preparation are essential to advancing the fundamental understanding of the biology of cancer and translating novel tools to diagnose and treat the disease.

We are interested in developing a portfolio of novel approaches to generate affinity reagents in cases where traditional methods have failed.

Visiting Scholar Opportunity

The Antibody Characterization Laboratory (ACL) is part of the Advanced Technology Program (ATP). ACL supports the Clinical Proteomic Technologies for Cancer (CPTC) program in its efforts to produce monoclonal antibodies to putative cancer targets. Each antibody is well characterized using standard operating procedures. All data and reagents are made available to the research community through the CPTC public portal http://antibodies.cancer.gov.

The first approaches taken by ACL focused on expression of soluble, full-length proteins in E. coli and the use of those expressed soluble proteins to induce production of monoclonal antibodies. This approach provided affinity reagents to the more readily accessible proteins (approximately 200 of the more than 1,200 candidates).

To generate affinity reagents for some of the more challenging targets (e.g., insoluble proteins or those having low-level expression), ACL researchers are currently using three strategies:

1. Harvest (from *E. coli* gene expression vectors) those full-length proteins that are soluble; immunize mice; harvest spleens to create hybridomas that produce monoclonal antibodies; and use the soluble peptides to screen for soluble proteins.
2. Synthesize 14–20 amino acid peptides thought to be immunogenic based upon computer modeling; immunize rabbits with synthesized peptides; harvest rabbit spleens to create hybridomas that produce monoclonal antibodies that are screened using the peptides. This approach is currently being used for 39 peptides in a pilot study.
3. Harvest (from *E. coli* gene expression vectors) those full-length proteins that are insoluble, in the form of "inclusion bodies"; immunize mice with the inclusion bodies; harvest mouse spleens to create hybridomas; screen for monoclonal antibodies with peptides that have been rendered semi-soluble by a novel technique developed at ACL.

While the peptide approach may be successfully applied to create affinity reagents to an additional limited number of biomarker targets, it is not known with what fidelity the computer-modeled, synthesized peptides actually reflect antigenic sites of cancer-relevant proteins. The other approaches should be able to create affinity reagents to a total of 400–500 of the estimated 1,200 potential biomarkers. This leaves a significant number of potentially high-value targets with no current approach demonstrated as feasible for raising affinity reagents.

Who Should Apply?

We encourage proposals for novel approaches to the development of affinity reagents. Applicants are encouraged to become familiar with the descriptions of the core facilities and points of contact to explore compatibility of their ideas with ongoing work.

Core facilities:

- Frederick National Laboratory Antibody Characterization Laboratory
- Protein Chemistry Laboratory
- Protein Expression Laboratory
- Advanced Technology Program (ATP). Depending on the approach, there may be a need for access to any of the other laboratories that support the ATP.

ACL activities support or have close working relationships with:

- Office of Cancer Clinical Proteomics Research
- Tissue Array Research Program
- NCI-Center for Cancer Research
- Advanced Biomedical Computer Center
- Industrial partners (often through cooperative research and development agreements)

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