INDO SWISS JOINT RESEARCH PROGRAMME (ISJRP)

JOINT UTILISATION OF ADVANCED FACILITIES

EXCHANGE GRANT REPORT

Grant No.: JUAF01

Part 1 - General Information

<table>
<thead>
<tr>
<th><strong>Project Title:</strong></th>
<th>Advanced training on application of proteomics in cancer biology</th>
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</thead>
<tbody>
<tr>
<td><strong>Keywords:</strong></td>
<td>Proteomics, mass spectroscopy, cancer, p53</td>
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<tr>
<td><strong>Research area:</strong></td>
<td>Cancer biology &amp; proteomics</td>
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<tr>
<td><strong>Start date:</strong></td>
<td>August 17, 2008</td>
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<tr>
<td><strong>Duration:</strong></td>
<td>3 weeks</td>
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</tbody>
</table>

Part 2 - Exchange Participant(s) Details

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Part 3 - Scientific & Technical Information

3.1 Purpose of visit

Proteomics is valuable in the discovery of biomarkers because the proteome reflects both the intrinsic genetic program of the cell and the impact of its immediate environment. Protein expression and function are subject to modulation through transcription as well as through posttranscriptional and posttranslational events. More than one RNA can result from one gene through a process of differential splicing. Additionally, there are more than 200 post translation modifications that proteins could undergo, that affect function, protein-protein and nuclide-protein interaction, stability, targeting, half-life, and so on, all contributing to a potentially large number of protein products from one gene. At the protein level, distinct changes occur during the transformation of a healthy cell into a neoplastic cell, ranging from altered expression, differential protein modification, and changes in specific activity, to aberrant localization, all of which may affect cellular function. Identifying and understanding these changes are the underlying themes in cancer proteomics. The deliverables include identification of biomarkers that have utility both for early detection and for determining of therapy.

Although proteomics traditionally dealt with quantitative analysis of protein expression, more recently, proteomics has been viewed to encompass the structural analysis of proteins. Quantitative proteomics strives to investigate the changes in protein expression in different states, such as in healthy and diseased tissue or at different stages of the disease. This enables the identification of state- and stage-specific proteins. Structural proteomics attempts to uncover the structure of proteins and to unravel and map protein-protein interactions.

MS has been helpful in the analysis of proteins from cancer tissues. Screening for the multiple forms of the molecular chaperone 14-3-3 protein in healthy breast epithelial cells and breast carcinomas yielded a potential marker for the noncancerous cells. This finding, in the light of the evidence that the gene for 14-3-3 was found silenced in breast cancer cells, implicates that this protein as a tumor suppressor. Using aMALDI-MS system, Bergman et al detected increases in the expressions of nuclear matrix, redox, and cytoskeletal proteins in breast carcinoma relative to benign tumors.

Stoeckli et al used imaging MS to examine protein expression in sections of human glioblastoma and found increased expression of several proteins in the proliferating area compared with healthy tissue. Liquid chromatography—MS and tandem MS (MS-MS) were used to identify thymosin β4, a 4,964 Da protein found only in the outer proliferating zone of the tumor. Imaging MS shows potential for several applications, including biomarker discovery, biomarker tissue localization, understanding of the molecular complexities of tumor cells, and intraoperative assessment of surgical margins of tumors.

3.2 Short description of work carried out during the visit

Technologies acquired:

1) Different parts of the LC/MS and MS/FS instruments and their functions
2) Learn the principles how to couple LC (liquid chromatography) to mass spectrometry and obtaining tandem MS/MS data
3) Purifying protein interactors by using GST-tagged yeast Npr1 protein kinase as a model system
4) In-gel digestion techniques for protein identification for mass spectrometric analysis
5) Application of bioinformatics tools (SEQUEST/Mascot, etc.) for protein identification/characterization
6) Application of the iTRAQ reagent for probing changes in the extent of phosphorylation in the yeast Npr1 kinase.
Additional techniques used on a daily basis like yeast cell culturing, preparative gel electrophoresis for phosphoproteomics, and technical matters of capillary chromatography were included in the training course.

Experiments carried out

First week:
- Introduction to the modules the and application of LC/MS and MS/MS instrumentation
- Introduction to spectral analysis and peptide identification based on tandem mass spectrometric data
- Setting up yeast cell cultures for obtaining GST-Npr1 for phosphorylation analysis

Second week:
- Performing the Npr1 pull down assays with Glutathione Sepharose
- Analysis of the pull down by SDS gel electrophoresis
- Excise the protein band followed by in-gel digestion to identify GST-Npr1
- Analysis of the digest by LC/MS/MS on a Thermo Finnigan LCQ ion trap
- Analyse and identify the digest with SEQUEST/Bioworks
- Tracking protein phosphorylation of GST-Npr1 by neutral loss analysis

Third week:
- Second GST-Npr1 pull down for protein quantitation.
- Quantitative analysis of differential protein expression by iTRAQ analysis
- Analysis of iTRAQ-labelled GST-Npr1 digest on an Orbitrap instrument.
- Data analysis and quantification by using SEQUEST and Bioworks.

3.3 Outcome

During my stay in the laboratory of Dr. Jenoe, his research interest around the yeast protein kinase Npr1 was used as a model system to demonstrate the use of mass spectrometry in aspects of protein phosphorylation related to growth control. The yeast TOR target Npr1 is a hyperphosphorylated protein that belongs to a fungus-specific family of Ser/Thr protein kinases dedicated to the regulation of plasma membrane transporters. Its phosphorylation is controlled by the nutritional input from the medium via the multisubunit protein kinase TOR1. Nitrogen starvation of yeast cells lead to rapid, though not complete dephosphorylation of Npr1. Even though, the sites of phosphorylation in Npr1 are well characterised, the functional consequences of phosphorylation, as in many aspects of cell growth, are not known.

To obtain a better understanding of the modulatory role of protein phosphorylation, the changes in phosphorylation following nitrogen starvation of yeast need to be quantitated and followed temporally. For this, first steps were undertaken in the use of the iTRAQ reagent (Zieske LR (2006). "A perspective on the use of iTRAQ reagent technology for protein complex and profiling studies". J. Exp. Bot. 57 (7): 1501–8.) to track changes of phosphorylation in signalling proteins quantitatively and temporally. The yeast Npr1 protein kinase is a suitable model protein due to extensive changes in phosphorylation following nitrogen limitation or drug treatment (Gander et al., (2008) Identification of the rapamycin-sensitive phosphorylation sites within the Ser/Thr-rich domain of the yeast Npr1 protein kinase, Rapid Communications in Mass Spectrometry, DOI: 10.1002/rcm.3790, ahead of publication).
The methodology applied to the yeast Npr1 kinase will allow us to use mass spectrometry in cancer related fields and widen our knowledge and learning to the basic understanding of the analysis of proteins many of which are unknown signaling targets. These proteins fall into multiple categories. Based on our findings, we can link oor signaling to translation (TrpRS), redox regulation (Prx II, thioredoxin), apoptosis (caspase-3, eIF5A), cell growth control (14-3-3σ, Nm23-H1), Ras signaling and the MAP kinase pathway (GRB2, MAPK8), proteasomal degradation (TBP-1).

3.4 Future collaboration with host institution

Further interactions by web discussions on data acquisition and anlysis to gather new inputs for my lab’s recent biochemical findings on the use of proteomic approach. This will allow us to recruit a leading team for discovery, characterisation so that new cues to cancer biology with potential for health and economic benefits can be exploited. This will be a major step and exchange and funding for collaborative exchanges will play an essential role in developing these interactions.

3.5 Various comments

It is not only my first time experience to learn mass spectrometry but also to get to know the principle, application and science of proteomics application in biology from the scratch. It was a useful training that opened my view for the future research in which I had no previous experience and expertise. Dr. Paul Jenoe’s expertise in this field is excellent. His continuous training gave me tremendous impetus in this area to explore my future research. Along with this LC/MS, MS/MS,Gel digestion, phosphoproteomics facility in single lab is an excellent advantage for a scientist that needs to be established in India. As a newcomer to this field, it was not possible to run cancer cell sample for my research work or to gather new data on my ongoing research but it is sure this exchange of idea and advanced training spurs new ideas of using proteomic analysis in cancer biology and cell signalling. It will give more analytical input in the results obtained in our laboratory. One thing I might add, though, is that the three week’s training allows only a small glimpse into the powerful tools proteomics techniques can offer and future visits should be planned in a longer time frame.