

## The Power of PCT

### Data Generated by Independent Researchers

### Published in Two Peer-reviewed Prestigious Journals

#### Paper Published in *Circulation: Journal of The American Heart Association*, September 2008

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#### **Pilot Proteomic Profile of Differentially Regulated Proteins in Right Atrial Appendage Before and After Cardiac Surgery Using Cardioplegia and Cardiopulmonary Bypass**

**Background** — Although highly protective, cardiac surgery using cardioplegia and cardiopulmonary bypass (CP/CPB) subjects myocardium to hypothermic reversible ischemic injury that can impair cardiac function which results in a greatly enhanced risk of mortality. Acute changes in myocardial contractile activity are likely regulated via protein modifications. We performed the following study to determine changes in the protein profile of human myocardium following CP/CPB.

**Methods and Results** — Right atrial appendage was collected from 8 male patients pre and post-CP/CPB. Atrial tissue lysates were subjected to 2-dimensional electrophoresis, total protein staining, gel averaging, and quantitative densitometry. Ten prominent spots regulated in response to CP/CPB were identified using mass spectrometry. Two hundred twenty-five and 256 protein spots were reliably detected in 2D-gels from pre- and post-CP/CPB patients, respectively. Five unique (i.e., not detected post-CP/CPB) and 17 significantly increased spots were detected pre-CP/CPB. Thirty-four unique and 25 significantly increased spots were detected in the post-CP/CPB group. Identified proteins that changed after CP/CPB included: MLC-2a, ATP-synthase delta chain and Enoyl-CoenzymeA hydratase, glutathione-s-transferase omega,  $\alpha$ -1-acid-glycoprotein, and phosphatidylethanolamine-binding protein.

Proteins were extracted in RIPA buffer with protease and phosphatase inhibitors using **Pressure Cycling Technology (Pressure BioSciences)**. The samples were placed in a NEP3229 Barocycler subjected to 40 cycles of high pressure for 30 seconds (**35,000 psi**) followed by atmospheric pressure for 10 seconds.

**Conclusions** — Cardiac surgery results in multiple consistent changes in the human myocardial protein profile. CP/CPB modifies specific cytoskeletal, metabolic, and inflammatory proteins potentially involved in deleterious effects of CP/CPB.

(*Circulation*. 2008;118[suppl 1]:S24–S31.)

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#### Paper published in *The American Journal of Pathology*, September 2008

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#### **A Comparison of Methods for Efficient Digestion of Protein Therapeutics Soluble Forms of the Notch Ligands Delta1 and Jagged1 Promote *in Vivo* Tumorigenicity in NIH3T3 Fibroblasts with Distinct Phenotypes**

We previously found that soluble forms of the Notch ligands Jagged1 and Delta1 induced fibroblast growth factor receptor-dependent cell transformation in NIH3T3 fibroblasts. However, the phenotypes of these lines differed, indicating distinct functional differences among these Notch ligands. In the present study, we used allografts to test the hypothesis that NIH3T3 fibroblasts that express soluble forms of Delta1 and Jagged1 accelerate tumorigenicity *in vivo*. With the exception of the full-length Jagged1 transfectant, all other cell lines, including the control, generated tumors when injected subcutaneously in athymic mice. Suppression of Notch signaling by the soluble ligands significantly increased tumor onset and growth, whereas full-length Jagged1 completely suppressed tumor development. In addition, there were striking differences in tumor pathology with respect to growth kinetics, vascularization, collagen content, size and number of necrotic foci, and invasiveness into the underlying tissue. Further, the production of angiogenic factors, including vascular endothelial growth factor, also differed among the tumor types. Lastly, both Jagged1- and Delta1-derived tumors contained phenotypically distinct populations of lipid-filled cells that corresponded with increased expression of adipocyte markers. The divergence of tumor phenotype may be attributed to ligand-specific alterations in Notch receptor responses in exogenous and endogenous cell populations within the allografts. Our findings demonstrate distinct functional properties for these Notch ligands in the promotion of tumorigenicity *in vivo*.

(*Am J Pathol* 2008, 173:865–878; DOI 0.2353/ajpath.2008.080006)

Total cell lysate for immunoblot and enzyme-linked immunosorbent assay (ELISA) analysis was prepared using the **Pressure Cycling Technology with the Barocycler NEP-3229 instrument (Pressure BioSciences, Inc., South Easton, MA.)**

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## CALENDAR OF EVENTS

### PLANT & ANIMAL GENOMICS

SAN DIEGO, CA  
JANUARY 10-14, 2009

Poster Presented at British Mass Spectrometry Society Meeting, September 7-10, 2008, by Dr. Paul Pevsner, Dept. of Pharmacology, New York University School of Medicine, New York, NY

## LCMS Identification of *In Vitro* Fertilization Culture Media Proteins

### Background and Significance

At the NYU Fertility Center 48% of cycles in women <35 years result in a live birth. Forty percent of these births in women <35 years at the NYU Fertility Center are twin deliveries. A strong criticism of assisted reproductive technologies (ART) is the high incidence of multiple gestations that increase fetal and maternal morbidity and mortality.

Grifo et al., recently described their clinic's progression to blastocyst transfer as a means to reduce the high-order multiple rate.<sup>1</sup> The ART community has addressed the need for more single embryo transfers (SET) but also recognizes the lowered pregnancy rates that may ensue. The ability to identify additional markers associated with embryo viability and competence has been the greatest challenge towards promoting SET.

In a recent study of 3 and 5 day growth media, we have identified gi|223976 haptoglobin Hp2, mass 41717. Two more proteins were identified in our latest report, gi|90108928 1 Chain H, Orally Available Factor7a Inhibitor, mass 28582, and gi|119573737 hCG1793647 [Homo sapiens] Mass: 6112. This study produced two new specific biomarkers unique to competent embryos: gamma-aminobutyric-acid receptor subunit and tetratricopeptide repeat protein 9.

### Materials and Methods

The IVF culture media from day 3 and day 5 embryos was extracted with organic solvent and high pressure using ProteoSolve® and the Barocycler® respectively (Pressure BioSciences, South Easton, MA). Trypsin digest of the proteins was completed in 45 minutes, shortened from 12 hours. The peptide digest was studied with LCMS (Hitachi NanoFrontier nLC, Dallas, TX).

### Results

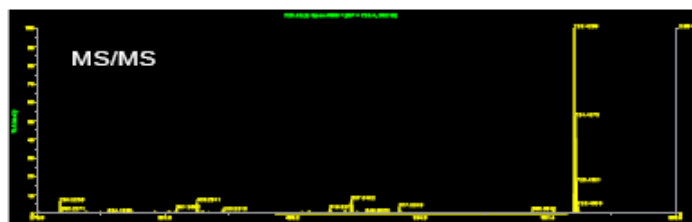
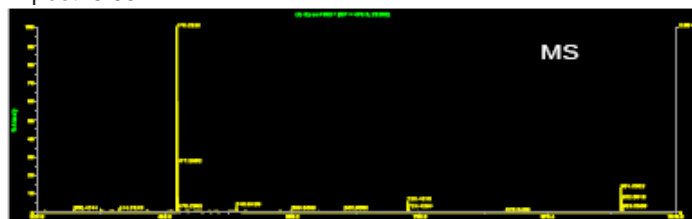
The precursor ions were identified with MS. MSMS of the precursor ions generated a mass list. Matrix Science Mascot® software query of the NCBI nr data base with this mass list identified two novel proteins that were only present in the implanted embryos that resulted in live births: TTC9\_HUMAN, Tetratricopeptide repeat protein 9 (TPR repeat protein 9) (Fragment) – Homo sapiens, Figure 1, and GBRE\_HUMAN, Gamma-aminobutyric-acid receptor subunit epsilon precursor (GABA (A) receptor subunit epsilon) – Homo sapiens, Figure 2. They were not present in the poor quality embryos from the same patient.

### Discussion

The Barocycler extraction resulted in a greater number of proteins identified than was noted in prior studies, and allowed a marked decrease in duration of trypsin digest from 12 hours to 45 minutes. Embryo quality is based on accepted microscopic morphologic criteria. However excellent morphology does not guarantee pregnancy. These new protein biomarkers of competent embryos should enhance embryo selection and improve live birth outcome with single embryo transfers.

Figure 1

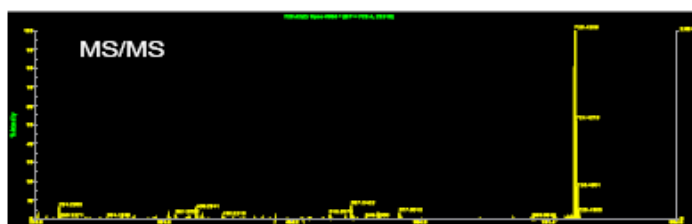
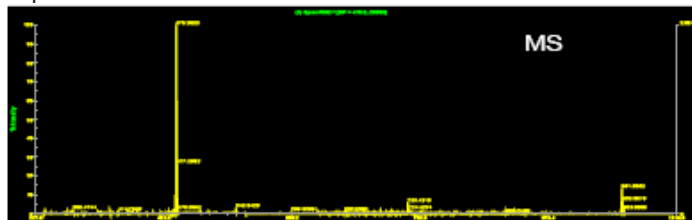
SpectraSample: Sample 17: 10387A ER 6/26/05 #3 25  
Protein: TTC9\_HUMAN, Tetratricopeptiderepeat protein 9 (TPR repeat protein 9) (Fragment) –Homo sapiens  
Peptide Sequence: EGENFK  
Mr(calc): 722.4150  
MS/MS: 723.4223 (+1 Charge State)  
Ions Score: 32  
Expect: 0.032



\*\*SAME ION DETECTED FOR GBRE\_HUMAN

Figure 2

SpectraSample: Sample 17: 10387A ER 6/26/05 #3 25  
Protein: GBRE\_HUMAN, Gamma-aminobutyric-acid receptor subunit epsilon precursor (GABA (A) receptor subunit epsilon) – Homo sapiens  
Peptide Sequence: WENFK  
Mr(calc): 722.3388  
MS/MS: 723.4223 (+1 Charge State)  
Ions Score: 32  
Expect: 0.032



\*\*SAME ION DETECTED FOR TTC9\_HUMAN