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# CANCER PROTEOMICS: A STRIDE THROUGH DISEASE PROTEINS

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**Abstract:**

Cancer, a dreaded disease with high mortality rate, is manifested in the form of genes translated into proteome. Extensive research has been undertaken to understand the mechanism of its onset, progress, and malignancy. The fatality of the disease has led researchers into developing improved diagnosis and targeted medicine. While some of the cancers are inherited and some acquired due to many known or unknown chemical or environmental effects or due to aberrant cellular mechanisms. The need to identify the genes and proteins remain paramount. The discovery of proteins and its PTMs are significant in cancer treatment and global and targeted proteomics play a very important role in these studies. In this study, we have tried to relate the importance of proteomic techniques and its importance in functionally characterizing proteins in various forms and stages of cancer along with study of various important PTMs. These may eventually help in early, better diagnosis and provide knowledge for discovery of targeted precision medicines.

**Keywords:**

Cancer, Proteomics, LC-MS, Biomarker, PTM

## Introduction:

Cancer is one of the leading causes of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018. It is a disease involving translation of genome into proteome. The term proteome demonstrates the complete arrangement of proteins encoded by a genome. Proteomics includes the global protein arrangement of a cell or life form. It is important to observe the level together with the action of proteins [1,2]. Proteomic data are helpful in characterizing cells and tissues in disease state and understanding different natural components. The arrangement, interactions, and activity of all proteins inside cells and living organism can be distinguished by using techniques for protein estimation. This field includes strategies that can be applied to serum and tissue to draw a significant biological data as biomarkers to help clinicians and researchers in understanding the unique science of their interest, for example, a patient with cancer [3].

Proteins are complex molecules composed of multiple amino acids each of which are unique and are the structural units of the living organisms. They modify themselves in their functions, stability, 3D designs, and are considered as the ultimate gene products of the greater part of the qualities. Any protein in an organism can be modified during translation or after translation. The post translational modifications (PTMs) of proteins manifest themselves in a form of physiological response during adverse conditions like disease including cancer. PTMs over 450 of them in the form of Phosphorylation, SUMOlyation, acetylation, glycosylation, ubiquitination changes the activity, turnover, longevity and interaction among proteins [4]. The change in post translational state of proteins involved in cell, survival, proliferation result in abnormal proliferation of cancer cells,

which occur due to activation of oncogenes and suppression of tumour suppressor genes (TSGs) [5,6].

Proteomics includes a vast range of techniques, for example, protein expression profiling, protein adjustments, interactions between proteins, structure of protein, and protein activity [7,8]. The outcomes obtained from such tasks can be utilized to interpret sickness processes, analyses diseases, help in drug advancement, and is the reason for biological discovery [9-12].

The proteomics strategies have become well-known in malignant growth studies. Proteomics-based advancements have empowered the identification of promising biomarkers and protein articulation arrangement that can be utilized to evaluate cancer diagnosis, classification of tumour, and to recognize promising responders for explicit treatments. This data can be acquired from several types of samples and be utilized to progress disease therapeutics [13-16]. Moreover, to comprehend the fundamental science of malignant growth, proteomics methods have been used to understand how the signalling pathways in tumour cells are changed and working on how to target different pathways for cancer treatment [17-20].

Malignant growth proteomics includes the identification and quantitative examination of differentially expressed proteins comparative with healthy tissue counterparts at various phases of infection, from preneoplasia to neoplasia. Proteomic advances can likewise be utilized to distinguish markers for diagnosis of cancer, to observe disease progression, and to recognize remedial targets. Proteomics is significant in the revelation of biomarkers because the proteome reflects both the natural genetic program of the cell and the effect of its nearby climate. Protein expression and activity are dependent upon balance through record as well as through posttranscriptional and translational events [21].

Even though proteomics generally managed quantitative examination of expression of protein, but recently, proteomics has been seen to comprehend the structure of proteins. Quantitative proteomics endeavours to explore the progressions in protein expression in various states, for example, in healthy and infected tissue or at various phases of the infection. This empowers the recognition of state-and stage-explicit proteins. Primary proteomics endeavours to reveal the arrangement of proteins and to unwind and identify protein-protein interactions [22].

**2. Proteomic techniques in cancer diagnosis and treatment:**

Scientific platforms for proteomics have been created to distinguish the entire arrangement of proteins in living beings and to uncover subjective and quantitative protein varieties upon assorted natural diversity. Additionally, far reaching research on proteins has become conceivable by building an amino acid sequence database on the arrangement of proteins [23].

The most interesting part of the proteomics field is its capacity to uncover novel biomarkers of infection. For instance, malignant tumour grows, changes in protein profiles and contrasts in protein dispersion both in tissues and body fluids, for example, blood can be analysed through quantitative examination. Proteomics facilitate the simultaneous qualitative and quantitative profiling of various proteins [24-27]

Different advances have been created to recognise proteomic estimation. Proteomic information is very helpful in grouping cells and tissues in disease states and comprehending different natural processes [28]. Scientists recognize the arrangement, interactions, and activity of all proteins inside the cells and organisms by using strategies for the protein estimation. The human proteome

data under a range of physiological and pathological conditions is measured and interpreted by the B/D-HPP (Biology/Disease centric). Significant discoveries continue to be made from all B/D-HPP teams across the world with personalized cancer immunotherapy and therapeutic modalities and with PTMs orchestrating many outcomes including response to therapy [29].

**2.1. 2D Gel Electrophoresis (2DE):**

Two-dimensional gel electrophoresis (2DE) or 2D-PAGE is a potential technique for proteomics work. It isolates the composite mixture of samples utilizing two distinct properties of the proteins. In the primary dimension, proteins are isolated by the pI value and in the second dimension it is isolated by the relative molecular weight. Although it was depicted way back in 1975 by O'Farrell, its relevance and adoptability were improved on account of the presentation of immobilized pH gradient strips, as they gave great reproducible outcomes and handling became simple. At first proteins are seen by labelling it with $^{32}P$ or $^{35}S$. Presently this has been interchanged by more sensitive strategies like SYBRO Ruby. Development was made in various phases of the 2D-PAGE strategy which can separate up to 10,000 proteins in a single gel. A 2D-PAGE gel picture is caught, and the analysis is done to observe the quantity of proteins expressed in a specific tissue. The identified proteins are cut and digested into fragments. These fragments are examined with high-resolution mass spectrometry. The digested protein fragments are then matched from known protein databases [30].

2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and surface-enhanced laser desorption/ionization with time-of-flight mass spectrometry (SELDI-TOF-MS) have been used for identification of tumour markers in bladder cancer [31]. 2D-DIGE with novel ultra-high sensitive fluorescent dyes (CyDye DIGE Fluor saturation dye) helps in efficient protein expression profiling of laser-micro dissected tissue samples. This allows accurate proteomic profiling of specific cells in tumour tissues [32]. 2D-DIGE and MS have revealed new bio markers of prostate cancer where secernin-1 and vinculin-1 were identified as biomarkers [33,34].

**2.2. Isobaric Tag for Relative and Isobaric Quantitation ( iTRAQ):**

iTRAQ is a shotgun-based quantitation method and is also known as bottom-up approach which permits the simultaneous identification and relative evaluation of many proteins in up to eight different organic samples in a single examination. Samples that are digested have been labelled with the 8-plex iTRAQ reagents and the sample is pooled and ready for MS/MS. iTRAQ-based quantitative proteomics is a promising methodology for worldwide examination of protein expression in small quantity of samples. iTRAQ is appropriate for biomarker applications as it gives both quantitation and multiplexing in a single reagent. In cancer biomarker studies, this procedure has been simply used to look for biomarkers in different types of cancer. Involving iTRAQ for urinary cancer biomarkers is a new advancing area and has potential for future breast cancer biomarker research for early diagnosis and monitoring progression [35].

**2.3. Protein arrays:**

A protein microarray is a piece of nitrocellulose covered glass slide on which various particles of protein have been bound at independent locations [36]. Protein arrays utilizes antibodies of known affinity and specifications, and they are pressed on the outer layer of aptamers. This technique permits the perception of the biochemical activity of thousands of proteins [37]. Protein microarray formats are divided into two principal classes: forward phase arrays (FPA) and reverse phase arrays (RPAs). In forward-phase arrays (FPAs), antibodies are arrayed and probed with cell lysates, while in reverse-phase arrays (RPAs), cell lysates are arrayed and probed with antibodies [38,39].

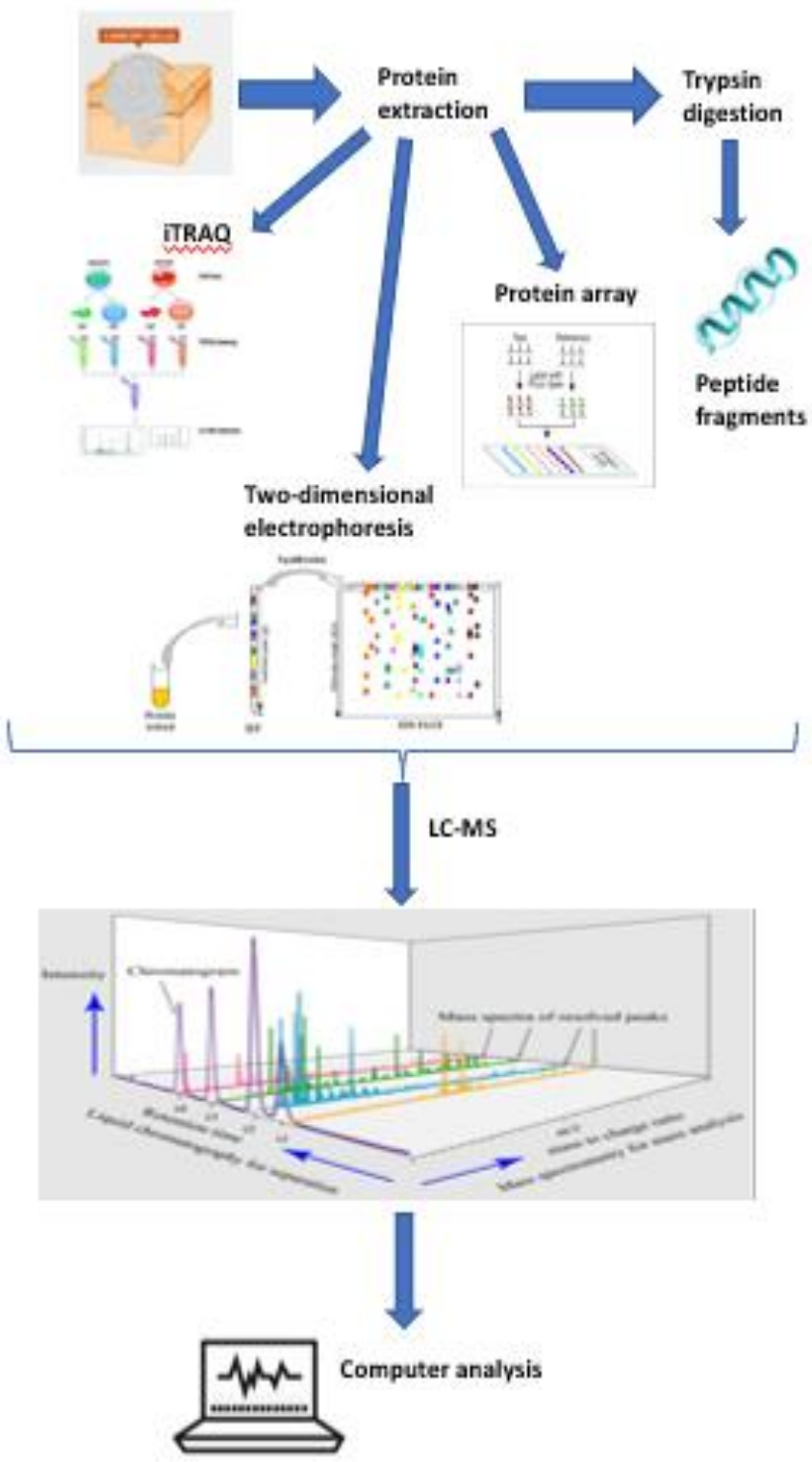


Fig 1: Figure showing different proteomic technologies involved in cancer: i. 2DE, ii. Protein array, iii. iTRAQ, iv. LC-MS, adapted: [108,109]

Prior information on molecules is expected to involve this technique for finding biomarkers. One part of the protein array approach that recognizes it from DNA microarrays is its capacity to distinguish protein isoforms that may be basic in identifying infection pathogenesis [40]. Discovering these explicit biomarkers is clinically helpful in demonstrating changes between a normal and infected sample. This data is useful to clinicians in the diagnosis of infection, as well as in the observing the response of patient towards the therapy [41].

**2.4. LC-MS:**

Liquid chromatography-mass spectrometry (LC-MS) is presently a unique technique with the advancement of electrospray ionization (ESI) giving a basic and strong interface. It may be applied to a wide arena of biological molecules and the utilization of multiple MS and stable isotope inside guidelines permits exceptionally delicate and exact test to be developed, even though some technique optimization is needed to limit ion suppression effect. Quick examining speeds permit a high level of multiplexing, and many mixtures can be estimated in a single scientific run. With the improvement of more reasonable and reliable instruments, LC-MS is beginning to assume a significant part in a few areas of clinical biochemistry and compete with traditional liquid chromatography and different methods like immunoassay [42].

LC-MS is an important technology for analysis in biomedical research. Its flexibility in approach to analyse proteome quantitatively as well as qualitatively is beyond doubt. Global quantitative proteomics for biomarker discovery and targeted quantitative proteomics for validation of biomarkers are two important LC-MS based proteomics approaches [43,44]. Label-free and stable isotope labelling approaches of global proteomics is dependent upon DDA (data dependent acquisition). Use of peak area or signal intensity can be used for the relative protein abundances or 'reporter ion' in case of isobaric labelling approaches like iTraq and tandem mass tags (TMTs). Global proteomics has revealed cancer biomarkers like prostrate, ovarian cancer and renal cancer [46-48]. iTRAQ labelling with SCX (strong cation exchange) is the most common method of analysing samples by LC-MS [48-50].

Targeted proteomics, in global proteomics using LC-MS/MS approach many low abundant proteins remain to be identified and has data reproducibility issues. To overcome this shortcoming SRM (selected reaction monitoring) also known as MRM (multiple reaction monitoring), PRM (parallel reaction monitoring) are being used [51,52]. These techniques involve 'spiking-in' of internal standards which are heavy labelled isotopes of peptides in the samples and give precise and accurate quantification of proteins. High end instruments like Triple quad (QQQ) for SRM studies, High resolution accurate mass (HR-AM) for PRM using Q-exactive instrument (Thermo corporation, USA) which uses an orbitrap mass analyser are used but they are limited to small number of proteins quantified in each run. PRM has been used for identifying aberrant proteins in lung cancer [53].

A new modification in technique known as DIA (data independent acquisition), it collects all the MS/MS scans irrespective of precursor ion selection from a full MS scan, it combines both the advantages of global proteomics and targeted proteomics. SWATH-MS a type of DIA provides high dynamic range, reproducibility, accuracy, and large-scale quantification [54]. This methodology has been used for analysis of the N-linked glycoproteome of prostate cancer and resulted in the verification of two glycoproteins as novel potential biomarkers for prostate cancer aggressiveness [55].

**3. Application of proteomics in cancer:**

Malignant growth is a multifaceted disease which results from dysregulated cellular signalling that control cellular behaviours, like

expansion and apoptosis, brought about by genomic, genetic, and epigenetic mutations at the cell or tissue levels [56].

Oncoproteomics is a part of proteomics which deals with the study of proteins and their interactions for a malignant growth cell by proteomic strategies. There is a trend to apply proteomics to encourage a better understanding of cancer infection, foster new tumor biomarkers for diagnosis of the disease, and early detection utilizing proteomic image of samples. Oncoproteomics has a promising role to alter clinical practices, including diagnosis of cancer and screening in view of proteomic stages as a complement to histopathology, individualized choice of therapeutic combinations that focus on the cancer-explicit network of protein, real-time evaluation of remedial efficacy and toxicity, and rational balance of treatment considering changes in the cancer protein network related with diagnosis and drug resistance [57].

**3.1. Early detection of cancer:**

Early detection is necessary to control and prevent cancer. Biomarkers help in this method by giving significant data about the status of a cell at a particular point in time. As a cell changes from non-infected to neoplastic, the changes can be recognized through the identification of the suitable biomarkers. Biomarker research has enhanced development technology like proteomics. Transformation of malignant tumour include mutations in protein expression with successive clonal multiplication of the transformed cells. These mutations can be checked at the protein level, both qualitatively and quantitatively. Protein marks in malignant growth give significant data that will help to more efficient treatment, prognosis, and reaction to therapy [58]

The capacity to recognise proteins inside complex organic fluids, for example, serum, plasma, nipple aspirate fluid and urine by proteomic innovations has reached a point where many species can be recognized fast, which brings the more prominent chance of distinguishing the ideal biomarkers of cancer [59,60]

**3.2. Metastasis:**

The variety of different cancers and the metastasis that occurs at the time of malignant growth are the major problem towards the successful therapeutics [61-63]. Metastasis is the most widely recognized aspect of malignant cancers; however, the precise technique by which the metastatic growth takes place is still not clear. Recently, several studies on proteomics have been performed to uncover the reason for the expanded metastasis found in cancer. In one such review, utilizing a few Omics like transcriptomics, proteomics, and phosphor-proteomics to analyse a patient-derived xenograft mouse model, TMT labelling analysis uncovered that an inflation in stress hormone levels during breast cancer development was found to cause an increase in the action of the glucocorticoid receptor (GR) at metastatic areas thereby reducing the rates of survival. It was also found that the increased GR action was involved in the initiation of various processes in metastasis and in the raised expression levels of the kinase ROR1, both of which relates to less endurance.

Scientists identified that expression of Bach1, a pro-metastatic transcription factor, through a multi-Omics analysis of the transcriptome and proteome. In lung adenocarcinoma, the deficiency of keap1 and subsequent Nrf2 initiation induced metastasis through the aggregation of Bach1, and this technique was related to a decrease in the survival rates of patients with lung cancer in a heme oxygenase-1-dependent way. Nrf2 was displayed to reduce the Fbxo22- mediated debasement of Bach1 in a heme oxygenase-1 dependent way, proposing that inhibition of heme oxygenase-1 is an efficient therapeutic technique for preventing cellular breakdown in the lung cancer metastasis [64].

**3.3. Drug resistance:**

Malignant growth can reoccur despite treatments like chemotherapy and surgery, propounding that recurrent cancer consists of cells that become resistant to anti-cancerous drugs [65,66]. The proteomics approach can be utilized to recognize the feature of drug resistant cancer cells and find targets that can defeat drug resistance that takes place during anti-cancer treatment. A few reports have shown that cells that endure therapy with anti-cancer agents in cancers like breast, pancreatic, and lung cancer presenting explicit protein expression and molecular techniques, corresponded with the lower survival rates of patients [67-69]. These investigations might give the feasibility to amplify the impact of chemotherapy utilizing additional drugs that control key proteins engaged with drug resistance. The features of drug resistant cancer cells are related to: stemness in development, progression, reoccurrence, metastasis [70,71]

Cancer stem cells (CSCs) derived from breast cancer cell lines show resistance towards drug, and proteomic analysis of these cells propose new explicit markers and therapeutic targets for CSCs [72]. Raffel *et al*. recommended that the significance of targeting on leukemic stem cells as the justification for the poor clinical results after treatment for acute myeloid leukaemia is because of chemotherapy-safe cells. Hematopoietic stem/progenitor cells and leukemic stem cell population investigation uncovered that IL3RA and CD99 could be markers of leukemic cells [73]. Proteomic

examination uncovered that the proteins with the highest growth in CSCs were related with metabolism of carbon, and the inhibition of synthesizing fatty acid with reduced viability of CSC, suggesting a key metabolic pathway controlling CSCs. The action of cancer stem cells (or CSCs) resistant to drug interferes with the therapeutic process, and tumours in which these cells exist are classified as incurable cancer that can't be dealt with conventional anti-cancer drugs. Hence, for an ideal disease treatment, it is important to distinguish explicit proteins in these cells and recognize new analytic and restorative targets [74

**Table 1. Markers used in cancer proteomics:**

| Cancer types | Sample types | Method of target discovery | Target | Biomarker/Target type | Characteristics of biomarker | Reference |
|---|---|---|---|---|---|---|
| LIVER | Patient's tissue | Proteomics<br>Phosphoproteomics | PYCR2<br>ADH1A | Prognostic | Reprogramming of HCC metabolism | 75 |
| Pancrease | Primary Pancreatic epithelial cells | Proteomics | LKB1 | Prognostic | Regulation of pathways associated with glycolysis, serine metabolism and DNA | 76,77 |
| | PDAC cell lines | Proteomics | MAP2 | Prognostic | Proteins used in microtubule synthesis are upregulated in gemcitabine-resistant cells | |
| Ovary | Patient's tissue | Proteomics | NNMT | Therapeutics | Central metabolic regulator of CAF differentiation and cancer progression in | 78 |
| Breast | Patient's tissue, breast cancer cell lines | Proteomics<br>Metabolomics | PYCR1 | Prognostic | The higher the expression of PYCR1,the lower the survival rate.PYCR1 expression helps to acquire resistance | 79 |
| | Breast CSCs, breast cancer cell lines | Proteomics | CD66c | Therapeutics | It is said to be a novel breast CSC marker by modulating the cell viabilityof CSCs under hypoxic condition | 80 |
| | Breast cancer cell lines | Proteomics | NEDD4 | Therapeutics | Presented as a new therapeutic target by regulating the expression of ALDH1A1 and CD44, which are characteristic of CSCs | 81 |
| Lung | EGFR-mutant cell lines | Proteomics<br>Phosphoproteomics | PI3K<br>MTOR | Therapeutics | Lung cancer is resistant to EGFR tyrosine kinase inhibitor. PI3K/MTOR inhibitor was used together to overcome resistance | 82 |

**4. Advances of Proteomics in Novel PTM Discovery:**

PTM of a protein can restore the entire downstream trafficking mutating the activity of protein and fate of the cell. Therefore, PTMs decide the ideal functionalities of a few proteins that are involved with Cancer. Phosphorylation is perhaps the most widely studied transformations and happens in a dynamic and quick, controlling different signalling pathways. In general, phosphorylation patterns of explicit proteins are seen in a few malignancies as proven in non-small cell lung cancer in the lungs (NSCLC) patient tumour sample, serum sample from patients suffering from breast and prostate cancer, patient derived intense myeloid leukaemia bone marrow cells (AML), human pancreatic duct tissue of Pancreatic ductal adenocarcinoma patients and renal cell carcinoma growths from kidney disease patients [83-87]

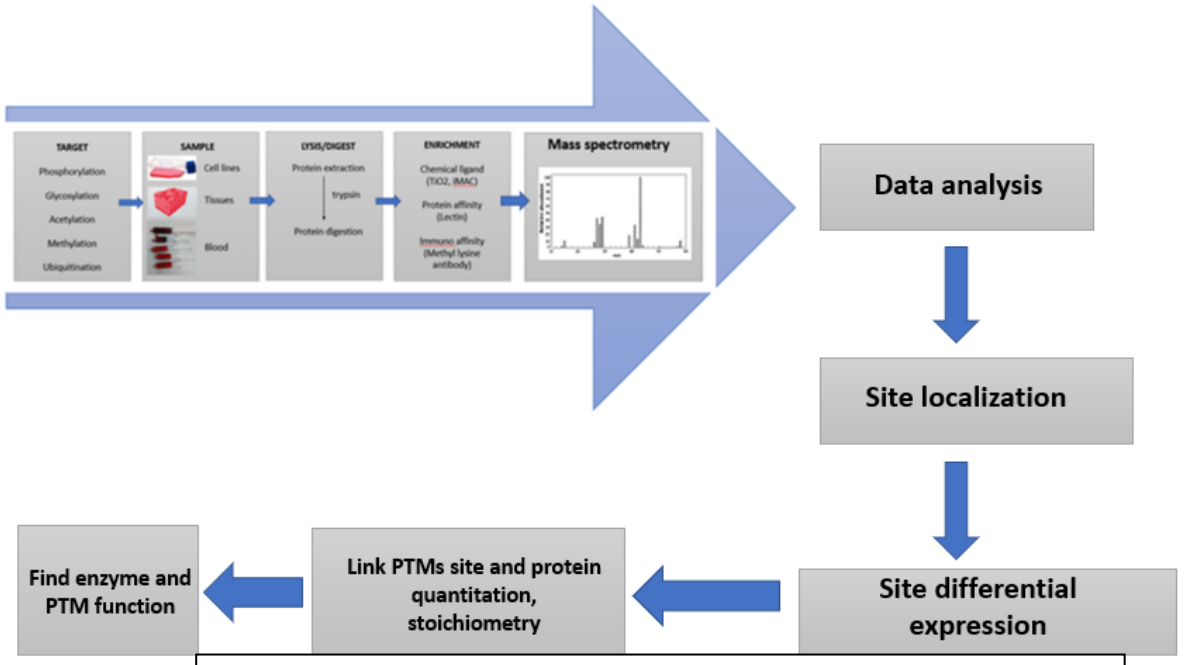


Fig 3: Post translational modification analyses. adapted [110]

Glycosylation is an extensive and complex form of protein post-translational modification (PTM), features of various cell surface and secreted eukaryotic proteins. Changes in protein glycosylation, which occur through varying the heterogeneity of glycosylation sites or changing the glycan structures of proteins on the cell surface and in body fluids, have been shown to interact with the advancement or progression, or both, of cancer and other disease states. Glycoproteins have also provided an ideal source for discovering biomarkers for disease detection, various clinical cancer biomarkers and therapeutic targets are glycoproteins including cancer antigen in gastrointestinal cancer, cluster of differentiation 340 (Her2/neu) in breast cancer and prostate-specific antigen (PSA) in prostate cancer. Differentially expressed glycosylation in serum taken from patients with pancreatic cancer has also been observed using liquid phase separation coupled with mass spectrometry (MS) analysis [88].

Another very important PTM, Sumoylation includes the covalent bonding of a small ubiquitin-like modifier (SUMO) to a residue of lysine of target proteins through the arrangement of an isopeptide bond. In people, there are three SUMO isoforms which are

joined to proteins through an enzymatic cascade like the process of ubiquitination. The significance of sumoylation in most cell processes includes the cell cycle, transcriptional regulation, and localisation of nucleus has been recently discovered [89-91]. SUMO-interacting motifs (SIMs) and recombinant SUMO-binding entities (SUBEs) has been adopted for the improvement and identification of endogenous poly-SUMO proteins [92,92]. SILAC, iTRAQ and LFQ have been utilized for quantitation of sumoylation [94-96]. Different bioinformatic instruments like SUMmOn, SUMOhydro, SumSec, and so on, has been presented for sumoylation site identification [97-100].

Acetylation and methylation are important PTMs that play roles in numerous cellular processes including cell signalling, metabolic pathways and especially, DNA-protein interactions. The acetylation of histone proteins is a vital process that impacts the accessibility of DNA to the transcriptional process. The transfer of an acetyl group to the α-amino group at the N-end of the protein is an irreversible modification, while acetylation at a lysine residue is reversible. Acetylation is catalysed by acetyltransferases and lysine acetylation might be switched by lysine deacetylases [103]. Methylation predominantly takes place on lysine and arginine amino acids. Nevertheless, different deposits like histidine, proline, and glutamine may be dependent upon methylation. Methylation is catalyzed by lysine or arginine methyltransferases and switched by demethylases [102].

The fundamental source of PTM identification is mass spectrometry-based proteomics, provided by assimilation with interactome and transcriptome approaches. The wide range is a significant challenge in plasma/serum proteomics, making recognizable proof of altered proteins rather restricted. This issue can be resolved by immunodepleting, but this method is less reproducible and requires large amount of introductory material to enhance modified proteins adequately. Though, these challenges lead to few misidentifications and altogether prevents examination of the specific role of explicit PTMs in oncogenesis. Hence, just a small part of PTMs is very much approved and relates to a few kinds of malignant growth, including glycosylation of COX-2 in colorectal cancer [103], citrullination of fibronectin during renal malignant growth [104], phosphorylation of PKM2 in thyroid malignancy [105], and deSUMOylation interceded by SENP1 in prostate malignancy [106].

**Discussion:**

Cancer is a disease which has been continually researched and many genomics projects have been completed and many currently underway. The sheer diversity in the various cancer types, stages and its outcome make the role of proteomics much more important. The available genomic data must be integrated with the proteomic data which is relatively new with the advent of new and robust proteomic technologies. The ‘proteogenomic’ study will provide a better insight into the disease. The proteomic data in a large-scale analysis of breast cancer had identified a GPCR not identified at mRNA level and highly phosphorylated kinases and consequences of 5q deletion have been studied [107]. The use of proteomic technologies like iTRAQ/iCAT and the intensive use of LC-MS have led us to better understanding of cancer at multiple levels involving protein as well as PTMs. The discovery of differential protein expression and PTMs along with histopathology and clinical data serve an important role in determining precision medicine and novel therapies. Large scale proteomics with reduced sample amount and increased sensitivity for low abundant proteins and peptides aided with high end computational data analysis provide hope for combating cancer better with early diagnosis and targeted treatment.

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