Milestones and Advancements on the Plan

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1. In project 1, Post-translational Modification of Vascular Proteins by Reactive Nitrogen Species, the ability to detect low abundance tyrosine nitration of specific proteins with antibodies adds a high degree of specificity and sensitivity not heretofore possible. This advancement adds to the project’s ability to assess oxidant stress in patients.

2. In project 2, Oxidation of Cardiovascular Protein Thiols, researchers used their own ICAT methods and methods available in the mass spectrometry instrumentation projects to quantify oxidant modifications of Ras. They performed FTMS and electrospray ionization MS/MS studies on oxidant-exposed recombinant Ras. Top down studies of intact Ras showed feasibility and after tryptic digestion 100% sequence coverage was achieved. Peroxynitrite-induced modifications were catalogued. Using their ICAT method they then quantified oxidation of reactive cysteines in the protein.

3. Project 3, Post-translational Modification of eNOS by Hypochlorite, met the milestone to obtain spectra of HOCl-modified eNOS, noting differences compared to controls.

4. In project 4, Post-translational Modification of Vascular Proteins by Homocysteine, the major advance is the progress made in generating cell culture models to assess the role of GPx-1 in thiol modification.

5. Project 5, Circulating Surrogate Target Cells of Oxidant Stress, began study of a second inflammatory platelet protein, TLR2. Pending resolution of the difficulties of studying TLR2 described above, investigators did not advance studies of the eNOS protein complex. As an initially unanticipated bonus, they began to obtain promising data on intracellular platelet Factor XIII A, identifying interacting proteins in resting platelets, and report that the analysis of interacting proteins in activated platelets is underway.

6. A number of novel approaches were reported by project 6, Reverse Engineering of Protein Networks, investigators. They validated their reverse engineering approach for reconstructing gene-protein networks by using expression data to correctly enrich for the known targets and associated pathways for several compounds of known mode of action. They validated this approach on PTSB, a novel growth inhibitory compound with a previously unknown mode of action, by predicting and validating thioredoxin and thioredoxin reductase as its target. They implemented a protein-gene network reconstruction approach based on mutual information that requires relatively few datasets and showed that this approach can be used to analyze networks of altered protein abundance. Lastly, they developed an approach for identifying functional network motifs, molecular interaction networks based on biological function, and applied the approach to protein interaction data from yeast, worm, fly and human.

7. Project 8, Circulating Endothelial Progenitor Cells, has met the milestone to begin in vivo endothelial progenitor cell studies in a transgenic model of reactive oxygen species (ROS) stress in GPx-deficient mice.
8. Project 9, **Impact of Oxidative Stress on Cardiovascular Diseases in the Community**, has met all analytical milestones regarding analysis of the clinical correlates of plasma myeloperoxidase and assessment of methods to assay asymmetrical dimethyl arginine (ADMA).

9. In project 10, **Proteomic Markers of Endothelial Damage and Oxidative Stress in Sickle Cell Disease**, researchers have used proteomics tools to observe differential protein expression and oxidative PTMs in proteins of patients with pulmonary hypertension and those with sickle cell trait co-existing with pulmonary hypertension.

10. The major advancement in project 11, **Post-translational Oxidative Modification of Vascular Proteins in Key Antioxidant Deficiency States**, is the full characterization of G6PD-deficient cell lines and the isolation of two target proteins for MS analysis. Two other advancements include the finding that aldosterone administration produces a G6PD-deficient phenotype in cells with a normal G6PD genotype and the characterization of oxidant stress in these cells. These findings are major, and novel, advancements with clinical implications and have allowed project investigators to use aldosterone infusion as a non-toxic method to pursue the effects of oxidant stress on post-translational modification of G6PD and eNOS in vivo.

11. The major milestones met in project 12, **Antioxidant Deficiency and the Heart**, were to identify post-translational modification of Ras and JNK in the regulation of myocyte hypertrophy and death in the setting of oxidative stress.

12. The **Bioinformatics** project developed a prototype protein identification server, the BUPID, opened it for beta testing, and invited the scientific community to join in the testing. They improved the software program with better scoring function and additional functionality and reported that initial results had sensitivity comparable to commercial systems yet offers the access and flexibility necessary for incorporation into further planned CPC software developments.

13. The **Core Laboratory** developed the capacity to combine multiple technologies for analysis of samples provided by the co-investigator labs and increased its capacity to handle larger numbers of samples. Technologies include Electrospray Fourier Transform MS (ESI FT MS) to provide the mass of intact protein samples as well as top-down sequencing and initial peptide mapping; Matrix-Assisted Laser Desorption/Ionization Time of Flight MS (MALDI TOF MS) for peptide mass mapping; MALDI FT MS for more accurate peptide mass measurement; and capillary Liquid Chromatography MS (capLC MS) to automate sequencing, variant identification, and identification. With this approach, Core investigators have identified proteins and oxidative post-translational modifications in the samples provided by the biological projects.

14. In the **High Throughput MALDI-CryoFTMS** project, data reduction programs, to be used in the construction of the hybrid cryoFTMS mass spectrometer, were developed and disseminated in scientific papers. XY stage control software met rigorous testing and automated spot sampling algorithms were implemented on the MALDI-FTMS instrument located at the Mass Spectrometry Resource, which can serve, for these evaluations, as a functional prototype of the instrument being constructed.