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PROTEOMICS IN PRIMARY BRAIN TUMORS

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1. ABSTRACT

Genomic damage present in tumors may lead to abnormal or altered expression of proteins. Some of the findings of genetic explorations of brain tumors have had their impact on making the diagnosis or are important with respect to therapeutic decisions. The identification of individual proteins or clusters of proteins expressed in neoplastic tissues potentially may have an even more important relevance for making the diagnosis, prognosis and treatment outcome. Diverse posttranslational modifications of proteins may be linked to neoplastic lineage or stage. For the rapidly developing fields of proteomics and its integration with genomics and transcriptomics (by now called operomics) the application of bioinformatics is crucial. This review addresses the nascent field of proteomics and its diagnostic potential in the feld of primary brain tumors. Various technical approaches to separate and identify proteins are described, including the most recent developments in the analytical proteomic technology.

2. INTRODUCTION

The incidence of primary brain tumors in European countries and United States is approximately 1-10 cases/100,000 persons per year with slight variations among different regions of the world (1-4). Survival rates of patients with primary tumors of the brain are still very poor, mainly due to a limited assortment of therapeutic options (5). Even the latest classification of primary CNS tumors by the WHO is still based on the morphological characteristics of the neoplasms. Although the classification was supplemented by genetic data for the various tumor categories, most genomic aberrations remain without clinical significance – with few exceptions. One of these exceptions is the particular genotype of oligodendroglial tumors. Oligodendroglial lineage, acknowledged by rather disputable morphologic characteristics, is genotypically characterized by the combined losses of the short arm of chromosome 1 and the long arm of chromosome 19 (6). The detection of these genomic characteristics has almost become part of making the diagnosis. It also became clear that tumors with either the classic oligodendroglial phenotype or the losses of 1p / 19q are sensitive to chemotherapy. Significant progression-free periods following the administration of drugs like PCV and more recently, temozolomide, have been witnessed (7-9). The delineation of such categories of primary brain tumors which are susceptible to certain drugs becomes of crucial importance, and thus their reliable identification becomes a primary focus of clinical and laboratory neuro-oncology today.

The genomic investigations in primary brain tumors have yielded a large variety of genetic changes

which result in the disruption or the blocking of the normal production or action of proteins. Changes at the DNA level, altered gene regulation or posttranslational modifications may lead to alterations in the expression, or may cause structural changes of proteins with an impact on cellular processes. The DNA sequence by itself does not reveal the possible post-translational modifications of the encoding proteins, which are essential for function and activity (10,11). Quantitation of mRNA does not provide information about the amount of active protein in a cell (12,13). The average number of protein modifications per gene in humans is generally no less than three, up to more than six (14). Among these modified proteins candidates for novel cancer markers may be present. The most common modifications of proteins include glycosylation, phosphorylation, lipidation, sulfation and proteolytic modifications. Protein glycosylation is a key post-translational modification relevant to a range of biological phenomena (15). Protein glycosylation is increasingly being recognized as one of the most prominent biochemical alterations associated malignant transformation and tumorigenesis (16,17,18). Protein phosphorylation cascades in malignant transformation may be linked with successive mutations in specific genes, leading to the activation of oncogenes and the inactivation of tumor suppressor genes (19,20). To date, other types of modifications have remained without correlating genotypical changes so far. Not surprisingly, the focus of researchers is changing gradually from genomic aberrations to the post-genomic events in the normal and abnormal situation. In tumors, changes in the rate of protein synthesis, altered post-translational modifications, inter-compartmental transports, and alterations in the degradation of proteins are encountered. Besides tracing tumor lineage-specific proteins, the identification of alterations in intracellular protein processing may yield relevant information as to therapeutic susceptibilities as well. Several strategies have been implemented to identify tumor-related proteins (21). Concerning primary tumors of the CNS, techniques have been developed for the comparative analysis of unfractionated lysates of tumor and normal tissues, the comparative analysis of secreted proteins and membrane fractions of different tumor cell lines, or the identification of tumor proteins inducing a humoral response (22-28). However, these studies have only to a limited extent accomplished the identification of single proteins as novel markers for diagnostic, prognostic or therapeutic targets so far.

With the aid of the developing tools of proteomics, additional information on cellular location of particular protein isoforms will become available (29). Disease-specific targets are to be identified, the monitoring of which will lead to novel endpoints for the evaluation of therapies like drug activity and toxicity. Investigations at the protein level is well on the way not only to characterize the different types of brain tumors, but perhaps more importantly, defining new therapeutic targets crucial for treatment options. The data obtained by applying proteomics, if linked to information from the major genome projects, will yield crucial information on cellular protein expression levels (13,30,31). Studies in protein-protein interactions within signaling networks will lead to the identification of molecules crucial for the control of normal biological processes and abnormal situations like cancer (10). Therefore, proteomics has the potential to identify novel targets for therapy and novel markers for (early) diagnosis (32).

This review addresses the nascent field of proteomics in primary brain tumors, including some powerful new techniques (most of which are still being developed). By now, there are only few studies on proteomics to primary brain tumors in the literature. Recent studies on the relatively large-scale identification and quantitation of proteins derived from whole- cell lysates of primary brain tumors will be summarized.

3. OVERVIEW OF PROTEOMICS

The term “Proteomics” has been defined as the global analysis of gene expression by a particular cell, organism or tissue type at a given time or under a specific set of environmental conditions using a combination of techniques to identify, quantitate or characterize proteins (33). Until recently, the goal of most studies of proteins was to characterize a single protein through the mass spectral analysis of its peptide digest fragments (34). An important target of the application of proteomics to cancer research is to obtain insight in the differences in protein expression between neoplastic and non-neoplastic conditions at the level of single cells or tissue types. The elucidation of differentially or uniquely expressed proteins or protein isoforms will provide clues as to the normal function of these proteins and will be crucial for the selection of cell or tissue type-specific biomarkers for clinical utilities.

The conventional procedures of protein expression analysis implies the extraction of proteins from cells, their separation and visualization by 1-dimensional or 2-dimensional gel electrophoresis (1D and 2D), the excision and in-gel digestion of the proteins, and the analysis of the resulting peptide mixtures by mass spectrometry–based techniques (35). However, the techniques are inadequate for the high-throughput measurements of a particular cell’s or organism’s proteome to reach this goal (36). Although 2D electrophoresis is a widely used method for global protein analysis by displaying several hundreds of proteins, it only succeeds in the visualization of the more stable and abundant proteins (36). Furthermore, 2D is an inadequate separation technique for hydrophobic proteins (37). In order to enhance the number of proteins to be detected, alternative separation techniques have been developed, such as high performance liquid chromatography (HPLC) (38-40), foam fractionation (41), capillary electrophoresis (CE) (42), capillary electrochromatography (CEC) (43), displacement chromatography (44), chip-based separation (45,46), tandem affinity purification (TAG) (47). Recently, simultaneous measurements of the expression of proteins in cells (including micro-organisms) (36,48,49), cancer cell lines (50) and tumor tissues (51,52) have been carried out by advanced LC-Fourier transform ion cyclotron resonance

(LC-FTICR) and protein microarrays. Coupling of laser capture microscopy (LCM) to surface-enhanced laser desorption ionization (SELDI) mass spectrometry using chip technology is another way of selectively comparing patterns of protein expression. Methods for the screening for tumor-related proteins of body fluids like serum or CSF have also been developed (53,54). The expression profiles obtained by the methods mentioned are complementary to data derived from investigations at the level of genomics and transcriptomics. The combined results of genomics, transcriptomics and proteomics will bring insights which are not, or only in part, feasible by the single use of one of these analytical approaches (55). Statistical programmes encompassing cluster analysis based on bioinformatics, play an important role. The new field of bioinformatics supplies a powerful tool for the analysis of the data obtained by proteomics and facilitates the rapid and efficient identification of proteins and protein networks (56). By now, information obtained by transcriptomics and proteomics is becoming more abundant and for the integrated molecular profiling of tissues or cell populations the term “operomics”, defined as the integration of data derived from genomics, transcriptomics and proteomics for profiling tissues and cell populations, has been launched (32). The comprehensive approach using the tools of genomics and proteomics is expected to lead to novel classifications of disease as well as novel diagnostic and therapeutic tools. The outcome of comprehensive profiling may yield a better understanding of the connections that exist between the three compartments DNA, RNA and proteins, allowing a deciphering of gene expression programs and the networking of proteins and their functions (57). The identification of previously unrecognized patterns of gene expression and better understanding of cellular responses to environmental factors and injuries will result from the application of bioinformatics (32).

Proteomics is a rapidly developing area of research and it is expected that it will lay a powerful foundation for the analysis of the entire set of proteins expressed by normal or transformed cells and tissues. It will be crucial for the identification of disease-specific targets as well as differentially displayed proteins, identifying specific parameters of disease and neoplasia in particular.

4. TECHNIQUES FOR PROTEIN SEPARATION/ PURIFICATION

4.1. Conventional techniques

Various techniques for the separation or purification of proteins have been widely applied over a long period of time. Many of these classical techniques were adequate for various applications in the field of biomedicine and biochemistry (58). Electrophoresis may serve as the most classical technique for protein separation. The technique has continuously been modified and improved. For approximately one hundred years, gelatin and agar gels have been applied in procedures for electrophoretic protein separation (59). Since the 1930s, gel electrophoresis has been widely used for the separation of ionized compounds such as amino acids, lipids, nucleotides, and charged sugars (60). The task of separating complex mixtures of proteins into individual components was revolutionized by the development of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (61). SDS-PAGE was not only applied to separate proteins, but also to estimate molecular weight (62). Since the early 1960's, microscale polyacrylamide gel electrophoresis was used and modified for the fractionation of proteins (63-65). Later, gel electrophoresis was found to be suitable for a variety of separation procedures (66). Some examples of the application of this technique in the field of neuroscience are found in the works of Hyden and Lange, who used gel electrophoresis for the analysis of protein changes in different brain areas as a function of intermittent training (64). The method was also employed by Griffith and La Velle for the analysis of the developmental protein changes in facial nerve nuclear regions (67). Ansorg and Neuhoff studied the effects of different extraction procedures on the patterns of brain proteins obtained (68).

Since direct comparisons of the electrophoretic gels of different samples is not possible, one-dimensional (1D)(61) and two-dimensional 2D slab gel techniques were developed (69-72). One dimensional slab gel electrophoresis is based on dansylated and nondansylated proteins/peptides in the relative molecular mass range (M_r) of 1,000 –100,000. Danysylated proteins/peptides are detected either directly in the gel or following electroblotting onto anion-exchange or polyvinylidene difluoride membranes, with a detection sensitivity of approximately 1 ng for the latter (66). Nondansylated proteins/peptides were either detected within the gels by colloidal Coomassie staining (73,74) or by electroblotting onto polyvinylidene difluoride membrane, followed by colloidal Coomassie staining (66). Successful separation by 2D electrophoresis requires an effective sample preparation procedure as described by Klose (70) or O’Farrell (71). Since the introduction of these preparation techniques, 2D has been become the most widely used proteomic tool for displaying protein expression patterns with high degree of resolution. The 2D electrophoresis technique implies first-dimension isoelectric separation of the proteins according to their charges, followed by second dimension separation according to the molecular weights. The resulting spot patterns are usually oriented with the low, acidic isoelectric points to the left and the low molecular weights at the bottom. However, the “classic” 2D is unsuitable to extremely basic proteins. An alternative (nonequilibrium pH gel electrophoresis) was designed for the very basic proteins (68). To address a series of problems from the “classic” 2D, different gel sizes, various pH ranges (75-77), and various staining methods (78) have been used. So far, up to 10,000 individual proteins have been resolved in a single gel (79).

4.2. Advanced techniques

Although 2D gel electrophoresis remains a powerful and versatile tool for the separation of proteins, there are some disadvantages of the technique. The method is relatively time-consuming, essentially non-quantitative, does not work well for hydrophobic proteins, and has a

limited dynamic range (80). Some alternative techniques for protein separation have recently been introduced (40,42,44-46,81). A significant advancement in the separation of proteins was reached by the introduction and development of high performance liquid chromatography (HPLC) (40). HPLC uses multidimensional liquid chromatography (LC) to separate peptide fragments which subsequently are processed by mass spectrometry. A series of HPLC-based coupling-techniques have been developed for the identification of proteins, such as LC-mass spectrometry (LC-MS), LC-electrospray tandem mass spectrometry (LC-MS/MS), LC-electrospray mass spectrometry (LC-ES/MS), and LC-Fourier transform ion cyclotron resonance (LC-FTICR). The major advantages of these techniques are the wide dynamic ranges, reduction of chemical noise, and last but not least, the avoidance of the time-consuming 2D PAGE procedure (80). A drawback of the LC-based techniques is that the coupling techniques are almost exclusively suited for spray or chemical ionization such as ESI or atmospheric pressure chemical ionization (APCI), and more or less exclude the use of the MALDI technique (82). So far, the technique is best suited for rapidly building an idiotypic proteomic database, rather than being applied in differential display proteomic assays (80).

Capillary electrophoresis (CE) is another modern analytical method with the advantages of a short procedure time and minimum consumption of both reagents and sample material (83). The technique implies a narrow 1D fused silica capillary, high voltage (30 kV), and on-column UV detection for separation (58). Various other methods such as capillary zone electrophoresis (CZE) (84), micellar electrokinetic chromatography (MEKC) (85), capillary gel electrophoresis (CGE) (86), capillary isoelectric focusing (CIEF) (87), capillary electrochromatography (CEC) (88) and capillary electroendoosmotic chromatography (89) are based on the principles of CE. CE is also a complementary tool to other separation techniques, such as high performance liquid chromatography (HPLC), slab gel electrophoresis (SGE), gas chromatography (GC) and thin-layer chromatography (TLC). The advantage of CE over these techniques is its applicability for the separation of widely different compounds, inorganic ions, organic molecules and large biomolecules, which require the highest resolving power of any liquid separation technique (58). However, special instrumentation is required for the application of this technique (58). It is anticipated that CE will become a technique for very high throughput complementary to other current methods already used in academic settings and industry.

Recently, a novel tandem affinity purification (TAP) was developed (47). TAP allows the rapid purification of complexes from a relatively small number of cells without prior knowledge of the complex composition, activity, or function (57). Because of its simplicity, high yield and wide applicability, TAP is a very useful procedure for the protein purification and exploration (90). More recently, affinity capture-release electrospray ionization mass spectrometry (ACESIMS) was developed (The details of this technique are described under section 5.6.) (91). Yet another novel strategy for protein separation is the free-flow protein separation technique based on isoelectric electrophoresis (92). Other methods recently developed for protein purification are affinity ultrafiltration (81), displacement chromatography (44) and foam fractionation (41).

5. TECHNIQUES FOR PROTEIN IDENTIFICATION

As to the identification of proteins, the traditional antibody-based approaches such as immunoblotting or enzyme-linked immunosorbent assays are still being used. Over the last decade, however, additional methods for the identification of proteins have advanced dramatically. The introduction of instrumentation sensitive enough to be applicable to biology systems and the development of mass spectrometric techniques made major contributions to the field (93). A series of mass spectrometric-based techniques have become most important for biosciences. Currently, differential display proteomics are being actively pursued based on multiplexed proteomics (MP) and difference gel electrophoresis (DIGE) (94,95). In addition, affinity capture release electrospray ionization mass spectrometry (ACESIMS) (91) and isotope-coded affinity tags (ICAT) (29) are recently developed techniques for the quantification of protein activity and content, and are applied to clinical enzymology and functional proteomics.

5.1. Chemical sequencing techniques

N-terminal sequence analysis by Edman chemistry continues to play an important role in the structural analysis of proteins and peptides. Improvements in the sensitivity of the method have been achieved mainly at the level of increasing the sensitivity of the on-line analysis of phenylthiohydantoins (PTH) amino acids by reverse phase high performance chromatography (RP-HPLC) at the 0.2-5.0 pmol range (96). C-terminal sequence analysis is an alternative technique to get amino acid sequence and configuration information. The most extensively studied and well-known C-terminus sequencing is the “thiocynate” method (97). An automated C-terminus sequence analysis has been introduced (98).

Two representative mass-linked sequencing techniques are ladder sequencing of polypeptides (99) and mass-coded abundance tagging (MCAT) (100). Ladder sequencing involves progressive N- or C-terminal amino acid truncation via chemical or enzymatic treatments. The particular advantage of ladder sequencing in relation to other techniques for sequence analysis is the high data acquisition rate and a very good sample throughput. Multiple determinations are carried out within minutes at high sensitivity and low sample consumption. The technique of MCAT was described for systematic proteome analysis based on differential guanidination of C-terminal lysine residues on tryptic peptides chromatography-electrospray tandem mass spectrometry. This approach is characterized by the automated, large-scale, and comprehensive de novo determination of peptide sequence and relative quantitation of proteins in biological samples in a single analysis. MCAT is simple, economic, and effective when applied to complex proteomic mixtures (100).

Sequencing-based techniques also allow to identify modified amino acid residues, characterized by phosphorylation, glycosylation, lipidation, sulfation or proteolytic modification (15).

5.2. Matrix-assisted laser desorption / ionization mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS)

The two mass spectrometric identification techniques which are most widely used by now are matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) (35,101), electrospray-ionization mass spectrometry (ESI-MS) (102) and combinations of both methods (103). Mass spectrometry essentially involves the highly accurate determination of molecular mass of proteins and peptides, which can be matched with masses of known amino acid sequences in databases, thereby identifying the proteins (104). Presently, mass spectrometry-based techniques are the methods of choice for protein identification and for the characterization of post-translational modifications (105). MALDI-MS is mainly used for peptide mass fingerprinting (PMF) (35). The masses of peptides derived from in-gel proteolytic digestions are measured and subsequently matched with a computer-generated list acquired from digestion of proteins using the same enzyme (usually trypsin) (105). The technique is suitable to characterize proteins from a series of completely sequenced genomes such as yeast (102). ESI-MS can be used to generate additional partial sequence information to complement the mass information (105). Recent developments are direct liquid chromatography/tandem mass spectrometry (LC-MS/MS) techniques, which allow fast and reliable mapping of very low amounts of peptides in an almost completely automated fashion (103). Mass spectrometry has also been used for the determination of the N-terminus part of protein molecules. The determination of the configuration of amino acids in peptides can be determined by the combined use of high-performance liquid chromatography (HPLC) and mass spectrometry (106).

5.3. Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS)

FTICR-MS is an unsurpassed techniques in terms of resolution (often exceeding 1,000,000) and mass accuracy (~1 ppm). The technique is based on electrospray ionization (ESI) and recently also upon MALDI with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) (107,108). FTICR-MS techniques enable the detection sensitivity at the level of zeptomoles (109). Such a high sensitivity enables specific investigations of mixtures of proteins, peptides, oligonucleotides and carbohydrate structures (36). In addition, the accuracy, resolution and sensitivity of the binding of proteins to RNA/DNA and protein-protein or protein-drug interactions are foremost improved by the use of FTICR-MS as compared to the other MS techniques. The identification and quantification of hundreds of different proteins in one sample has become feasible. More than 60% of the proteins expressed in the microorganism Deinococcus radiodurans could be detected by using LC/FTICR-MS (110). Identification of protein expression patterns of tumor tissues and body fluids obtained from cancer patients (including brain tumor patients) by FTICR-MS opens new windows for molecular diagnosis, prognostication, prediction of treatment response, individualization of treatment and the identification of new targets for specific drug treatment.

5.4. Surface enhanced laser desorption (SELDI)

Protein chips systems have been developed and applied since the mid 80s (111). Surface-enhanced laser desorption / ionization (SELDI) utilizes affinity surfaces to retain proteins based on their physical or chemical characteristics, followed by direct analysis by TOF-MS (45,112,113). The underlying principle in SELDI is surface-enhanced affinity capture through the use of specific probe surfaces or chips (114). This protein biochip is the counterpart of the array technology in the genomic field and also forms the platform for Ciphergen's ProteinChip ® array SELDI MS system (113). The technique allows the rapid profiling of the masses of the proteins in very small samples of 1-2 µl per analysis, ideal for small biopsies or microdissected tissues (115). Advantages of this approach include the simple sample preparation before the application of MS and the ability to capture trace amounts of proteins directly from a biological fluid (116). The information about hydropathicity, total charge, isoelectric point, phosphorylation, glycosylation and primary structure can also be tracked (113). The technique has been applied to cancer proteomics (oncoproteomics)(114), including prostate, (117), ovary (53,118), breast (51,119), head and neck (120), kidney and uterus (112).

5.5. Imaging mass spectrometry (IMS)

Imaging Mass Spectrometry (IMS) is a new technique for direct mapping and imaging of biomolecules present in tissue sections by MALDI-MS (121). This technique has been applied to both animal and human tissues (121-123). While MALDI-MS was targeted for the analysis of peptides and proteins present on or near the surface of tissue sections, IMS is focussed on the spatial distribution of proteins in tissues. Tissues can be represented as frozen sections, microdissected individual cells, or imprints blotted on a membrane. For instance, in order to compare the expression of specific proteins by glioblastoma cells with surrounding normal glial cells, a target area is selected prior to analysis by MALDI-MS (124). Comparison of the MALDI mass spectra taken from different locations within the tumor and at the tumor - surrounding tissue interface yields peaks with distinct spatial distribution. In this way areas of vital, ischemic and necrotic tumor tissue can be compared at the protein expression level. More importantly, comparison of the expression level of proteins specifically expressed by tumor cells with that of pre-existing normal cells becomes feasible. Additionally, applications aiming at the intra-operative assessment of tumor margins may become possible by this approach (124).

Table 1. Comparison of current tools for proteomics

Tools	Main functions	Main advantages	Main limitations
2D	Analysis and separation	▪ High resolution	Limited dynamic ranges, time-consuming
HPLC	Separation	▪ Wide dynamic ranges, possibilities for automation	Coupling with MALDI not yet described
CE-based variants	Separation	▪ Short procedure time ▪ Minimum consumption of both reagents and sample material ▪ Applicability for the separation of widely different compounds	Requires special instrumentation
MALDI	Identification	▪ Fast, sensitive, reasonable tolerance to buffer salts and detergents.	
LC-based MS	Separation and Identification	▪ Generation of additional sequence information ▪ Operation in a full automated fashion possible	
FTICR-MS	Identification, structure elucidation	▪ Unsurpassed resolution and mass accuracy ▪ Capable of resolving isobaric components ▪ An additional aid to structure elucidation	operator necessary
SELDI-MS	Structure elucidation, profiling of heterogeneous protein mixtures	▪ Small samples per analysis, simple, fast ▪ Generate information about structure and protein-protein interactions	Not yet widely used Limited ability for identification
ACESIMS	Quantification	▪ Analysis of enzymatic reactions with MS	Difficult monitoring isomerizations
ICAT	Quantification	▪ Relative quantification of protein expression	Limited resolution of proteins
IMS	Comparative analysis	▪ Analysis of in situ tissue sections	Limited reproducibility
MCAT	De novo sequencing, quantification	▪ Automated, large-scale, simple, economic, effective	Latest technique, disadvantages not yet described
Bioinformatics	Computation for processing and structuring data	▪ automation speed, standardization available	Developing field of science
Data mining	Extraction of information from the existing data	▪ Automatically up-to-date or filter relevant information	Requirement of relevant databases

5.6. Affinity capture-release electrospray ionization mass spectrometry (ACESIMS) and isotope-coded affinity tags (ICAT)

ACESIMS and ICAT are the latest techniques developed for the quantification of proteins. Quantification of proteins is part of the field of functional proteomics, which investigates changes in cellular protein expression and post-translational modifications in response to an external impetus such as response to a drug, environmental stress or cell signalling (125). The principal features of these methods are their usage of biotinylated tags that function as molecular handles for highly selective and reversible affinity capture of conjugates from complex biological mixtures such as cell homogenates and subcellular organelles (126). Both methods use conjugates labeled with stable heavy isotopes as internal standards for quantitation. ACESIMS uses synthetic substrate conjugates specifically for targeting cellular enzymes, while ICAT relies on the selective conjugation of cysteine thiol groups in proteins, followed by enzymatic digestion and quantitative analysis of peptide conjugates by mass spectrometry (29).

5.7. Biomolecular interaction analysis mass spectrometry (BIA/MS)

Biomolecular interaction analysis mass spectrometry (BIA/MS) is a two-dimensional analytical technique that quantitatively and qualitatively detects analytes of interest. In the first dimension, surface plasmon resonance (SPR) is utilized for detection of biomolecules in their native environment (127). SPR detection is non-destructive, analyte(s) retained on the SPR-active sensor surface can be analyzed in a second dimension using MALDI-MS (see further under Section 6).

5.8. Bioinformatics in proteomics

The field of bioinformatics is rapidly growing. Bioinformatics is the science of computation for processing and structuring of data obtained in the fields of genomics, transcriptomics, proteomics, evolution and neighboring research areas (128). The use of bioinformatics is essential to trace networks or cascades of interacting proteins (129). Currently, the application of bioinformatics in proteome analysis is based on the use of data mining. Data mining is the process of extracting useful information from massive observational data sets. Regarding the field of proteomics, the automated extraction of information from existing databases may result in the elucidation of interactions between various proteins (130,131). The development of specific software programs for gel image matching, spot identification, standardization of 2D databases, the analysis of differential expression patterns and cluster analysis are prerequisite for the successful application of the most recent techniques described in this review. While the application of techniques like nanoLC-FTICR-MS enable to overcome problems of irreproducibility of 2D analysis in heterogeneous samples, still bioinformatics are irreplaceable to handle the gigabyte files generated by such advanced techniques to generate useful and reliable databases. A proteomics working flowchart and the main proteomics-base techniques are illustrated and summarized in Figure 1 and Table 1, respectively.

6. PERSPECTIVES OF THE APPLICATION OF PROTEOMICS IN THE FIELD OF NEURO-ONCOLOGY

The central tool for displaying protein expression of brain tumors is still two-dimensional electrophoresis

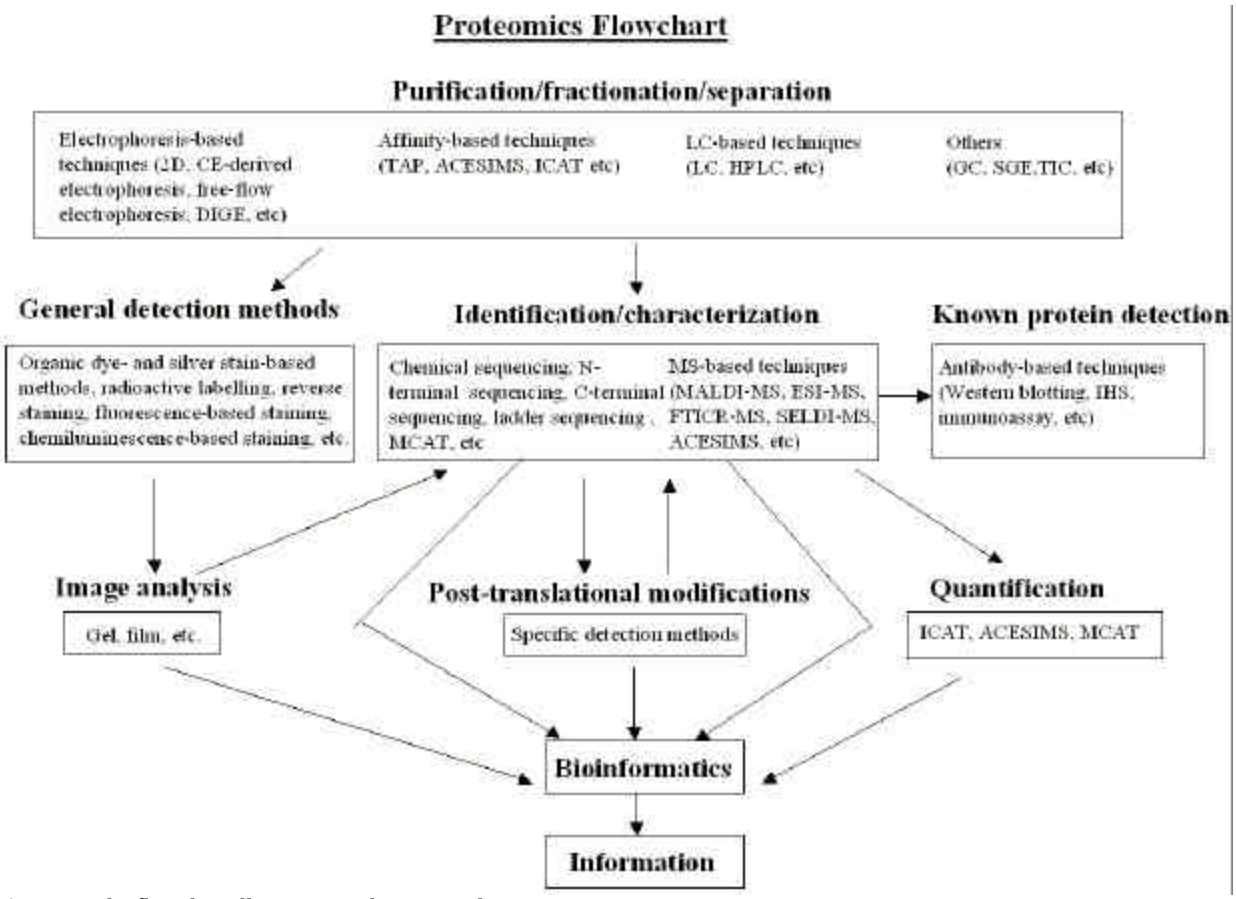

Figure 1. The flowchart illustrates working procedures in proteomics.

(2D) (132). A 2D map of normal parietal cortex has been established (132). In this map approximately 400 spots were identified corresponding to 180 different brain protein components, which may be useful as a reference database to study changes in protein level caused by varions diseases. Based on the application of 2D techniques, several protein expression maps of healthy and diseased human brains have been established (132-137). However, these studies were basically restricted to 2D based-protein pattern comparisons between various primary brain tumors and normal human cortex (138), human glioma cell lines (26), neuroblastoma cell lines (137) and glioma cell lines of different species (139). Meanwhile, IMS-based protein maps of normal brain tissue and a human glioblastoma xenograft have updated the existing data (121). More recently, a relatively large cohort of quantitative 2D PAGE studies was undertaken to search for diagnostic and prognostic relevant proteins in glioma subgroups and medulloblastomas (21). The results of these studies clearly indicate that each tumor type has its characteristic protein profile (spot format or mass spectrometric image). The findings represent powerful sources for further identifying tumor-specific-proteins. Also by using the proteomics-based techniques, tumor-related proteins such as Tß4, oncoprotein 18 (Op18) and nucleoside diphosphate kinase A (NDPKA), have been identified in glial tumors (21,121). Unfortunately, these studies were based on pattern comparisons and semi-quantitative analyses. The identification of specific proteins from the patterns has not been undertaken yet. In addition, the studies quoted did not specifically purify the target cells before applying proteomics, that is, the tissues investigated still contained vessels, stromal cells, etc. Laser capture microdissection (LCM) or manual microdissection appears to be the best choice to get a purified target tissue or cell type. Moreover, future studies should also take into account the fact that lineage-specific proteins may also be determined by various abnormal post-translational modifications like glycosylation or phosphorylation (131).

Proteomics, i.e. the high-throughput separation, display and identification of proteins, may become a powerful tool in drug development (140). Currently, this application is in its early stage but the near future will bring a merger of biology, engineering and informatics with a far broader impact on society than pure genomics has had so far (141). The identification of the 'right' target(s) is now a critical part of the process of drug discovery (142). However, the challenge of characterizing the structures and functions of all proteins in a given cell demands technological advances beyond the classical methodologies of protein biochemistry (141). The recent development of surface plasmon resonance (SPR)-based biosensor technologies for biospecific interaction analysis (BIA)

enables the monitoring of a variety of molecular reactions in real- time (143,144). SPR-based BIA offers many advantages over most of the other methodologies available for the study of biomolecular interactions, including full automation, availability of a large variety of activated sensor chips that allow immobilization of DNA, RNA, proteins, peptides and cells, and the innecessity of labeling procedures (143-145). Aside from the analysis of all possible combinations of peptide, protein, DNA and RNA interactions, this technology can also be used for screening of monoclonal antibodies and epitope mapping, analysis of interactions between low molecular weight compounds and proteins or nucleic acids, interactions between cells and ligands, and real-time monitoring of gene expression (143). Importantly, the use of MALDI in concert with SPR-BIA is currently feasible (146). The coupled techniques may be applied to biological fluids (127).

An alternative source for proteomic analysis of brain tumors is the cerebrospinal fluid (CSF). Normally, various metabolites of intracerebral cell populations may enter the CSF. Proteins detected in CSF may mirror the metabolic state of the cerebral tissues under various circumstances, including disease (147). The concentration of background proteins in CSF is relatively low and therefore, tumor-related proteins may be detected more readily. A normal human CSF 2D protein database has been established (148,149). To date, a few 2D protein maps of non-neoplastic brain diseases have been published (150,151).

The application of proteomics in Neuro-Oncology is still in its infancy. It is expected that the use of proteomics will rapidly gain attention in this field and certainly will lead to a significant enrichment of the latest classifications of primary brain tumors and their grading. More importantly, proteomics will enable to trace specific tumor markers and provide crucial data for drug development and other treatment modalities for brain tumors.

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Abbreviations: 2D, two dimensional electrophoresis; CE, capillary electrophoresis; DIGE, difference gel electrophoresis; TAP, tandem affinity purification; ACESIMS, affinity capture-release electrospray ionization mass spectrometry; LC; liquid chromatography; HPLC; high performance liquid chromatography; GC; gas chromatography; SGE, slab gel electrophoresis; TLC, thin-layer chromatography; MCAT, mass coded abundance tagging; MALDI-MS, matrix-assisted laser desorption/ionization; mass spectrometry; ESI-MS, electrosprayionization mass spectrometry; FTICR-MS, Fourier-transform ion cyclotron resonance, mass spectrometry; SELDI-MS, surface enhanced laser desorption; ICAT, isotope-cold affinity tags; HIS; immunohistochemical staining

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