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Applicant’s Division:  Clinical Chemistry
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(Must hold a primary appointment in Pathology)
Appointment Category:  _____ House Staff  x  Clin Fellow  _____ Research Fellow
               _____ Medical Student  _____ Graduate Student (Program: __________)
Register for:  _____ Clinical Research  __x__ Translational Research  _____ Basic Research

Full Poster Title:  Targeted proteomics: Biomarker discovery and validation for the early detection of breast cancer

Where has the work been presented?
Meeting Name  Partial data presented at HUPO, Oct 6-10, 2007
Meeting Date  ____________________________________________________
Not Previously Presented __________________________________________
Where is this work being published?
Journal Name, Volume, Page, Date  ________________________________________________
In Preparation (Y/N)  - Where?  Y  Cancer Res/Clinical Cancer Res.
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*INCLUDE A ONE-PAGE ABSTRACT (including title and all authors) OF THE WORK
YOU WILL BE PRESENTING

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E-mail COMPLETED Registration form and abstract to:  
Stacey Morgan (smorgan9@jhmi.edu) on or before  
Friday, March 14th, 2008

If you have questions or problems regarding your submission, please contact
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Objective/Relevance: Our goal is to develop a multi-biomarker panel for the early detection of breast cancer. Nipple aspiration fluid (NAF) is chosen as the starting material since biomarkers discovered in proximal fluid have the desired tissue specificity and the likelihood to be released into serum.

Strategy: To achieve this goal, we will follow a four-step study plan: biomarker screening in NAF, validation in tissue, development of multiplexed immunoassays and quantitative evaluation in NAF and serum.

Methodology: NAF has limited sample volume and protein content. One of the proteomics technology we have used successfully is SELDI-MS (requires 1 µg of protein). By comparison of mass spectra from a small cohort of NAF, we have discovered a panel of three candidate biomarkers among them an unidentified 4.7 kD peptide BF5 (Clinical Cancer Research, 2005). To validate BF5, and to screen for additional biomarkers using a different proteomic platform, we have prospectively collected additional samples, and adopted a novel technology (Antibody Microarrays, Clontech). In combination with nanoscale two-color labeling, we were able to compare on the same array the relative abundance of 512 antigens in matched NAF (left-and-right) from 18 cancer cases, using 1 µg of protein.

Specimens: Bilateral aspiration was performed on 42 women with unilateral breast cancer/disease (locally invasive, 31; DCIS, 6; ADH, 5) and 31 controls. This collection yielded 123 NAF samples. The tissue microarray used for immunohistochemistry study consists of 180 cases of infiltrating breast cancer, representing the spectrum of clinical stages and histologic variants. For future validation, we have a multicenter serum collection consisting 480 specimens from 4 diagnostic groups (healthy, benign, DCIS and locally invasive breast cancer).

Results: 1) We have validated the cancer associated expression of BF5 in NAF on SELDI-MS, and determined its protein identity as 41/42-aa C-terminal peptide of alpha-1-antitrypsin (AAT). We demonstrated that C-41/42 can be generated in vitro by action of MMP-7 from the full length AAT which is elevated along with C-41/42. Therefore, elevated C-41/42 is likely the combined effect of elevated AAT synthesis and the activity of specific MMPs present in the tumor. The C-terminal fragments of AAT are functionally active. A C-36 fragment has been shown to serve as a tumor-derived suppressor to the host immune-system, a significant and distinct biological activity not exhibited by the full length protein. 2) We have identified by antibody array analysis 17 additional biomarkers. These proteins were elevated in at least 4 cancer cases out of 18 studied. 3) We have performed validation on one of the additional marker P116Rip. This protein has not previously been associated with breast cancer. We showed that P116Rip is selectively expressed in highly invasive “stromal like” breast cancer cell lines, and we observed positive cytoplasmic staining in breast cancer tissues.

Conclusions: We have completed biomarker screening in NAF using both SELDI-MS and antibody microarrays. We validated our result using an independent cohort and independent methods. Further tissue validations and development of multiplexed immunoassays will allow us to evaluate the potential clinical utility of these markers in NAF/serum.