

2-DE Proteomic Profiling of Neuronal Stem Cells

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Key Words

Proteome profiling · 2-DE map · Mass spectrometry · Neuronal stem cells · Neuronal differentiation · Peroxiredoxin · Transgelin · Glial fibrillary acidic protein

Abstract

Proteomics has become a powerful tool in neuroscience studies. Although numerous human neural stem cells are available for research purposes since many years, there exists only limited information on proteomic data from stable neural stem cell lines. Profiling and functional proteome studies of neuronal stem cells will help to describe the protein inventory as well as protein activity and interactions, subcellular localization and post-translational modifications. The proteomic analysis of neuronal differentiation processes will elucidate the complex events leading to the generation of different phenotypes via distinctive developmental programs that control self-renewal, differentiation, and plasticity. Using the ReNcell VM197 model, a cell line derived from human fetal ventral mesencephalon stem cells, we studied the protein inventory of the stem cells by 2-DE gel electrophoresis and mass spectrometric protein identification and constructed a 2-DE protein map consisting of

more than 400 identified protein spots. This proteome reference database constitutes the basis for further investigations of differential protein expression during differentiation. A profiling of the neuronal differentiation-associated changes displayed the large rearrangement of the proteome during this process, and the proteomic techniques proved to be a valuable tool for the elucidation of neuronal differentiation process and for target protein screening.

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Introduction

Replacement of lost neurons by transplantation of neural stem cells (NSC) constitutes a promising new approach in the treatment of progressive neurodegenerative diseases. Stem cells are unspecialized cells and capable of dividing and renewing themselves for long periods of time and to develop into many cell types. Using directed variations in *in vitro* culture conditions and/or genetic modifications, immature stem cells can differentiate into different neuronal progeny. Despite the fact that immortalized NSC lines have been established [1–3] and that some data exist from different investigations regarding phenotype, differentiation capacity and protein changes

during development, it is still difficult to draw consistent conclusions from studies because different cell types, preparation protocols as well as cultivation conditions have been applied. The differentiation as well as maturation of stem cells involve significantly complex events leading to the generation of different phenotypes via distinctive developmental programs. In contrast to transgenic technologies and microarrays [4–8], with proteomic approaches important issues such as protein amount, protein stability, subcellular localization of proteins, post-translational modifications and protein-protein interactions can be elucidated. Nowadays, the combination of high-resolution two-dimensional gel electrophoresis and identification of proteins by mass spectrometry (MS) is broadly applied to proteomic profiling. Nevertheless, up to now human NSCs have not been subjected to profound proteome analysis, although they have a great therapeutic capacity [9–12]. Applying proteome approaches, the programs that control self-renewal, differentiation, and plasticity will be accessible at a comprehensive scale and new specific markers for neuronal development and factors driving the differentiation of stem cells in defined directions can be identified, as shown by recent studies. Maurer et al. [13] presented a proteomic database for NSCs isolated from the hippocampus of adult rats and cultured for 10 weeks. In a 2-DE proteome profiling, they identified about 260 proteins of which 128 were regulated during neuronal differentiation [14]. More recently Wang and Gao [15] published a proteomic study of mouse embryonic stem cells, using 2-DE followed by liquid chromatography MS. They generated a proteome reference map of stem cells and dopaminergic neurons and identified 23 proteins with altered expression or phosphorylation during neural differentiation. Kadota et al. [16] used mouse embryonic stem cells with a single human chromosome 21 in order to study early neuronal differentiation in Down syndrome (DS). Another study, performed by Schrattenholz et al. [17], enabled the enrichment of phosphoproteins of neuronal derivatives of murine embryonic stem cells exposed to chemical ischemia in a differential and quantitative proteome analysis. In our 2-DE proteomic approach with multipotential, nestin-positive human fetal midbrain stem cell line (ReNcell VM197), we were able to construct a 2-DE map of proteins from early differentiating cultures and to detect differential expression of specific proteins during the neuronal differentiation process [18].

2-D Gel Electrophoresis and MS for Proteomic Investigations

The term ‘proteome’ describes the protein inventory of an organism, organ or tissue, and proteomic investigations also include structural and functional characterization of proteins at a large scale. For the elucidation of changes at the proteome level and the identification of new target proteins for therapeutic agents, screening techniques are necessary that cover large sets of proteins and that are highly reproducible. High-resolution 2-DE protein separation with its unsurpassed separation power combined with high sensitivity MS has evolved into a leading-edge proteomic technique [19] (fig. 1). In general, a protein preparation from a crude homogenate is followed by 2-DE protein separation in order to display a complex set of expressed proteins under defined physiological conditions [20]. The first dimension is an isoelectric focusing of the proteins under denaturing conditions on strips with immobilized pH gradient. The second dimension separation is completed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), i.e. the separation of the proteins according to their apparent masses. After the gel run, a fixation of the proteins is done that depends on the staining or labeling method used for the detection of the protein spots.

Usually, protein amounts in the range of 50 µg to 1 mg are used for the 2-DE gel separation. By applying staining with silver-nitrate or a fluorescence labeling (Sypro Ruby, Invitrogen, Karlsruhe, Germany) a protein detection limit of some nanograms can be achieved. However, preparative gels for protein identifications usually require higher protein amounts. Staining with Coomassie Brilliant Blue G-250 (CBB) (Serva, Heidelberg, Germany) is highly compatible with MS and therefore it is the preferred (nonfluorescence) visualization method for spot isolation with subsequent mass spectrometric protein analysis. For documentation of gel images, scanners or CCD cameras are used and the pictures are loaded to gel analysis programs for spot detection, evaluation of protein expression and spot labeling.

Alternatively, to the comparison of gel-pairs of parallel 2-DE analyses of different protein samples the differential in-gel electrophoresis (DIGE) method can be applied [21]. Using this method, two samples are labeled with different fluorescence markers before 2-DE separation. A mixture of the samples is applied to the same 2-DE gel, and after separation of the proteins by 2-DE the different excitation and emission wavelengths of the fluorophores are used to record sample-specific images. Thus, the tech-

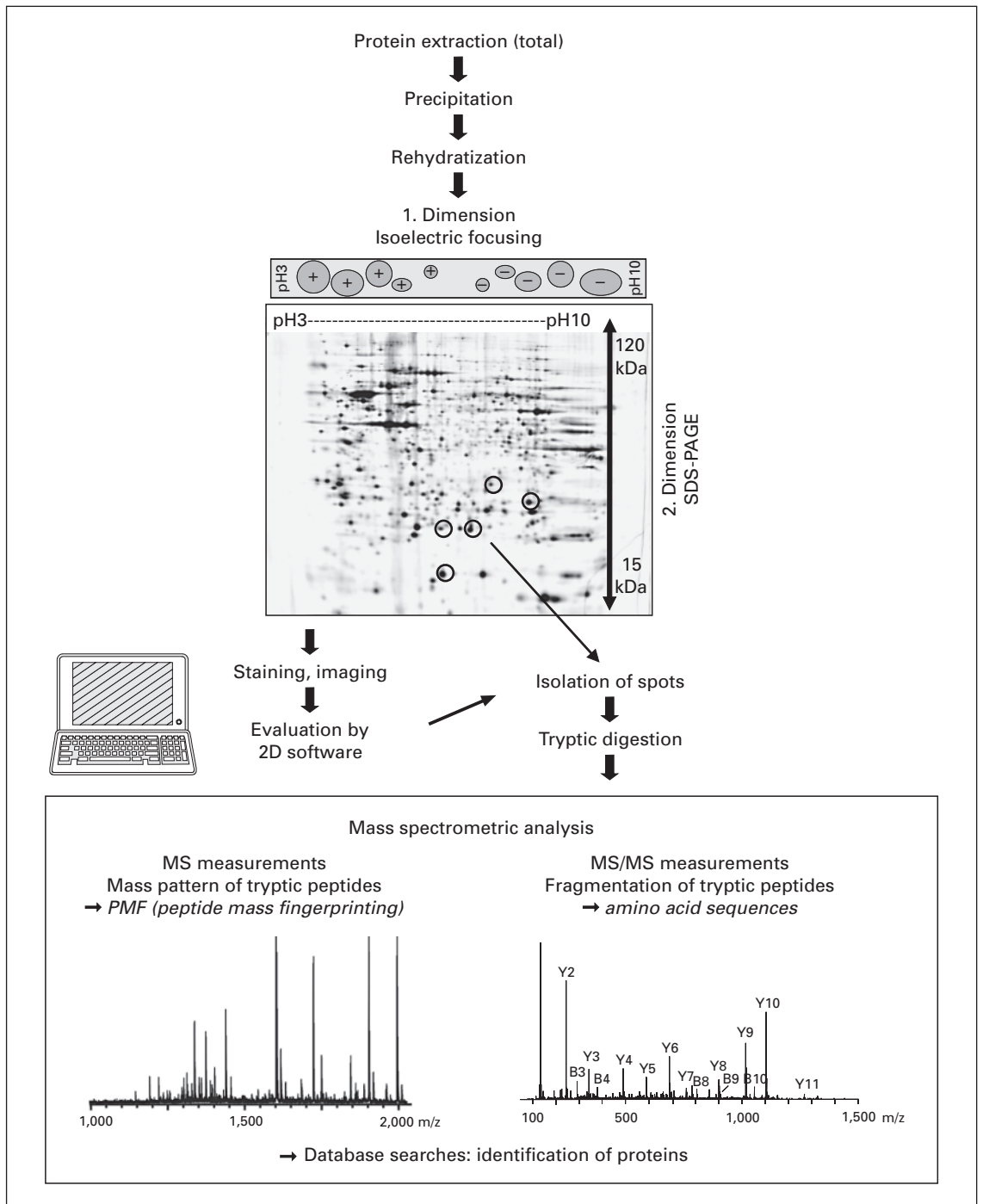


Fig. 1. Workflow of standard proteomic profiling by two-dimensional gel electrophoresis and mass spectrometric protein identification.

nical difficulties that are associated with crossgel comparisons of independently processed gels are dramatically reduced. The technology can be further improved by including a mix of both samples that was labeled with a

third dye as a pooled standard, thus generating an internal standard for each individual protein and dramatically improving the reliability of the analysis.

Furthermore, prefractionation of protein samples from cytosol, nuclei, membranes or organelles is a useful selection step for the analysis of more distinct sets of proteins. Other approaches enable the enrichment of modified proteins for 2-DE gel analysis, such as phosphoproteins or glycoproteins [17, 22, 23]. For the identification of interacting proteins, as they are found in receptor complexes, signal cascades or transcription complexes, coimmunoprecipitation of the complexes before proteome analysis is appropriate.

For identification by MS, protein spots are isolated from 2-DE gels and digested by trypsin (or other proteases), generating a sequence-dependent mix of peptides of defined masses. During the MS analysis, the masses of the tryptic fragments are determined with high accuracy. This results in a so-called peptide mass fingerprinting, i.e. a mass pattern of the set of measured tryptic peptides of the digested protein. For the protein identification, the experimentally determined mass spectrum is matched to theoretical protein digests with the aid of dedicated search engines such as Mascot (Matrix Science) [24] and protein database information (SwissProt, TrEMBL, GenBank).

Most protein spots of 2-DE gels can be successfully identified by peptide mass fingerprinting using matrix-assisted laser desorption/ionisation MS instruments. Yet another level of confidence can be obtained by applying MS/MS techniques for identification [19] (fig. 1). In these approaches, the peptide mass fingerprint is substituted by peptide fragmentation patterns that are generated by fragmentation of selected peptides in the mass spectrometer predominantly at their peptide bonds and subsequent recording of high-accuracy fragment spectra. The acquired spectra provide information about the amino acid sequence of a peptide. MS/MS techniques and the development of more precise and sensitive instruments improved not only the protein identification but also the localization of posttranslational modifications such as phosphorylation, acetylation and glycosylation [25].

Recently, gel-free approaches that combine cation exchange separation (SCX, Strong Cation Exchange) with reverse-phase liquid chromatography followed by 'on-line' MS (electrospray ionization MS) detection and identification have made tremendous advances and came more into fashion. These methods also allow to identify hydrophobic proteins and proteins with extreme pH values, which thus far escaped conventional 2-DE-based proteomics techniques. The techniques are certainly complementing and partially substituting gel-based proteomic approaches. In combination with stable isotope labeling such as the semiquantitative ICAT method (iso-

tope-coded affinity tag), such approaches permit simultaneous identification and quantification of proteins [26]. Also chip-based methods with antibodies or peptides as more focused techniques should be mentioned in the context of proteomics methods. Though the specificity and sensitivity of the approaches still have to be improved, the protein chip techniques are a highly promising completion of MS methods for elucidation of the expression and function of defined sets of proteins [27–29].

Proteomics in Neuroscience ('Neuromics')

Proteomic Investigation of the CNS and Cerebrospinal Fluid

Proteomics has become a powerful tool for the elucidation of neurological structures and processes as well as disease-related molecular abnormalities.

The recent development of extremely sensitive MS methodologies provides the requisite tools for 'neuromic' analyses [30], especially since many neurological diseases are very complex and multigenic in origin and many crucial proteins are of low abundance. The first proteomic investigations in neurology have not been performed on nervous tissue but rather on cerebrospinal fluid (CSF) [31] and blood. An overview of disease-specific proteins characterized by proteomics approaches of human CNS and/or CSF from patients with neuropsychiatric disorders is given by Rohlf [32]. Recent progress in proteomics approaches and future promises for the investigation of schizophrenia, Alzheimer's disease and Parkinson's disease are summarized by Kim et al. [30].

The high complexity of neural protein samples can be reduced for MS analysis by fractionation methods, such as subcellular or organelle fractionation or affinity techniques [20, 33, 34]. The analysis of multiprotein complexes, enriched by ligand affinity or coimmunoprecipitation, has proven to be a useful tool for the characterization of protein functions, biochemical pathways and networks, as shown for the N-methyl-D-aspartate receptor complexes [35]. Similarly, this method allows to deplete proteins such as albumin, haptoglobin, IgG and transferrin from CSF or blood samples that mask proteins of interest [32]. Altogether, the 'profiling proteomics' and 'functional proteomics' will give a more complete description of neuronal protein inventory as well as conclusions about protein activity, interactions and the presence of posttranslational modifications, as recently reviewed by Choudhary and Grant [33].

Proteomic Investigations of Neuronal (Stem) Cell Lines

Neuronal cell lines derived from mature tissues have been frequently subjected to proteomic investigations with diverse techniques during the past years. Most approaches are based on the 2-DE technique combined with MS identification of specific, differentially expressed proteins or whole sets of protein spots [36–40]. Other techniques like coimmunoprecipitation for the identification of interacting proteins, ICAT or LC-MS/MS have also been applied in the investigation of neuronal cell lines [41, 42]. A general comparison of the proteome of six cell lines, one of these a neuroblastoma line, was performed by Schirle et al. [43]. After one-dimensional SDS-PAGE and digestion, the identification of the entire set of proteins was performed by LC-MS/MS and between 260 and 1,100 proteins were identified in the different lines. When comparing the data from the six different cell lines, it was surprising that only 104 of the total number of 1,543 nonredundant cytoplasmic protein clusters were found to be shared between all cell lines analyzed. Cell line-specific cytoplasmic clusters (i.e. proteins that were exclusively found in a single cell line) account for 36% of all identified proteins in HEK293, for example. The authors reported that there are numerous examples of highly abundant proteins that are found in a cell line-specific manner. Examples include the dopamine monooxygenase precursor (IPI00012890.1) and the neuron-specific calcium-binding protein hippocalcin (IPI00145135.1) [43].

However, up to now human fetal NSCs have not been subjected to profound proteome analysis, although they have a great therapeutic capacity, as shown, e.g., for Parkinson's disease [9, 11], epileptic disorder [10] and injured neurons [12]. Using a number of variations in culture conditions and/or genetic modifications, cultured neuronal stem cells can differentiate into different neuronal progeny. The complex events leading to the generation of different phenotypes via distinct developmental programs can be studied systematically with transgenic technology and microarray techniques [4–6, 8]. However, ultimately the regulatory functions are carried out by the cellular protein constituents, and proteomic approaches are most appropriate to determine protein content, stability and subcellular localization, posttranslational modifications and protein-protein interactions.

First proteomics studies on neuronal differentiation of neuronal stem cells using 2-DE gel methodology have been reported in 1999, when Pearce and Svendsen published a short communication about their differential 2-

DE gel approaches of human brain cells treated with epidermal growth factor (EGF) and fibroblast growth factor (FGF) [44]. Guo et al. [45] identified 12 out of 24 differentially expressed proteins from human embryonic stem cells after retinoic acid induction of neuronal differentiation using 2-DE analysis. Importantly, nine of the identified proteins were previously known to be involved in the process of neuronal differentiation or neuronal survival.

For the identification of aberrant and specific genes involved in the early differentiation of DS neurons, Kadota et al. [16] used an *in vitro* neuronal differentiation system of mouse embryonic stem cells with a single human chromosome 21. The 2-DE proteomics results provided a set of specific gene products altered in early differentiating DS neuronal cells.

Maurer et al. [13] presented a proteomic database for NSCs isolated from the hippocampus of adult rats, cultured for 10 weeks. In a 2-DE proteome profiling approach, the authors were able to map about 1,100 protein spots of which 266 were identified. In a consecutive publication, they reported results of the 2-DE expression profiling of differentiation of the adult hippocampus stem cells, where 128 out of 367 regulated proteins were identified [14]. In both studies, identified proteins were assigned to functional categories. A large proportion of the proteins were allocated to the functional groups of metabolism, folding-associated proteins, cytoskeleton and transcription. In our 2-DE gel proteomics investigation of a human NSC line we identified by MS more than 400 spots out of 950 that displayed about 320 different proteins [18]. We assigned the proteins to more specific functional categories, although the functional distribution was generally similar to that of Maurer's group (for details see the following section of this article). Recently, a further generation of a proteome reference map of stem cells has been published by Wang and Gao [15], based upon MS identifications of 2-DE-separated proteins of mouse E14 cells and derived dopaminergic neurons. Twenty-three proteins with altered expression or phosphorylation after neuronal differentiation were identified. The downregulation of the translationally controlled tumor protein and the upregulation of alpha-tubulin were confirmed by Western blotting.

Also recently, Schratzenholz et al. [17] used the combination of isotopic labeling and fractionation of phosphoproteins together with 2-DE and MS for the identification of proteins associated with effects of chemical ischemia of neuronal derivatives of murine embryonic stem cells.

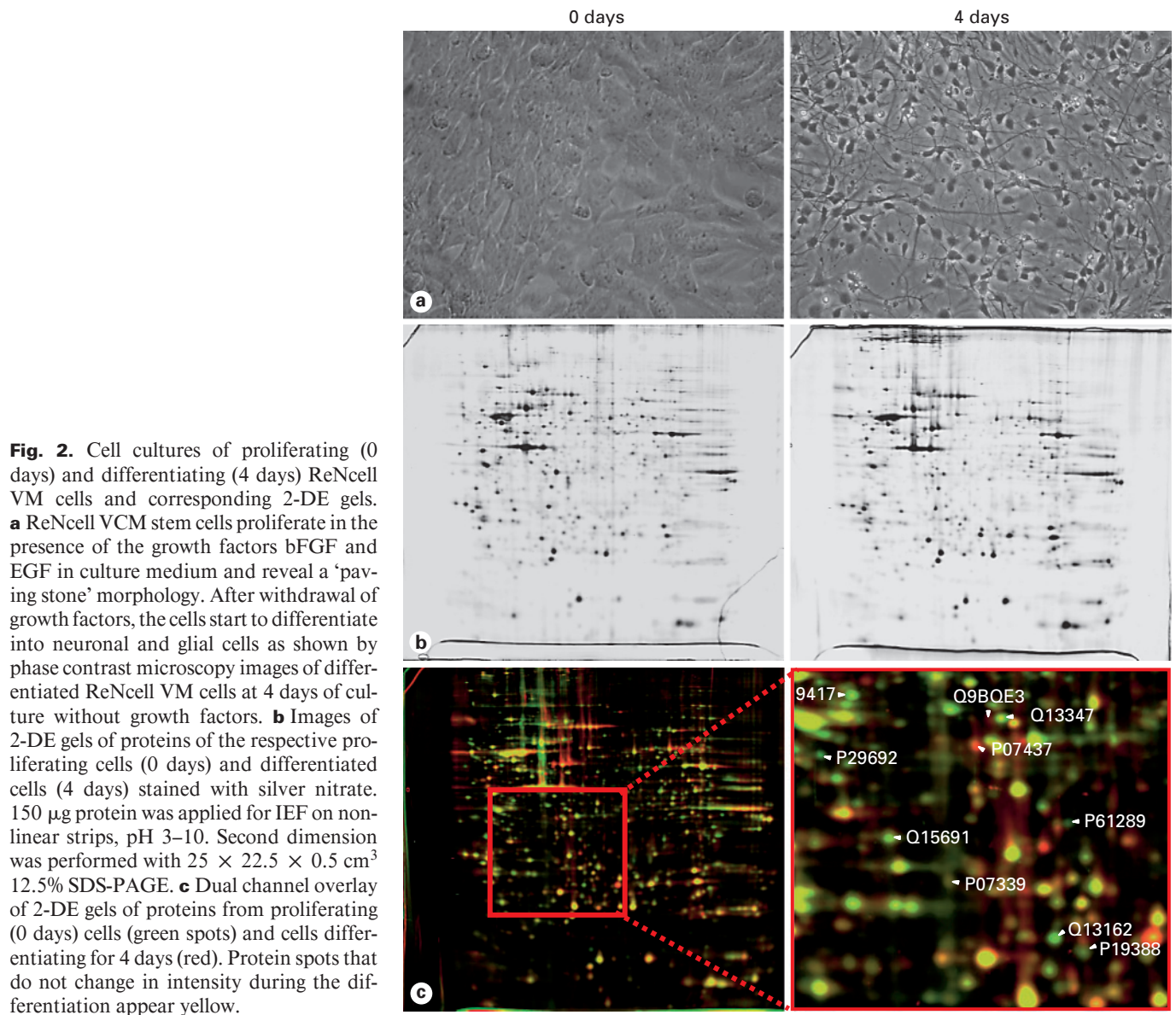


Fig. 2. Cell cultures of proliferating (0 days) and differentiating (4 days) ReNcell VM cells and corresponding 2-DE gels. **a** ReNcell VCM stem cells proliferate in the presence of the growth factors bFGF and EGF in culture medium and reveal a ‘paving stone’ morphology. After withdrawal of growth factors, the cells start to differentiate into neuronal and glial cells as shown by phase contrast microscopy images of differentiated ReNcell VM cells at 4 days of culture without growth factors. **b** Images of 2-DE gels of proteins of the respective proliferating cells (0 days) and differentiated cells (4 days) stained with silver nitrate. 150 µg protein was applied for IEF on non-linear strips, pH 3–10. Second dimension was performed with 25 × 22.5 × 0.5 cm³ 12.5% SDS-PAGE. **c** Dual channel overlay of 2-DE gels of proteins from proliferating (0 days) cells (green spots) and cells differentiating for 4 days (red). Protein spots that do not change in intensity during the differentiation appear yellow.

The different 2-DE protein maps provide a very useful basis for further detailed differential gel-based analysis of the specific stem cell lines during their neural differentiation applying gel-based proteomics approaches. Altogether, only a few initial proteomic investigations of neuronal progenitor cells are available so far. But it becomes obvious that the proteomic approaches are promising steps towards a comprehensive understanding of the developmental processes in stem cells.

2-DE Proteomic Investigation of the Human Neuronal Stem Cell Line ReNcell VM197

Method

In a proteomic study, we investigated the protein inventory of a proliferating human stem cell line by 2-D gel electrophoresis and identified changes in the protein profile during neuronal differentiation after 4 and 7 days [18]. The analysis was performed with a stable human fetal midbrain stem cell line, ReNcell VM197 (ReNeuron Ltd., Guildford, UK) as a representative model for cultured human NSCs. The cell line was derived from the

ventral mesencephalon of 10-week-old fetal neural tissue and was immortalized with the v-myc oncogene. The ReNcell VM197 cell line was selected after in vitro assays for stable karyotype, proliferation and neural differentiation as well as robustness (freeze-thaw viability) and long-term survival after grafting into the rat brain. A standard differentiation protocol was used in our study to turn the undifferentiated stem cell monolayer of 'paving stone' morphology into a differentiated cellular neural/glia network (fig. 2a). After expansion of the ReNcell VM197 cells for 3–4 days at 37°C in the presence of EGF and basic FGF, a differentiation of the cells was achieved by withdrawal of the growth factors. Within 2–3 days after removal of EGF and basic FGF, the cells started to change to a more differentiated morphology. Cells were harvested for proteomics analysis either immediately prior to the withdrawal of EGF and basic FGF (undifferentiated) state or 4 and 7 days after the start of differentiation, respectively, and prepared for 2-DE. Aliquots of protein homogenates were isoelectric focused on 24-cm nonlinear pH 3–10 immobilized gradient strips, and the second dimension separation was performed with 12.5% SDS-PAGE. Analytical gels were stained using silver nitrate, and preparative gels for MS analysis were stained with colloidal CBB. After gel digitization, spot detection and quantitation were performed with the Delta2D software, version 3.2 (Decodon, Greifswald, Germany). For the identification of the differentially expressed protein spots, replicate gel images of each of the time points were analyzed. After spot detection and editing, the intensities of the normalized spots were determined and induction/repression ratios between spots of the different time points (0, 4 and 7 days of differentiation) were calculated. For the protein identification, spots were excised from colloidal CBB-stained 2-D gels, digested by trypsin and applied to MS protein identification. For some proteins, the expression pattern was validated by quantitative Western blot analysis.

Proteome Map of ReNcell VM197 NSCs

The evaluation of the differential protein expression revealed a high number of up- and downregulated protein spots (fig. 2b, c) reflecting the striking morphological changes during the time of differentiation. As the basis for the 2-DE proteomics analysis of the cell line ReNcell VM197, it was our first aim to identify as many spots as possible present in undifferentiated cells and to generate a 2-DE proteome map of this cell line. We were able to map a total of 956 protein spots; 318 unique proteins were identified in 402 spots. These proteins were classified into

Table 1. Relative quantitative distribution of functional protein categories

Functional protein categories	Proteins, %
Neurotransmitter metabolism	0.3
Development	0.6
General transport	0.6
Neuronal differentiation	0.6
Protein transport	0.6
Cell death, apoptosis	0.9
Extracellular matrix	0.9
Ion transport	0.9
Alcohol and aromatic compound metabolism/transport	2.2
Miscellaneous	2.2
Lipid metabolism/transport	3.1
Energy metabolism	4.4
Transcription	4.7
Cell cycle, cell division	6.0
Metabolism, enzyme	6.3
Carbohydrate metabolism/transport	6.9
Signal transduction	8.5
RNA and other nucleic acids metabolism and transport; nuclear proteins	9.1
Stress response	9.1
Cytoskeleton	10.7
Protein synthesis/metabolism/processing/degradation	21.3

Proteins of ReNcell VM cultures were isolated from 2-DE gels and identified by MS. Functional protein categories were defined based on information from Gene Ontology and ExPasy databases.

21 functional categories based upon information from Gene Ontology (www.geneontology.org/) and Ontoglyph database (www.blueprint.org/products/ontoglyphs/) (table 1). Most of the proteins (about 21%) were related to protein synthesis/metabolism/processing and degradation, followed by the groups of cytoskeleton and the stress response proteins. A complete table of identified proteins has recently been published [18]. The 2-DE-protein map of the human ReNcell VM197 stem cells constitutes our basis for a detailed differential analysis during neural differentiation of this model cell line applying gel-based proteomics approaches.

Differential Expression of Proteins during Neural Differentiation

After withdrawal of growth factors from culture medium, ReNcell VM197 cells stopped proliferating and started to differentiate. The typical 'paving stone' mor-

Table 2. Selection of differentially expressed proteins of ReNcell VM cells of proliferating state compared to cells after 4 and 7 days of differentiation

Protein name	Expression (2-DE gel)	Accession No. (SwissProt, NCBI)	Function category
Nucleosome assembly protein 1-like 1	down	P55209	cell cycle, cell division
Proliferating cell nuclear antigen	down	P12004	
DNA polymerase delta subunit 2	up	P49005	
Heterogeneous nuclear ribonucleoprotein K	down	P61978	RNA/other nucleic acids metabolism and transport, nuclear proteins
Nucleoporin 50 kDa	up	Q9UKX7	
Ubiquilin 1	down	Q9UMX0	protein synthesis/metabolism/processing/degradation
Elongation factor 2 (C-terminal fragment)	up	P13639	
Serine-threonine kinase receptor-associated protein	down	Q9Y3F4	signal transduction
Stress-70 protein, mitochondrial precursor (GRP75)	up	P38646	stress response
Peroxiredoxin 1	up	Q06830	
Peroxiredoxin 4	down	Q13162	
Creatine kinase, B chain	up	P12277	energy metabolism
Glial fibrillary acidic protein, astrocyte	up (4 spots)	P14136	cytoskeleton
Transgelin-2	up	P37802	
Tubulin beta-2 chain (N-terminal fragment)	up	P07437	
Vimentin	down (7 spots) up (1 spot)	P08670	

phology of proliferating cells turned to the polymorphic phenotype of neuronal and glial cells. The morphological changes were reflected by the changes of the silver-stained 2-DE gel protein profile of the cells (fig. 2). We found 77 spots out of the total of 956 spots downregulated after 4 and 7 days of cell differentiation. Thirty of these proteins were identified by MS of proteins extracted from spots excised from Coomassie stained gels. Sixty-nine spots were upregulated after 4 and 7 days of cell differentiation, and 19 out of them could be identified by MS.

Table 2 summarizes selected results of identified proteins that were up- or downregulated after 4 and 7 days of differentiation [for a complete table, see 18]. In order to validate the results of proteome analysis, quantitative Western blotting was performed for two upregulated proteins, transgelin-2 [46] and peroxiredoxin 1 [47, 48], and two downregulated proteins, peroxiredoxin 4 [47, 48] and proliferating cell nuclear antigen [49]. For all four proteins, the results of 2-DE analysis have been confirmed by Western blot analysis.

The following candidate proteins for the elucidation of the neural differentiation process are representative of the large spectrum of functional groups and may participate in the cessation of cell division and induction of

apoptosis, establishing of cell adherence junctions, growth cone development, neurite elongation and synapse formation. The nucleosome assembly protein 1-like 1 protein, the heterogeneous nuclear ribonucleoprotein K and ubiquilin were downregulated during ReNcell VM197 cell differentiation, whereas the nuclear migration protein nudC, the DNA polymerase delta subunit 2, nucleoporin 50 kDa, the elongation factor 2 and the creatine kinase B chain were upregulated.

Furthermore, besides the above-mentioned peroxiredoxins five stress-related proteins were found to be differentially regulated in our study (upregulated: GRP75 and HSP-105; downregulated: HSP-70, HSP 90-alpha and HSP7C). Generally, reassembling of cytoskeletal proteins is an important feature of morphological changes during neural differentiation [50]. Therefore, it is not surprising that more than 10 out of 45 differentially regulated proteins belong to components of microfilaments, microtubuli and intermediary filaments such as actin, ezrin, tubulins, glial fibrillary acidic protein, laminin and vimentin.

The comparison of the results of our approach with those of proteomics screening of Maurer et al. [14] in NSCs from the adult rat hippocampus shows only a few

corresponding protein expressions. Similarly, gene expression microarray studies in different systems often show only limited overlap [4–8].

This indicates a lack of common features of NSCs with regard to proteome (and genome) profiling. In this context, the origin of the cells is of major relevance, but also genetic modifications, culture conditions and the number of all passages must be considered when profiling and functional proteome studies of neuronal stem cells of different studies are compared. A higher conformity of proteomic studies between different groups of investigators with regard to the stem cell lines and the application of standard operation procedures would facilitate the comparability of proteomics results. In addition, the establishment of unique databases for the broad set of information deriving from further in-depth proteomics of neuronal stem cells will be indispensable.

Conclusions

2-DE proteomic profiling turns out to be a powerful method for the investigation of complex events in various systems. Improved techniques of 2-DE and especially MS

facilitate the elucidation and characterization of structures and processes of neuronal specimens at the proteome level. For the analysis of neuronal stem cell development, proteomic approaches will help to describe those pathways which are involved in differentiation and neural plasticity, indicating a functional role of newly identified proteins in human NSCs. Using the 2-DE gel technology and MS protein identification, we generated a 2-DE proteome map of the human NSC line ReNcell VM197, derived from the ventral mesencephalon (www.neuromics.med.uni-rostock.de). The database provides a protein inventory, which will help to specify changes in the protein expression pattern due to specific pathways activated or suppressed during proliferation and differentiation of NSCs.

Acknowledgements

We thank Carla Biedermann and Nicole Deinet for technical assistance with cell culturing, protein preparation and Western blotting, Dr. Stefan Mikkat and Dr. Christian Scharf for MS analysis, Dr. Matthias Berth for valuable help in proteomics data analysis and Dr. Eilhard Mix for critical comments on the manuscript.

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