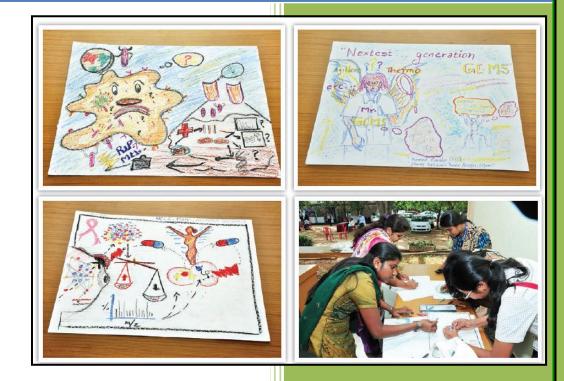


Proteomics Society, **India** (**PSI**) PSI News Letter Vol. 2 (No.1) April 2014



Editors

Abhijit Chakrabarti, K Dharmalingam Utpal Tatu

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From the Editorial Desk

Dear Members,

This News letter comes to you after a long gap. For several reasons it has not been possible to communicate with you. We look forward to interact with you more often and at scheduled times.

Proteomics is becoming an important tool in different areas of biology. In this issue we bring to you information on Plant Proteomics, Histone PTMs and Cancer and Biomarkers for Nasopharengeal cancer. Each of the Research Highlights summarize the role of proteomics in these different scientific fields.

The 5th Annual Meeting of the Proteomics Society was held in IISc during 28th -30th Nov 2014. The report provides you a glimpse of the sessions that were covered, the poster presentations, panel discussions and equipment exhibitions. Links have been provided to PSI website for a more detailed report.

The 6th Annual Meeting of the Society is to be held during the first week of Dec 2014. A short write up gives you a glimpse of the events planned. Do block your calendar for the dates and make all efforts to attend. A link is provided to the meeting website.

The life of the News Letter is very dependent on your contributions. We urge you to send Research Highlights, reports of events in your Institutes pertaining to Proteomics, your achievements such as awards, etc, upcoming events, crosswords, puzzles and so on. This will ensure that all of us stay connected. We are sure you will not disappoint us.

With Best Wishes

Editors

PS: Please send your contributions for the News Letter to abhijit.chakrabarti@saha.ac.in



Greetings from the President, PSI

Dear Members,

This is my first communication with you all after the new Office Bearers have assumed office in Jan 2014.

The Proteomics Society, India was established in 2009 with a mandate to bring the scientific community engaged in Proteomics together and to increase the awareness of proteomics tools for biological research. This was possible with the focused and persistent efforts of Dr Ravi Sirdeshmukh (past President), Dr MAVijayalakshmi and Dr Shantanu Sengupta, (past Vice Presidents) and other colleagues. They steered the Society through its fledging years to bring it to the point it has now reached.

With the Society reaching its first milestone, the new Office Bearers are now poised to take the Society to greater heights with the participation of each and every member of the PSI.

It is apparent that there are increasing number of Indian scientists who are now engaged in Proteomics research using the latest tools to answer biological queries related to disease, plants, microbes and pursue basic hypothesis driven research. Some are involved in developing technologies and tools contributing to the changing requirements vis a vis, specific separation technologies, sensitivity, increasing high throughput and mulplexing etc. Bioinformatic analysis and statistical tools are also receiving attention.

Our Annual meetings have brought together several of the scientists using Proteomic technologies. They have shared their science with each other and contributed to educating young students and faculty who are considering using proteomics tools in their research. Through these meetings we have met the mandate of the Society. Some efforts have also been made to hold workshops and teacher training programs to keep the students and teachers updated in the ever growing and developing proteomic technologies.

There is however much more to be done and our efforts have to be increased towards spreading the information about Proteomics. It is here that I look forward to the PSI members to come forward with ideas for programs which they can conduct in and around their base and reach out to those who do not have the opportunity to learn and use proteomics technologies. Our target audience should be

the teachers and senior students who will themselves learn and then pass on the information to their students and colleagues. Programs to network between faculties with a view to generating collaborative programs will also be very effective.

Another area in which PSI members can contribute to the growth of the Society is by encouraging student and faculty members interested in proteomics research to join the Society. Do make efforts towards this end.

The upcoming 6th Annual meeting and the accompanying International Conference on "Protemics from Discovery to Function" is being organized by Dr Sanjeeva Srivastava at IIT, Bombay during 7-9th Dec 2014. We will have the opportunity to meet and listen to the globally acknowledged scientists in Proteomic research and learn about the directions in which the Proteomics community is targeting itself to identify all the proteins coded by the genome. The post conference workshops will give an opportunity for those proposing to use Proteomics technologies to learn from the experienced members of the Proteomics community.

The Proteomic Society Newsletter is our organ providing information about our activities and interesting areas of research to our members. I request each member to consider contributing articles for the newsletter, upcoming meetings, scientific crosswords, information on proteomics related technologies etc. It is only with your input we can interact with each other. We welcome participation from industry in our efforts.

I look forward to hearing from you with your ideas.

With Best wishes for 2014

Regards

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Research Highlights

Plant Proteomics: current status, challenges and future prospects

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The term "proteomics" was first used in the late 1990s to describe systematic analysis of proteome, the protein complement of genome. Past decade has witnessed an explosion in the field of proteomics due to significant advances in mass spectrometry enabling smarter protein characterization ranging from localization, quantification, post-translational modification to protein-protein interactions. Plant proteomics is considered to be in relative infancy as compared to proteomics analyses of prokaryotes, yeast and humans. In case of plant research, the tremendous potential of proteomics is quite far from being fully exploited, and the second-, third- and fourth-generation proteomics techniques are still used limitedly. Nevertheless, plant proteomics has made tremendous contributions in understanding the complex processes of plant biology and elucidation of the molecular circuitry.

Over the past decade, proteomic studies have gained importance in plant research. A critical survey of literature (NCBI PubMed database was searched using the key words "proteom*" and "plant*.") indicated that the number of publications in plant proteomics is increasing rapidly every year (Figure 1A). Despite the economic importance of crop plants, majority of plant proteomics research has been focused on the non-crop model plant, Arabidopsis (*Arabidopsis thaliana*). Next comes rice (*Oryza sativa*), a cereal model, followed by crop species such as wheat, maize, soybean, and potato (Figure 1B). The sequencing of its genome in 2000 has made Arabidopsis the plant paradigm for molecular biology and various 'omics' approaches. Rice genome was sequenced in 2002 followed by the genomes of important crop species including potato, maize, tomato and soybean as well as the so-called orphan crops such as sorghum, millet and cassava. These have been released in public databases and despite the limited annotation of most, the availability of genome sequences provides new opportunities to study plant stress responses using rapidly expanding proteomic technologies.

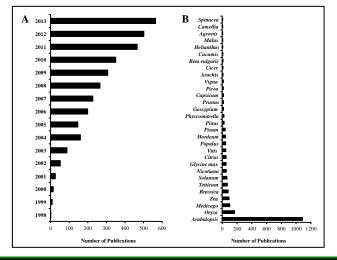


Figure 1: Number of publications per year related to plant proteomics. (A) Number of publications per year and (B) Number of publications for different model plants and crop species over the period 1998-2013.

Initially, plant proteome analysis was largely a qualitative approach, developing proteome maps and constructing databases of expressed proteins for various organs, tissues and subcellular components. The common challenges encountered during proteomic analysis of plant tissue comprise the requirement of cell wall disruption to extract proteins, removal of polyphenols, and the hindrance of high dynamic range of protein abundances in detection of low-abundant proteins (for instance Rubisco can yield ~ 40% of total protein content in green tissue). Advanced protein fractionation strategies have facilitated subcellular and organellar proteomics, a promising strategy that enables the visualization of low abundant proteins and also provides important information about protein localization and pathway compartmentalization.

Recent advances in mass spectrometry and high-throughput analysis have boosted the application of proteomics to study protein quantification and modification(s). The avenues awaiting for further exploration include protein cataloguing deeper into the proteome by identifying low-abundant proteins, relative protein quantification, post-translational modification, assigning of function(s), protein localization into organelles, validating previous proteomes and eliminating false-positive data, and discovering potential biomarkers for tissues, organs, organelles, beside screening of transgenic plants and food-safety evaluation.

Protein chemistry has been a forte for Indian biological research for many years and with this scientific background, proteomics effort has evolved at the turn of the century. Proteomics being a technologydriven and equipment intensive science has extensive financial demands. Trained man-power is also required to handle proteomics platform, as per the need of research and biological questions. In an effort to accelerate progress in plant proteomics research, a concerted national emphasis must be placed on these issues. Currently several Government funding agencies, for example, Department of Biotechnology (DBT), Council of Scientific and Industrial Research (CSIR), Department of Science and Technology (DST), and Indian Council of Agricultural Research (ICAR), provide financial support to promote proteomics research and development of necessary infrastructure. The critical mass of proteomics scientists has gradually grown in the country and a number of centers have initiated proteomics programs and the trend seems to be gradually increasing. The first Proteomics Conference in India was held at CCMB, Hyderabad in 2003. As the research efforts expand and technologies evolve, it is important to promote interactions among the proteomic communities and help them share knowledge. To facilitate these objectives, a consortium of the Indian proteomics scientists, Proteomic Society India (PSI) was formed in 2009. Plant proteomics still has a long way to go, and it underscores the need for greater funding and integrated approaches to obtain a deeper understanding of plant biology. Indeed, International Plant Proteomics Organization (INPPO) is a great worldwide initiative to bring together the plant proteomic communities and foster cooperation, collaboration, and education among the fraternity.

Making biological sense of a proteomic experiment requires proper experimental design, data validation, and comprehensive analysis. Much of the proteomics studies focussed on *Arabidopsis* has been recently amassed in a single portal, MASCP. Previously, the Rice Proteome Database was created containing 23 reference maps of proteins from different tissues and subcellular compartments besides stress-responsive proteomes. The Soybean Proteome Database is also a valuable proteome database. The development of such collective searchable database resources shall aid rapid dissemination of proteomic information from

various studies on several species to the scientific community at large. This shall pave the way for technological applications in relation to these findings and targeted manipulation of novel candidates, thereby facilitating the generation of improved plant varieties. Indeed, translational proteomics is being considered a meaningful way of proteomics research with the main objective of delivering useful applications to address societal problems. Translational proteomics approaches may be used to improve crop tolerance to abiotic and biotic stresses, to achieve yield enhancement in agricultural production, to advance sustainable development of plant-based bioenergy feed stocks, and to optimize food safety as well as food quality controls.

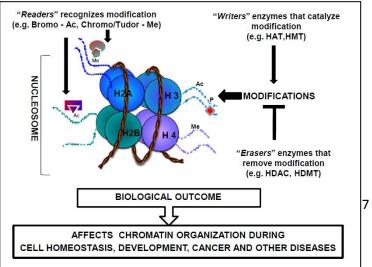
Histone Post-translational Modifications as biomarks in cancer: Dream or Reality ?

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Cancer is a pleiotropic and multifaceted disease in which initiation and progression are attributed to innumerable known and unknown factors. During the past decade, the concept of cancer as a disease of epigenetic, as well as genetic alterations has gained substantial impetus, thereby adding to the complexity. Epigenetics is the study of inherited phenotypes, which are not encoded by the DNA sequence. The term epigenetics commonly refers to changes in DNA methylation, microRNAs, histone post-translational modifications, and other chromatin elements that can alter gene expression. DNA codes for genetic information, that compacts itself around an octameric nucleosomal core of histone proteins and forms a dynamic chromatin structure, the nucleosome. The nucleosome consists of two each of the four core histones: histone 2A (H2A), histone 2B (H2B), histone 3 (H3), and histone 4 (H4), and one linker histone, H1. Histones are highly conserved basic proteins that can undergo post-translational modification (PTM), phosphorylation, acetylation, methylation, ubiquitylation, summoylation, deamination, ADP ribosylation, and proline isomerisation, at specific amino acid residues located on their N- and C- terminal tails. The addition or removal of PTMs from histone tails is dynamic and achieved by various histone modifying enzymes.

The enzymes involved in so called "writing" and "erasing" these reversible marks include, histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases (HDMs), histone ubiquitinating enzymes as well as deubiquitinating enzymes.



The specific PTMs on over hanging histone tails, serve as a "reader" for respective binding domains in proteins, to recognize and interact at the site and thereby modulate gene regulation.

In recent years, the complexity of the post translational modifications and histone/chromatin associated proteins is receiving increased attention. Information about the PTMs and the related modifying enzymes is available in the database *HI*stome: The *H*istone *I*nfobase (http://www.actrec.gov.in/histome/). The increased need for sensitive and accurate identification of epigenetic determinants of disease is one of the many driving forces behind Mass spectrometry -based proteomics for understanding the role of histone PTMs. MS offers an unbiased method for discovering, screening and quantifying histone **PTMs** as well as to examine the combinatorial nature of PTMs in a high-throughput fashion. Mass spectrometry, specific protein enrichment protocols and several proteomic methods have also emerged as versatile tools to understand chromatin biology and dynamic association of chromatin bound proteins involved in replication, gene expression and a variety of biological states including cancer.

The fundamental roles of histone modifications in gene regulation and expression, and with their impact on genomic stability is well established, so it is not amazing that aberrant patterns of histone marks will play an important role in cancer biology and serve as notable predictors of disease subtypes, progression and patient survival. Recent studies have shown that there is global loss of histone PTMs such as histone 4 acetylated lysine 16 (H4K16ac) and histone 4 trimethylated lysine 20 (H4K20me3) in almost all cancer types. A decrease in H4K20me3 levels in various cancer cell types is associated with diminished expression of specific histone modifying enzyme, Suv4-20h2. Loss of H4K16ac mark leads to an altered chromatin conformation, which might contribute to genome instability and has been shown to influence tumor progression and cancer cell sensitivity to chemotherapy. Reduction in H4K16ac correlates with downregulation of hMOF (human orthologue of the *Drosophila melanogaster* males absent on the first gene),a major enzyme that acetylates H4K16, in primary breast carcinoma and medulloblastoma and it is also a biomarker for clinical outcome for the latter. Further, H4K16ac is also deacetylated by the NAD-dependent HDAC sirtuin1. SIRT1 is reported to exhibit increased expression in various tumors, based on which it is proposed as a prognostic indicator.

Interestingly, few histone modifications show tumor specific alteration patterns, such as breast cancer cells show low levels of histone 3 dimethylated lysine 4 (H3K4me2) and histone 3 acetylated lysine 9 (H3K9ac), whereas lung cancer cells show low H3K4me2 but high H3K9ac levels. These rather contradicting data suggest that the same histone modification can predict opposite prognosis in different cancer types. Distinct patterns of modification are also seen in other cancers. Reduced level of histone 3 dimethylated lysine 4 (H3K4me2) and histone 3 acetylated lysine 18 (H3K18ac) level correlates with poor prognosis of patients having adenocarcinoma. H3K4 methylation is established by the SET1 and mixed lineage leukemia (MLL) family of HMTs and removed by the lysine-specific histone demethylase 1 (LSD1) and jumonji AT-rich interactive domain 1 (JARID1) family of HDMs. These enzymes show altered activity in cancer. Expression levels of LSD1 are significantly elevated in bladder, lung, colorectal carcinoma and neuroblastoma and correlate with adverse outcome. The JARID1 family of HDMs has the ability to reverse both the H3K4me2 and H3K4me3 modification state. Higher levels of trimethylation of lysine27 on H3 (H3K27me3) associates with the aggressiveness of tumors and poor progression-free survival of patients in samples studied in prostate, breast, oesophageal, liver and renal cell carcinoma.

system and has been implicated in the formation of repressive chromatin domains. Recent studies have shown that overexpression of Enhancer of zeste homolog 2 (EZH2) is observed in diverse cancers, including prostate, breast, renal and ovarian cancers, as well as glioblastoma multiforme. Generally, EZH2 overexpression in cancer cells seems to result in an EZH2-dependent increase in H3K27me3. On the contrary, H3K9me3, another transcriptional repressive mark, has been reported to decrease significantly in various malignant conditions such as in prostate cancer and in AML patients and associates with their poor prognosis. Though, in case of breast and gastric adenocarcinoma the level has been reported to increase significantly. More recently, it has been shown that subtypes of prostate cancers can be classified by the pattern of H4K20me1, me2 and me3. Overexpression of G9a, a H3K9 HMT, has been reported in prostate, lung, liver, colon and breast cancers, which is in line with the increased H3K9me3 and loss of its counterpart H3K9ac in many cancers. Further, higher levels of G9a in patients with lung and liver cancer are associated with poor prognosis. Thus, there is now evidence for both increased and decreased activity of enzymes controlling histone methylation in cancer, demonstrating that a precise balance of histone methylation is critical for normal cell growth.

In addition to being prospective molecular biomarkers of disease, histone marks may also serve as potential targets of epigenetic cancer therapies. Unlike genetic mutation, histone modifications are dynamic and reversible in nature and occur in a targeted manner by site-specific modifiers. This specificity generates the potential for targeted inhibition of the specific histone modifiers, e.g. HDAC and HAT which in turn modulate tumor growth, inducing apoptosis and indirectly regulating other genomic output through variety of mechanisms. HDAC inhibitors (HDACi) are being broadly used for the treatment of auto immune diseases other than cancer. HDACi act synergistically or in an additive manner with different anticancer agentssuch as radiation therapy, chemotherapy, and differentiation agents. Chemotherapeutic agents which have additive or synergistic effects with HDACi therapy include antitubulin agent docetaxel; topoisomerase II inhibitors doxorubicin, etoposide, and ellipticine; and DNA cross-linking reagent, cisplatin. Histone targeting drugs, such as, histone deacetylase (HDAC) inhibitors, suberoylanilide hydroxamic acid (SAHA, Zolinza), and romidepsin (Istodax) have already been approved for clinical use by the FDA, and many more continue to be developed and evaluated. Vorinostat is the first HDACi to be approved for clinical use in treating patients with cutaneous T-cell lymphoma. Similarly, importance of HAT has been reported in B cell non-Hodgkin lymphoma that majorly occurred due to inactivating mutations in acetyl transferase genes causing reduction in HAT dosage. Interestingly, some of HAT inhibitors (HATi) have been shown to prevent growth of cancer cells. Anacardic acid, isolated from cashew nut shell liquid, has been identified as a potent non-competitive inhibitor of both p300 and PCAF HAT activity in vitro. Also, the inhibition of p300 acetyltransferase activity by the newly synthesized HATi, CTK7A has been shown to arrest the tumor growth in the xenograft nude mice system, particularly in oral squamous cell carcinoma.

Collectively, it is now well established that histone modifications are altered in most cancers, but it is still unclear whether these changes are causative or a consequence of tumourigenesis? However, the imbalance of histone modifications with their modifying enzymes is one of the major hallmarks of most, if not all, types of cancers which hold futuristic possibility for prediction, prognosis and treatment of diseases 'as and when' the story unfolds. From the recent literature reports which use proteomics for unravelling the complexity of the dynamic chromatin machinery it is becoming apparent that proteomics is likely to play a major role in answering the emerging questions.

Further Reading:

- 1. Rothbart SB, Strahl BD; Interpreting the language of histone and DNA modifications. *BBA -Gene Regulatory Mechanisms*. (2014) http://dx.doi.org /10.1016/j.bbagrm.2014.03.001.
- Dillinger S, Garea AV, Deutzmann R, Németh A. Analysis of Histone Posttranslational Modifications from Nucleolus-Associated Chromatin by Mass Spectrometry. Functional Analysis of DNA and Chromatin: Methods in Molecular Biology. (2014) Volume 1094, 277-293
- 3. Thompson LL, Guppy BJ, Sawchuk L, Davie JR, McManus KJ; Regulation of chromatin structure via histone post-translational modification and the link to carcinogenesis. *Cancer Metastasis Rev.* (2013) Dec; 32(3-4):363-76.
- 4. Arnaudo AM, Garcia BA. Proteomic characterization of novel histone post-translational modifications. *Epigenetics & Chromatin.* (2013), 6:24, 1-7.
- 5. Chervona Y, Costa M; Histone Modifications and cancer: biomarkers of prognosis? *Am J Cancer Res.* (2012) 2(5): 589–597.
- 7. Khare SP, Habib F, Sharma R, Gadewal N, Gupta S[†], Galande S[†]; HIstome: a relational knowledgebase of human histone proteins and histone modifying enzymes. *Nucleic Acids Research* (2011) Database issue doi:10.1093/nar/gkr1125). [†]Corresponding Authors
- 8. Füllgrabe J, Kavanagh E and Joseph B; Histone onco-modifications. *Oncogene* (2011) 30, 3391–3403; doi:10.1038/onc.2011.121.
- 9. Cohen I, Poręba E, Kamieniarz K, Schneider R; Histone modifiers in cancer: friends or foes? *Genes Cancer*. (2011) Jun;2(6):631-47.
- 10. Izzo A, Schneider R; Chatting histone modifications in mammals. *Brief Funct Genomics*. (2010) Dec; 9(5-6):429-43.
- 11. Arif M, Vedamurthy BM, Choudhari R, Ostwal YB, Mantelingu K, Kodaganur GS, Kundu TK. Nitric Oxide-Mediated Histone Hyperacetylation in Oral Cancer: Target for a Water-Soluble HAT Inhibitor, CTK7A. *Chemistry and Biology: Cell Press.* (2010) 17:8, 903-913.

Proteomics for identification of early biomarkers of Naso-pharyngeal carcinoma

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Nasopharyngeal cancer (NPC) is a rare disease in most parts of the world. However, this is much more common in South East Asia, some parts of Africa and the Arctic, in comparison to the Western world, where it is a rare malignancy. NPC is much more frequent in Southeast Asia and Chinese population (18%) compared to North America (0.25%) [1]. In India, NPC is also rare and it is seen in some hilly areas of Northeast India, particularly Nagaland, Manipur and Mizoram. In northeast India, incidence of NPC ranges over complete spectrum from lowest (0.5 to 2.0/100 000 among Caucasoid) to the highest (about 20/100 000 among Cantonese/Zhongshan dialect Chinese) [2].

Nasopharyngeal carcinoma usually refers to squamous cell carcinomas that arise from either stratified squamous or pseudo stratified columnar epithelium of the nasopharynx. The nasopharyngeal mucosa also contains other structures including salivary and lymphoid tissue that can rarely give rise to lymphoma, salivary gland tumours or sarcomas. The three possible etiological factors contributing to the high incidence of NPC in various populations are (I) The Epstein Barr virus (EBV), (II) Genetically determined susceptibility and (III) Associated environmental factors. It is widely accepted that factors other than EBV are also important determinants of the risk for NPC. The association of NPC with the Epstein-Barr virus (EBV) was firmly established as early as 1973. Yet, the role for the virus in the pathogenesis of NPC is still debated and needs further investigations.

Disease biomarker identification is one of the most important areas of research today, as the identification of reliable biomarkers has an enormous impact on disease diagnosis, selection of treatment regimens, and therapeutic monitoring. NPC is highly sensitive to radiotherapy, which is the preferred treatment, but its prognosis is dependent on the stage of disease. Unfortunately, early diagnosis of NPC is still difficult because of non-specific symptoms and deep location. This emphasizes the need to identify biomarkers for early diagnosis, predicting metastasis, recurrence and therapeutic responses. Various techniques are used in the biomarker discovery process but the most common technique is proteomics mainly because of its high throughput nature. This short note mainly describes investigations using proteomics for identification of biomarkers for early detection of NPC.

Serum proteomics

Serum proteomics is an approach of choice for biomarker discovery for early diagnosis as blood samples are quickly and easily obtainable non-invasively with less trauma and complications. Some of serum proteomics studies have used the SELDI-TOF-MS for comparing serum from NPC patients and individuals without cancer and identified differentially expressed peaks or masses of proteins as biomarkers [3-7]. Ho et al. showed that six differentially identified peaks e.g. m/z 6692, 6811, 6862, 7979, 9176, 10272 could correctly classify 83% of NPC patients and 82% of the normal individuals. When EBNA1 IgA test was combined with serum protein profiles, a higher sensitivity (99%) and specificity (96%) was obtained [3]. Wei et al identified 4 biomarker protein serum peaks with higher sensitivity and specificity, of which two peaks (m/z 4581 and 7802) were able to predict 80% of stage I and II NPC patients and 86% stage III and IV patients with NPC [4]. Huang et al. selected three serum biomarkers to establish a decision tree model. Using this model with a blind testing set, 95.0% sensitivity, 83.33% specificity and 90.63% accuracy was obtained [5]. Few other differentially expressed serum protein peaks were also identified by Cao et al (m/z 8605, 5320, 5355, 5380, 5336, 2791, 7154, and 9366) and Huang et al (m/z 2019.691, 2223.114, 2244.074, 2467.500, 2491.888, 7977.352, 15931.654, 31988.322, 39347.955) [6, 7]. No further studies have reported the use of these protein peaks as biomarker in NPC patients so far.

There are several studies which have used 2D electrophoresis (2DE) with MS/MS as an approach wherein serum profiles for NPC and non-cancer samples have been compared to detect differentially expressed protein spots. These spots of interest were then identified with one of the MS/MS technique and fewer significant proteins involved in early diagnosis of NPC were obtained [8-11]. Liao et al described three serum proteins, HSP70, sICAM-1, Serum amyloid A1 protein discovered by 2D electrophoresis-based

serum proteome analysis and validated by ELISA and immunohistochemistry as biomarkers for lymph node metastasis in nasopharyngeal carcinoma [9]. In another study, Liao et al identified Transferrin, ZNF544 protein, transthyretin, FAD-synthetase, NM23-H1, 12-lipoxygenase, SAA1 protein precursor, cytochrome P450, soluble intercellular adhesion molecule-1, cathepsin G, lysine-specific histone demethylase 1 as differentially expressed proteins [11]. The study of Xiao et al resulted in identification of three antigens, cytokeratin 19, Erb3-binding protein, Rho GDP dissociation inhibitor- β proteins which elucidated antibodies in more than 36.8% of NPC patients compared to healthy controls. They further showed that autoantibodies against these proteins has utility in screening and early diagnosis of NPC [8]. In another serum proteomics study, α -2 macroglobulin and complement factor B protein were identified by Seriramalu et al [10].

Plasma proteomics

Plasma has also been used as the sample for investigation in combination with various proteomics techniques. Cheng et al used plasma protein fractionation with MALDI TOF-MS and found 12 biomarkers with sensitivity (32 to 83%) and specificity > 90% indicating that combined use of these increases the diagnostic efficiency [12]. In another study, Cheng et al found and validated Chloride intracellular channel 1 as a potential plasma biomarker with 63% sensitivity and 77% specificity [13]. In a quantitative plasma proteome study, Peng et al identified Kallikrein, thrombin-antithrombin III complex as a potential biomarker [14].

Tissue proteomics

Various proteomics techniques were used in combination for detection of biomarkers in early diagnosis of naso-pharyngeal carcinoma using tissue as a sample. Li et al published the first two dimensional proteome map of human naso-pharyngeal carcinoma wherein they identified 28 differentially expressed proteins between normal naso-pharyngeal epithelial tissue and naso-pharyngeal carcinoma tissue [15]. In another study, Cheng et al compared micro dissected normal naso-pharyngeal epithelial tissue and naso-pharyngeal carcinoma and found that 36 proteins were differentially expressed. Of these, few proteins like annexin1, stathmin, 14-3-3 σ and cathespin D were associated with TNM (Tumor, Node, metastasis) stage of naso-pharyngeal carcinoma [16, 17]. Using 2DE proteomic analysis, Tang et al identified Galectin-1 as a novel biomarker in nasopharyngeal carcinoma [18]. Chen et al used 2DE western blot approach for identification of differentially expressed tyrosine-phosphorylated proteins between nasopharyngeal carcinoma (NPC) and normal nasopharyngeal epithelial tissues and 13 proteins were identified of which, Raf kinase inhibitor protein (RKIP) was successfully validated [19].

Advanced quantitative approach, isobaric tag for relative and absolute quantitation (iTRAQ) labelling, and two-dimensional liquid chromatography, tandem mass spectrometry (2D LC-MS/MS) was employed by Xiao et al where they used flash frozen paraffin embedded NPC tissue with various degrees of differentiation. This study resulted in identification of 730 unique proteins, of these 141 were up regulated and 140 down regulated. The relative expression levels of cathepsin D, keratin 8, SFN, and stathmin1 which were identified and quantified were successfully validated by immunohistochemistry [20].

Li et al used 2DE analysis of the stroma-related proteins in nasopharyngeal carcinoma and normal nasopharyngeal epithelial tissues and identified sixty differentially expressed proteins of which, expression of CapG protein was confirmed by western blotting and immunohistochemical analysis [21]. In another study, 2D-DIGE was used with similar samples and twenty differentially expressed proteins were identified, of which two proteins L-plastin and S100A9 were successfully validated by Western blotting and Immunohistochemical analysis [22]. Ge et al carried out comparative proteomic analysis of secreted proteins from nasopharyngeal carcinoma-associated stromal fibroblasts and normal fibroblasts and identified 11 significantly differentially expressed spots including galectin-1 protein [23].

Ruan et al used the 2D western blot approach to identify and quantify the tyrosine phosphorylation level of 16 proteins in TGF- α -treated CNE2 human NPC cells [24, 25]. Among these identified proteins, ANXA3, KRT8, and KRT18 were validated to be novel tyrosine-phosphorylation targets of EGFR signalling by IP-western blotting [24]. Our group is also working with various samples of NPC patients using proteomics approach and we are expecting interesting results in near future.

Various studies have identified and validated many proteins as potential biomarkers, however there is no protein or group of proteins which has come up as a diagnostic marker for early diagnosis of NPC. This emphasizes the need to validate the utility of the markers in large cohorts of patients.

References:

- 1. Wee, J.T., et al. Chin J Cancer, 2010. **29**(5): p. 517-26.
- 2. Kataki, A.C., et al. Chin J Cancer, 2011. **30**(2): p. 106-13.
- 3. Ho, D.W., et al. Cancer, 2006. 107(1): p. 99-107.
- 4. Wei, Y.S., et al. Cancer, 2008. **112**(3): p. 544-51.
- 5. Huang, Y.J., et al. J Exp Clin Cancer Res, 2009. 28: p. 85.
- 6. Huang, X.W., et al. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi, 2011. 46(6): p. 509-12.
- 7. Cao, S.M., et al. Chin J Cancer, 2010. 29(8): p. 721-8.
- 8. Xiao, Z.Q., et al. Proteomics Clin Appl, 2007. 1(7): p. 688-98.
- 9. Liao, Q., et al. Clin Exp Metastasis, 2008. 25(4): p. 465-76.
- 10. Seriramalu, R., et al. Electrophoresis, 2010. **31**(14): p. 2388-95.
- 11. Liao, Q.L., et al. Nan Fang Yi Ke Da Xue Xue Bao, 2008. 28(2): p. 154-8.
- 12. Chang, J.T., et al. Clin Biochem, 2006. **39**(12): p. 1144-51.
- 13. Chang, Y.H., et al. J Proteome Res, 2009. 8(12): p. 5465-74.
- 14. Peng, P.H., et al. J Proteomics, 2011. **74**(5): p. 744-57.
- 15. Li, F., et al. Int J Oncol, 2007. **30**(5): p. 1077-88.
- 16. Cheng, A.L., et al. J Proteome Res, 2008. 7(6): p. 2415-26.
- 17. Cheng, A.L., et al. Clin Cancer Res, 2008. 14(2): p. 435-45.
- 18. Tang, C.E., et al. Oncol Rep, 2010. **24**(2): p. 495-500.
- 19. Chen, Y., et al. Med Oncol, 2009. **26**(4): p. 463-70.
- 20. Xiao, Z., et al. J Histochem Cytochem, 2010. 58(6): p. 517-27.
- 21. Li, M.X., et al. Med Oncol, 2010. 27(1): p. 134-44.
- 22. Li, M.X., et al. J Cell Biochem, 2009. 106(4): p. 570-9.
- 23. Ge, S., et al. Exp Ther Med, 2012. **3**(5): p. 857-860.
- 24. Ruan, L., et al. Med Oncol, 2010. 27(4): p. 1407-14.
- 25. Ruan, L., et al. Proteome Sci, 2011. **9**: p. 35.

Past Events

5th Annual Meeting of the Proteomics Society, India Developments in Proteomics & Metabolomics in India: An Organizer's Perspective

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The 5th annual meeting of the Proteomics Society was held at IISc, Bengaluru during 28th-30th Nov. 2013. About 450 participants from various scientific disciplines including medical, pharmacy and engineering and biotech professionals attended the conference. They included lecturers and senior research scientists from academic as well as from biotech and pharmaceutical organizations.

The conference was inaugurated by Prof. P. Balaram, Director, Indian Institute of Science, Bangalore. The scientific sessions were initiated by an outstanding talk by Prof. S. K. Brahmachari, Director General, CSIR on the use of Omics technology in improving healthcare.



The theme of 5th Annual Meeting of Proteomics Society- India was Medical Proteomics. Each day of scientific sessions was dedicated to Nobel laureates namely - Prof. Frederick Sanger, Prof. James Rothman and Prof. Koichi Tanaka respectively.

The scientific sessions included 38 plenary talks by leading national and international scientists. They provided their research views on topics ranging from proteomics, genomics, metabolomics and volatilomics. In addition there were presentations on new technology developments in mass spectrometry. A panel discussion entitled "Applications of Mass spectrometry in Medical Research" highlighted the concept of personalized medicine. The poster presentations were held for two days (28-29th) wherein participants who were Ph.D students, post docs and young scientists from academia and industry were allowed to showcase their research. Over 75 entries were received and 68 posters were presented. The abstracts for all the entries were printed in a conference proceeding which was distributed amongst registered participants.

The other highlights of the meeting were a) Q&A, b) Science & Sketch and c) Science and society sessions. These informal sessions provided ample opportunity to exchange scientific ideas amongst the young scientific community. The above deliberations were aptly judged and best presentations were provided cash prizes.

On the second day of the meeting, a special scientific presentation with audio visual effects was organized by Thermo Scientific at The Lalit Ashok which was followed by dinner. The presentation by Thermo scientists included new development in orbitrap technology in mass spectrometry. Once again these sessions were followed by Q & A. These deliberations provided an opportunity for the delegates to network amongst themselves and facilitated exchange of scientific ideas.

To balance the grilling scientific deliberation a cultural programme which comprised Indian classical music, Kathak, Bharatanatyam, Odissi and Rajasthani folk dance were organised. These cultural performances provided a good relief to young scientists after the heated scientific schedule. The success of the meeting can be seen from the testimonials which were provided by scientists from academia and industry who had participated in the event.

A Pre-Meeting Workshop on Mass Spectrometry and Metabolomics was held on 27th Nov 2013.

C-CAMP organized this workshop in collaboration with the 5th Annual Meeting of Proteomics Society India in order to create a better understanding of the field of Metabolomics and its applications. A total of 39 participants attended the workshop. The workshop covered topics on introduction to Metabolomics, quantitative metabolomics and volatile organic compounds followed by a hands on training session on quantitative metabolomics. The overall feedback from the participants was very positive and most of the participants found the workshop to be well structured and course material to be relevant.

More detailed information about the meeting is available on the Proteomics Society, India website http://www.psindia.org

Upcoming Events

Proteomics Society, India 6th Annual Meeting and International Conference in IIT Bombay, Dec 6-11, 2014

Dear Colleagues,

The Proteomics Society, India (PSI) has been annually bringing together the proteomics community to encourage exchange of ideas, increase collaborations and enhance innovations at the National and International level. We are delighted to announce that the 6th Annual Meeting of PSI and International Conference on "Proteomics from Discovery to Function" is going to be held at the Indian Institute of Technology (IIT) Bombay from 7th – 9th Dec 2014. Pre-conference event is scheduled on 6th December and post-conference workshops are scheduled on 10th – 11th Dec 2014. The PSI-2014 Annual meeting and International proteomics conference will feature many distinguished speakers, including Dr. Pierre Legrain (HUPO President), Dr. Mark Baker (HUPO President-elect), Dr. Gilbert Omenn (Ex-HUPO President), Dr. Samir Hanash (Ex-HUPOPresident), Dr. Catherine Costello (Ex-HUPO President), Dr. Joshua LaBaer (US-HUPO President-elect), Dr. Concha Gil (Chair, HUPO-2014 Spain), Dr. John Yates, Dr. Brenda Andrews, Dr. Philip Andrew, Dr. Chung Ching Ming Maxey, Dr. Andrew Link, Dr. Robert L. Moritz, Dr. Juvan Calvete and others who will address large gatherings.

Following the tradition of Proteomics Society, India to give an opportunity to the younger PSI members, Dr. Rapole Srikanth, Dr. Sanjeeva Srivastava, Dr. Harsha Gowda and several others will give talks during the conference, and participants will also get an opportunity to listen to the PSI senior members Dr. Surekha Zingde, Dr. Ravi Sirdeshmukh, Dr. M. A. Vijayalakshmi, Dr. K. Dharmalingam, Dr. Akhilesh Pandey, Dr. Abhijit Chakrabarti, Dr. Rakesh Mishra, Dr. Utpal Tatu, Dr. Shantanu Sengupta and Dr. Mahesh Kulkarni among others, during the meeting. There will be 9 themed sessions in the conference, which include few core themes on Cancer biomarkers, Biomarkers of biofluids, Post-translational modifications and few new themes on Advances in MS technology, Interactomics and microarrays, Proteomics and Systems Biology and Human Proteome Project. In addition to the plenary and invited talks, there will be short talks from the young investigators and students. There will be four parallel post-conference workshops on Gel-based proteomics, Mass spectrometry, Protein Microarrays and Surface Plasmon Resonance, which will be two-days long and highly structured to provide advance knowledge and hands-on experience to the participants. The details of workshops and pre-conference event will be updated soon.

The PSI-2014 in Mumbai will provide a unique opportunity to share your proteomics research with wide scientific community and a forum for the in-depth analysis of the challenges involved in studying the dynamic proteome. In addition to the highly stimulating scientific talks; the interactive sessions, panel discussions and Mumbai sightseeing activities are also scheduled in the pleasant weather of Mumbai in December. Please join us in Mumbai to explore a plethora of information on latest research in proteomics and to network with fellow academicians, industrial researchers and editorial board members of prestigious journals. Block your calendar now and register to have a stimulating, enjoyable and unique

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experience of this extravaganza in proteomics. Seats are limited and abstract submission is currently accepted. For more information about this event and for the registration related queries, please visit http://www.bio.iitb.ac.in/~sanjeeva/psi2014/ or contact me at: sanjeeva@iitb.ac.in

We are looking forward to welcome you to the beautiful campus of IIT Bombay !

Sincerely yours, Sanjeeva Srivastava Convenor, 6th PSI Meeting & International Conference IIT Bombay, Mumbai

Upcoming National and International Proteomics conferences / meeting / workshops

Upcoming National and International Proteomics meeting 62nd ASMS Conference on Mass Spectrometry and Allied Topics June 15-19, 2014 Baltimore Convention Center, Baltimore, MD Short Courses, June 14 - 15, 2014 http://www.asms.org/conferences/annual-conference/annual-conference-homepage **ICPB 2014: International Conference on Proteomics and Bioinformatics** July 18-19, 2014 in Oslo, Norway http://www.waset.org/conference/2014/07/oslo/ICPB 88th OMICS Group Conference 4th International Conference on Proteomics & Bioinformatics August 04-06, 2014 in Hilton Chicago/ Northbrook, Chicago, USA http://www.proteomicsconference.com/ **AOHUPO 2014** Frontiers in Protein and Proteomic Research August 06-08, 2014 Miracle Grand Convention Hotel, Bangkok, Thailand http://www.aohupo2014.com/ Eleventh International Symposium on Mass Spectrometry in the Health and Life Sciences: **Molecular and Cellular Proteomics** August 17-21, 2014 in Hotel Nikko, San Francisco http://msf.ucsf.edu/symposium/ 20th International Mass Spectrometry Conference (IMSC) August 24 and 29, 2014. Geneva, Switzerland http://www.imsc2014.ch/ 10th Siena Meeting - From Genome to Proteome 20 years of proteomics August 31 - September 04, 2014 in Siena, Italy http://www.congressi.unisi.it/proteome/

HUPO 13th Annual World Congress The proteome quest to understand biology and disease October 5 – 8, 2014 in Madrid, Spain <u>www.HUPO2014.com</u> OurCon II 2014 Imaging Mass Spectrometry Conference and Congress of the Turkish Proteomics Society. November 18-21, 2014: in Antalya, Turkey <u>http://www.ourcon2.org/</u>

Proteomics Society, India

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