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Stem cell proteomes: A profile of human mesenchymal stem cells derived from umbilical cord blood

Multipotent mesenchymal stem cells (MSCs) derived from human umbilical cord blood (UCB) represent promising candidates for the development of future strategies in cellular therapy. To create a comprehensive protein expression profile for UCB-MSCs, one UCB unit from a full-term delivery was isolated from the unborn placenta, transferred into culture, and their whole-cell protein fraction was subjected to two-dimensional electrophoresis (2-DE). Unambiguous protein identification was achieved with peptide mass fingerprinting matrix-assisted laser desorption/ionization – time of flight – mass spectrometry (MALDI-TOF-MS), peptide sequencing (MALDI LIFT-TOF/TOF MS), as well as gel-matching with previously identified databases. In overall five replicate 2-DE runs, a total of 2037 $\pm$ 437 protein spots were detected of which 205 were identified representing 145 different proteins and 60 isoforms or post-translational modifications. The identified proteins could be grouped into several functional categories, such as metabolism, folding, cytoskeleton, transcription, signal transduction, protein degradation, detoxification, vesicle/protein transport, cell cycle regulation, apoptosis, and calcium homeostasis. The acquired proteome map of nondifferentiated UCB-MSCs is a useful inventory which facilitates the identification of the normal proteomic pattern as well as its changes due to activated or suppressed pathways of cytosolic signal transduction which occur during proliferation, differentiation, or other experimental conditions.

Keywords: Database / Human mesenchymal stem cells / Proteomic profiling / Umbilical cord blood
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1 Introduction

In principle, mesenchymal stem cells (MSCs) are highly attractive candidates for tissue engineering approaches in mesenchymal tissue regeneration because they can easily be obtained and cultivated and are not ethically stigmatized. Their main source has traditionally been the bone marrow (BM). BM-MSCs exhibits a multipotent differentiation potential and can give rise to mesodermal derived tissue such as bone, cartilage [1], tendon [2], and muscle [3]. Further on, differentiation of MSCs to cells of adipoid [4], hematopoietic, neural [5], and stroma type [6] was described. The major detriments of BM as source of MSCs, however, are the invasive collection procedure, high degree of viral exposure [7], and significant decrease in cell number, proliferation, and differentiation capacity with progressing age [8, 9]. This has spurred an ongoing search for alternative sources of MSCs for clinical application.

Human umbilical cord blood (UCB) is an increasingly accepted source for hematopoietic stem cell transplantation for children as well as for adults [10, 11]. Whether UCB also might be an acceptable source of MSCs is still under discussion. Some groups reported that MSCs are not present in UCB from term deliveries and exist only in fetal blood [12–15]. Other reports claim that cells comparable to BM-MSCs can be obtained from UCB of full-term deliveries [16, 17]. In a recent study, we found that the frequency of UCB-MSCs is indeed extremely low but that the quality of each UCB unit significantly correlates with the cells' isolation efficacy [18]. By comparing UCB-MSCs with BM-MSCs, significant differences concerning expansion and differentiation potential

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Abbreviations: ACTH, adrenocorticotropic hormone; **BM**, bone marrow; **FACS**, fluorescence-assisted cell sorting; **HCCA**, α-cyano-4-hydroxycinnamic acid; **MNC**, mononuclear cells; **MSCs**, mesenchymal stem cells; **nph3**, neuropolypeptide h3; **PMF**, peptide mass fingerprinting; **PK**, pyruvate kinase; **PKC**, protein kinase C; **PS**, peptide sequencing; **UCB**, umbilical cord blood

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were observed, whereas the immune phenotype after culture was very similar. The need for further detailed characterization of UCB-MSCs and their differences towards BM-MSCs then prompted us to focus on the molecular determinants of their cellular properties. We thus employed proteomic technology to systematically analyze the protein expression of UCB-MSCs. Albeit proteomic methods have been used in MSC investigations [19, 20], MSCs isolated from human UCB have not been subjected to profound proteome analysis. On the other hand, genomic approaches have recently been published which analyze BM-MSCs and UCB-MSCs using gene expression profiling methods [21–24]. In this study we propose for the first time an expandable database of UCB-MSC proteins which will serve as a starting point to generate a comprehensive reference map and reference database of their proteome. Proteomic databases have proven to be effective tools in basic and applied stem cell research as they facilitate the identification of changes in signal transduction components [25] with regard to plasticity, proliferation, or differentiation [19, 26, 27]. The knowledge of the cellular proteome also provides a useful instrument for the assessment of differential protein expression changes under disease conditions, for the analysis of functional protein interactions, and for the clinical treatment protocols [28, 29]. These possibilities emphasize the necessity of a reliable and verifiable protein analysis as the basis of a proteomic reference database. Such an analysis should fulfill the following criteria: (i) it should exhibit an excellent analytical resolution in the separation of proteins, (ii) it should account for the extensive molecular variety of proteins based on their processing *via* alternative splicing, mRNA editing, or co- and post-translational modifications, (iii) it should possess a high accuracy in protein identification, and (iv) it should allow for a methodological performance in a high-throughput fashion. These demands are met by protein separation *via* 2-DE and subsequent protein identification *via* MS, namely MALDI-TOF-MS and MALDI LIFT-TOF/TOF-MS. They have proven effective in the analysis and identification of proteins from silver-stained SDS-PAGE gels. The proteomic profile of UCB-MSCs set forth in the present study should help to further explore UCB-MSCs intrinsic biological properties and to support the development of their therapeutic applications. Furthermore, the identified proteomic profile can be expanded step by step in the future by the inclusion of additional specifically isolated subcellular fractions.

2 Materials and methods

2.1 Isolation of stem cells from UCB

UCB-MSCs were isolated from UCB as previously described [18]. Briefly, one UCB unit obtained from a full-term delivery was collected from the unborn placenta with informed consent of the mother into a bag system containing 17 mL citrate phosphate dextrose anticoagulant (Cord Blood Collection System, Eltest, Bonn, Germany). The unit was stored at 22 $\pm$ 4°C prior to processing. After 1:1 dilution of the UCB unit with PBS (Nexell, Baxter, Unterschleissheim, Germany and Merck, Darmstadt, Germany), mononuclear cells (MNCs) were isolated by a Ficoll-Hypaque density gradient centrifugation (Amersham, Freiburg, Germany) for 30 min at 435 $\times$ *g* and room temperature. MNCs were removed from the interphase, washed, and counted using an automated cell analyzer (CellDyn 3700, Abbott, Wiesbaden, Germany). To isolate the MSCs, UCB-derived MNCs were then seeded onto fetal calf serum-precoated culture plates (Falcon, Becton Dickinson, Heidelberg, Germany) in MSCGM medium (MSCGM CellSystems, St. Katharinen, Germany) at a density of 1 $\times$ 10^6/cm^2. After incubating at 37°C in humidified atmosphere containing 5% CO_2, fresh medium was added and the nonadherent cells were discarded. Cultures were then maintained and screened continuously to detect developing fibroblastoid cells. Between day 16 and 20 after plating, their rapidly expanding colonies became visible and were harvested by using 0.04% trypsin/0.03% EDTA (PromoCell, Heidelberg, Germany). Recovered cells were replated at densities of 4–5 $\times$ 10^3 cells/cm^2 as passage 1 (P1) cells and thereafter. In order to determine their immune phenotype, the surface expression of typical marker proteins was analyzed using fluorescence-assisted cell sorting (FACS) flow cytometry. For this, cells were labeled with the following antihuman antibodies: CD14-FITC, CD34-PE, CD73-PE (also referred to as SH3 [4, 30]), CD90-Cy5 (Becton-Dickinson, Heidelberg, Germany), CD29-PE, CD44-FITC, CD45-PerCP, HLA-class I-FITC, HLA-class II-FITC (Beckman Coulter, Krefeld, Germany), CD133-PE (Miltenyi Biotech, Bergisch-Gladbach, Germany), CD105-FITC (SH2 [4, 30], and CD106-PE (Immunokontakt, AMS Biotechnology, Wiesbaden, Germany). Mouse isotype antibodies served as respective controls (Becton-Dickinson; Beckman Coulter). Ten thousand labeled cells were then acquired and analyzed using a FACSCalibur flow cytometer running the CellQuest-Software (Becton-Dickinson). Finally, the cells' multipotent MSC characteristics were thoroughly evaluated using osteogenic, adipogenic, and chondrogenic differentiation assays as described in detail in [18].

2.2 Sample preparation

In order to obtain high-yield protein extracts from the UCB-MSCs, a modification of our previously developed specific protocol for comprehensive characterization of stem cell proteomes [26] was employed. After three passages (P3), the cells were removed from the culture,

washed three times, and dissolved in a detergent lysis buffer containing 7 M urea, 2 M thiourea, 4% w/v CHAPS, 0.5% v/v Triton X-100, 0.5% v/v IPG buffer pH 3–10 (Amersham Biosciences, Uppsala, Sweden), 100 mM DTT, and 1.5 mg/mL Complete protease inhibitor (Roche, Mannheim, Germany) for 60 min at 18°C in an orbital shaker. The lysate was then centrifuged at 21 000 × *g* for 30 min and its supernatant's protein content determined by the Bradford assay [31, 32].

2.3 2-DE

UCB-MSC protein extracts from one healthy individual were separated by 2-DE, essentially using standard protocols [33, 34]; five replicates of the sample were run. For IEF (first dimension), 500 μg of the protein lysate was run in 6 M urea, 2 M thiourea, 1 M DTT, 2% w/v CHAPS, and 0.5% v/v IPG buffer on 18 cm immobilized nonlinear pH 3–10 gradient IPG strips (Immobiline DryStrip pH 3–10NL, Amersham Biosciences) in the IPGphor apparatus (Amersham Biosciences) using the following protocol: after 12 h of reswelling time at 30 V, voltages of 200, 500, and 1000 V were applied for 1 h each. Then voltage was increased to 8000 V within 30 min and kept constant at 8000 V for another 12 h, resulting in a total of 100 300 Vh. For the subsequent SDS-PAGE (second dimension), the proteins were transferred to 20 × 18 × 0.4 cm^3 polyacrylamide gels and separated by their mass in a 12.5% acrylamide matrix. The protein spots in five different experiments (replicate gels) of the protein extracts were visualized by ultrasensitive silver staining [35] and detected *via* the Phoretix Expression software v.2005 (Nonlinear Dynamics, Newcastle-upon-Tyne, UK).

2.4 Protein identification by MS

Protein identification was achieved with peptide mass fingerprinting (PMF) using MALDI-TOF-MS, peptide sequencing (PS) using MALDI LIFT-TOF/TOF-MS, and gel-matching through polynomial image warping. For PMF, protein spots were automatically located using the HT-Analyzer software (Genomic Solutions, Ann Arbor, MI, USA), excised using an automated spot-picker Flexys (Genomic Solutions), and destained as described in [36]. In-gel digestion with trypsin (Promega, Madison, WI, USA) was employed using a modified protocol as described in [37]. Samples were loaded onto prespotted AnchorChip targets which are stainless steel supports coated with hydrophobic material equipped with an array of 384 circular interruptions (anchors) of 600 μm diameter (Bruker-Daltonics, Bremen, Germany). They were prepared using α-cyano-4-hydroxycinnamic acid (HCCA) as matrix, whereby 0.3 μL of analyte solution and 1.2 μL of matrix solution (0.3 g/L HCCA in ethanol:acetone = 2:1) were applied onto the anchors using an Investigator ProMS MALDI Spotting Robot (Genomic Solutions). Samples were then allowed to air-dry at room temperature. Peptide mass spectra were obtained using an Ultraflex TOF/TOF (Bruker-Daltonics) in the fully automated reflectron TOF operation mode controlled by the flexControl software. The mass spectrometer was equipped with a SCOUT-MALDI source for multisample handling, a pulsed UV laser, a two-stage gridless reflector, a 2 GHz digitizer, a LIFT-TOF/TOF unit to analyze fragment ions of selected peptide ions (see below), and multichannel-plate detectors for linear and reflector mode measurements. All measurements were carried out in positive ionization mode using a reflector voltage of 20 kV. The external instrument calibration was achieved using signals from $[M + H]^+$ ions of the following reference standards (*m/z*): Bradykinin clip (1–7) mono 757.39916; Angiotensin II mono 1046.5418; Angiotensin I mono 1296.6848; Substance P mono 1347.7354; Bombesin mono 1619.8223; Renin substrate mono 1758.93261; adrenocorticotropic hormone (ACTH) clip (1–17) mono 2093.0862; ACTH clip (18–39) mono 2465.1983; and Somatostatin clip (28) mono 3147.4710. Fragment mass-*per*-charge spectra were obtained by integration over up to 2000 successive laser pulses (*f* = 50 Hz). The spectra were internally mass calibrated using trypsin autodigestion peptide signals (*m/z* 842.5094, 1045.5637, 2211.1040, and 2283.1802) as reference values and were the basis of mining the NCBInr database for protein identification *via* Mascot query (Matrix Science, London, UK) with the following parameters, enzyme: trypsin; missed cleavages: 1; allowed modifications: carbamidomethyl (fixed) and methionine oxidation (variable); tolerance: 75 ppm, *i.e.*, mass measurement accuracies were typically $\leq$ 75 ppm. The Mascot-delivered probability based score was regarded as a quality parameter for the correct identification [38]. Protein spots that could not successfully be identified with the previous method were additionally analyzed by PS with the same device, Ultraflex MALDI LIFT-TOF/TOF. Here, a high-resolution timed ion selector to separate selected peptide ions, a LIFT device for raising the potential energy of fragment ions, a velocity focusing stage with subsequent postacceleration, and a postLIFT metastable suppressor device [39] were used. For the analysis, maximally five precursor ions were selected, insofar as sufficient quality precursors were present. The ions were initially subjected to acceleration with 8 kV in MS step one, then selected using a timed ion-gate, and finally energy-lifted to a voltage of 19 kV. Fragmentized ion species were then accelerated in the second ion source and analyzed in MS step two, running in reflector mode. Mascot analysis of the obtained spectra was performed with 0.8 Da tolerance,

one missed cleavage, and carbamidomethyl and methionine oxidation as allowed modifications (Section 2.4). The Mascot-delivered probability based score was again observed as the quality factor for accurate identification. Additional spots were identified by gel-matching through polynomial image warping as described by us recently in [27] using unpublished proteomic reference material from human BM-MSC as well as the proteome of adult rat brain stem cells [26].

3 Results and discussion

3.1 Morphology and phenotype of UCB-MSCs

The UCB-derived fibroblastoid cells (UCB-MSCs) were identified as distinct colonies at day 14–16 after initial plating. The adherent cells displayed a consistent spindle-shaped elongated morphology. Surface marker expression analysis using FACS flow cytometry was performed at passage 4. Clearly, the cells showed no signs of hematopoietic marker expression such as for CD14, CD34, and CD133 and stained negative for HLA-class II (Fig. 1). But they were positive for CD29, CD44, CD73, CD90, as well as for HLA-class I. However, only subpopulations of the cells did express the markers CD105, also known as SH2 (53.6%), and CD106 (72.4%). In summary, immunophenotype of UCB derived fibroblastoid cells revealed the typical pattern also described for BM-MSCs.

3.2 Protein expression in UCB-MSC

UCB-MSC protein expression patterns of the five 2-DE replicate gels as obtained by electrophoretic separation (pH 3–10), silver staining, and densitometric image analysis were matched to a reference gel and included in the construction of the database. Also, several gels were run without sample material but with only medium and FCS

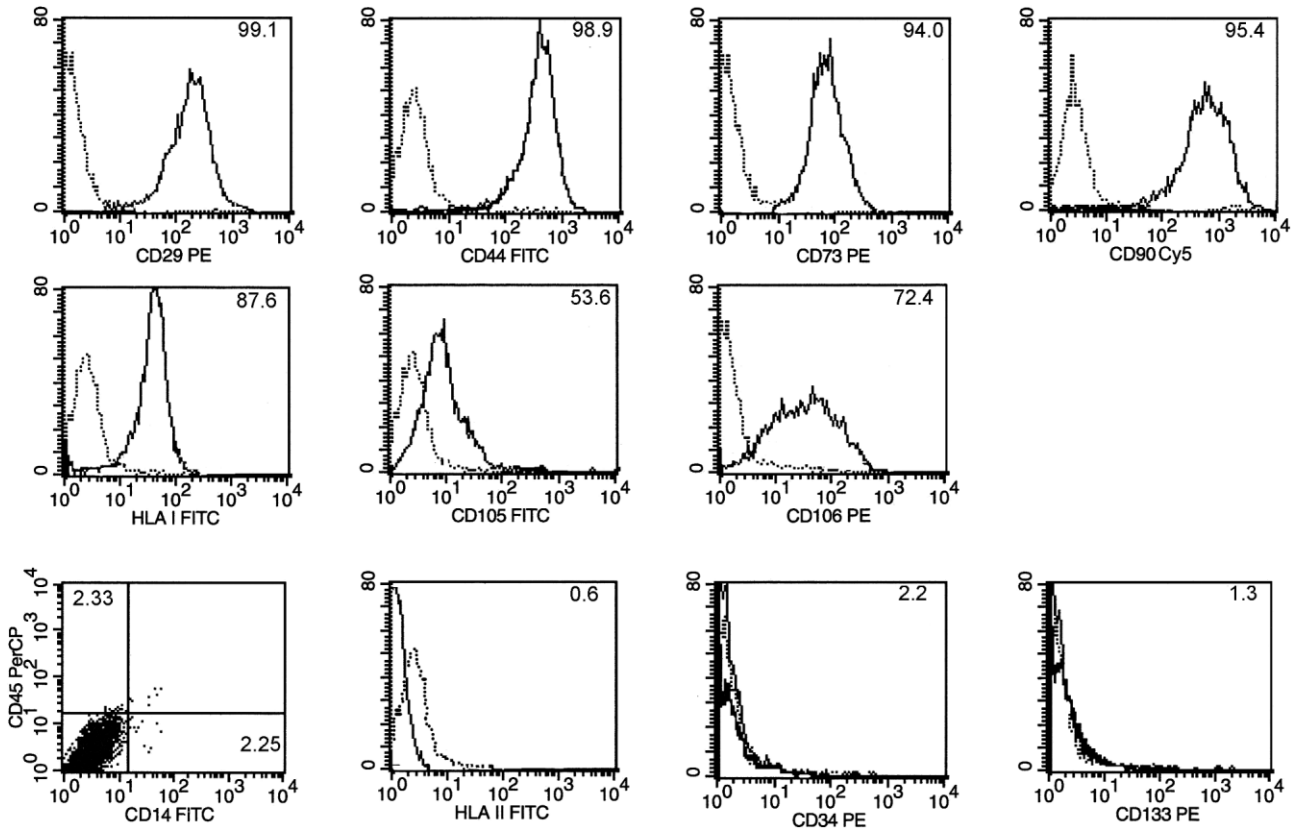

Figure 1. Immunophenotype of UCB-MSC. Fibroblastoid cells from UCB at passage 4 were trypsinized, labeled with antibodies against the indicated antigens and analyzed by flow cytometry. The respective isotype control is shown in the dotted line, a representative example of 12 UCB-MSC clones in the bold curve. The values represent the mean percentage of all assessed cells positively stained by the respective antibodies. The cells are positive for CD29, CD44, CD73, CD90 and HLA-class I (upper lane and middle lane left window), negative for CD14, CD34, CD45, CD133 and HLA-class II (lower lane) and show only fractional expression of the markers CD105 and CD106 (middle lane center and right window).

showing no interfering protein staining (data not shown). The five technical replicate gels showed a high reproducibility when run under identical conditions of separation. In order to identify the detected spots in the gels, each spot was assigned a unique arbitrary number during the matching process. The derived 2-D reference pattern of UCB-MSCs is shown in Fig. 2. We were able to map an average of 2037 $\pm$ 437 (N = 5) protein spots displaying the whole-cell proteome of UCB-MSCs, which ranged from 1674 to 2572 flagged spots. The database contains 2572 unique spots being present in at least one of the five gels.

3.3 Database of UCB-MSC proteins

Excision of a large number of spots and their in-gel trypsin digestion was performed in preparation for PMF using MALDI-TOF-MS or PS using MALDI LIFT-TOF/TOF-MS and subsequent bioinformatic data mining *via* the Mascot platform. Including the spots identified through gel-matching, a total of 205 protein spots representing 145 different proteins and 60 of their isoforms or post-translational modifications could hereby be identified unambiguously (65 spots by MALDI-TOF, 11 spots by MS/MS, and 136 spots by database gel-matching). Spot labeling numbers were assigned to the gel in Supplementary Fig. 1. All proteins are listed along with their respective database accession information (Swiss-Prot/TrEMBL database: http://www.expasy.ch/sprot and NCBI-Entrez database: http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi), experimental and theoretical molecular masses, p*I*, and the analysis data acquired from MS and LIFT-MS in Supplementary Table 1. For PMF, this includes the numbers of obtained and matched peptides, the calculated sequence coverage and the Mascot mining scores (http://www.matrixscience.com). For PS, the number of analyzed peptides and the Mascot score are specified. Figure 3 shows a representative fragment spectrum of the MS/MS analyses. Only Mascot database query results

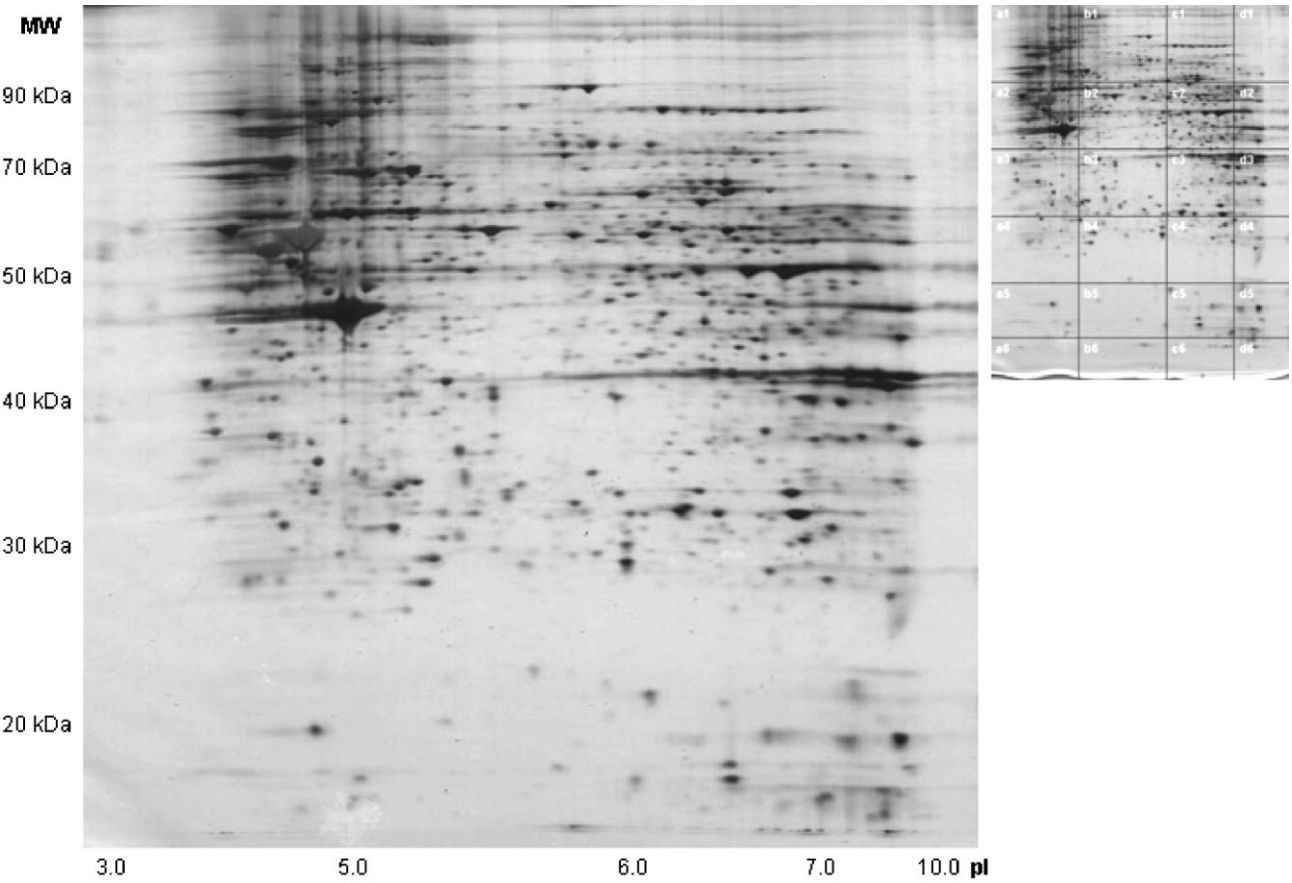

Figure 2. 2-D protein map of the whole-cell lysate from UCB-MSC. Protein extraction was achieved with detergent lysis, and protein separation was performed with immobilized non-linear pH 3–10 gradient IPG strips and subsequent SDS-PAGE in a 12.5% matrix followed by silver staining for spot visualization. The protein scout on the right allows easy detection of the annotated spots. The proteins are listed in Supplementary Table 1 (UCB-MSC database) and Supplementary Fig. 1.

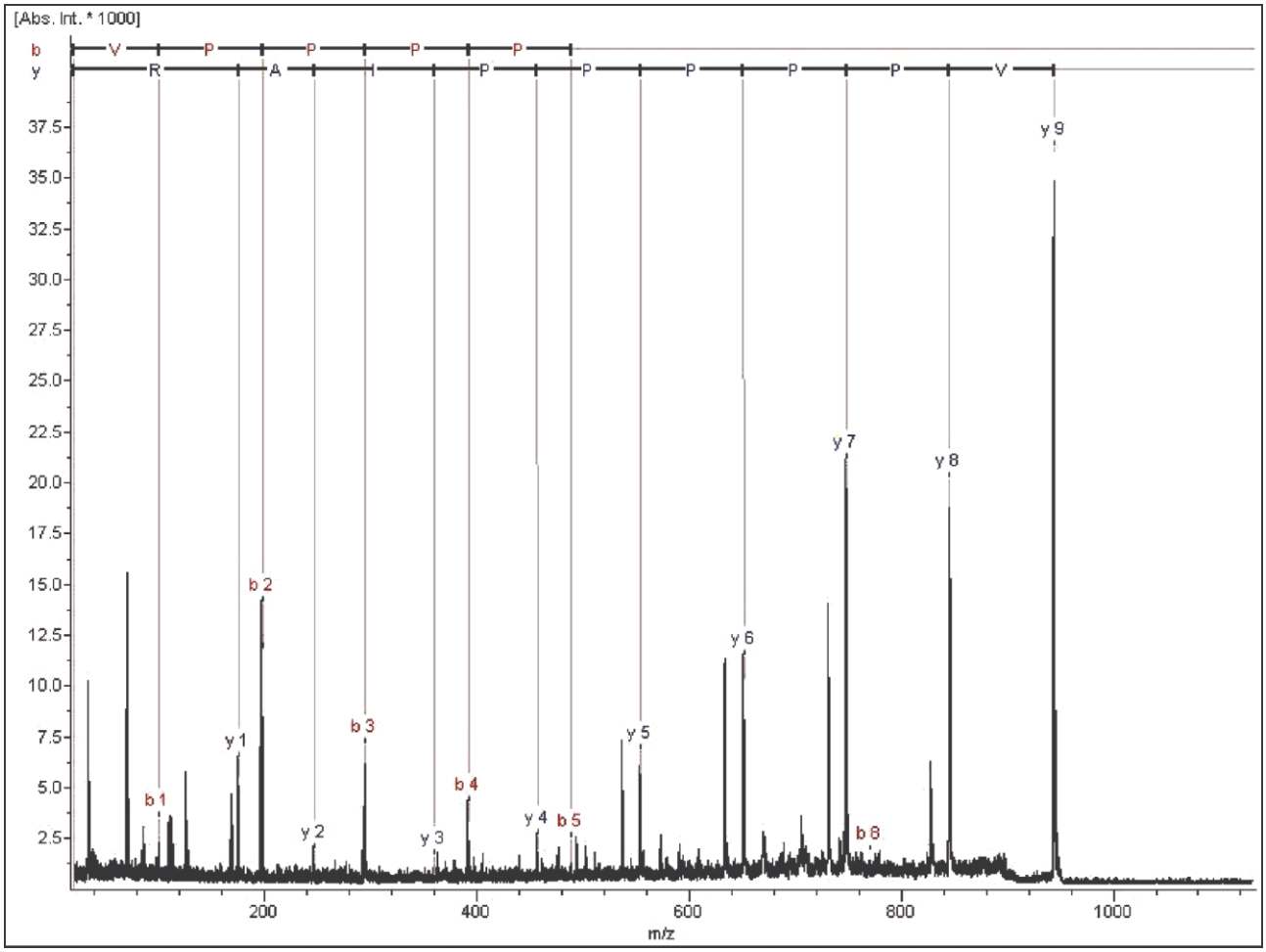

Figure 3. MS/MS fragmentation spectrum. Fragmentation of VPPPPPIAR found in NCBI Entrez Protein accession No. gi|13097279, HNRPC protein [Homo sapiens]. The protein corresponds to spot No. 1975 in Supplementary Table 1.

that were statistically significant at the 5% level were considered ($p < 0.05$). Corresponding to every detected protein, Supplementary Table 1 also shows the total number of spots whose identification has yielded the same result. Here, protein representations through the occurrence of multiple spots may have resulted from existing isoforms, post-translational modifications, alternatively spliced or otherwise truncated forms of a protein. Thirty different proteins were represented by more than one spot, 115 only by one spot.

The present protein study includes molecules belonging to diverse cellular compartments. Their isolation with the above-described protocol preferably discriminates lipophilic candidates associated with different types of membranes. Hydrophobic protein lysis from cellular compartments for 2-DE remains to be an unconquered problem in proteomics today and can presently only be accomplished in part with the help of specifically different isolation protocols [40–42]. The intention of this database, however, is to serve as an initial starting point for the establishment of a comprehensive reference map and reference database for UCB-MSC which can be expanded step by step by the inclusion of additional specifically isolated subcellular fractions. Furthermore, precaution is required when using the p*I* corresponding to each individual protein. As database mining yields the virtual peptide chain of the best-matching candidate for the protein, the associated p*I* is usually calculated from the amino acid composition. But proteins and their constitutive peptide chains in the cell lysate may sometimes exist in modified forms, which can deviate from the databases' candidates and may reflect a possible deviation of their p*I* from the predicted value.

The present study also assessed the dependability of gel-matching in proteomic stem cell profiling with the help of reference material from intraspecies human BM-MSCs as

well as interspecies rat brain stem cells. The identification results of seven proteins as successfully matched with the human BM-MSCs were verified with PMF and yielded a consilience rate of 100%. In contrast, as checked by PMF, only 27% of the protein identities (11 proteins) could accurately be concluded from the gel position of the whole-cell proteome of rat neural stem cells. This rather unreliable gel-matching for cross-species protein identification has also been confirmed recently [43].

3.4 Functional classification of identified proteins in UCB-MSC

The identified proteins could be grouped into several functional categories (Fig. 4). The largest group comprised proteins that belong to the cellular metabolism. This includes pathways such as Krebs cycle, amino acid metabolism, cellular housekeeping, or protein biosynthesis. Associated with this were also proteins involved in energy metabolism and cellular respiration. Candidates of another group are known to play roles in the folding of proteins and included chaperones, chaperonines, foldases, and heat shock proteins. In addition, protein members of the cellular cytoskeleton could be found whereby not only constitutive but likewise modifying and rearranging components were detected. Further groups comprised participants of the genomic transcription pathway such as RNA processing, signal transduction (including calcium-mediated signaling), and protein degradation (including proteasome and proteolytic components of the endoplasmic reticulum). Apart from cellular detoxification and vesicle/protein transport, further categories featured proteins with tasks in cell cycle regulation, apoptosis, and calcium homeostasis or hypothetical function. Altogether, 39% of all detected proteins (57 of 145) have been identified as catalyzing enzymes.

We could identify the protein vimentin in the stem cells, which is in accordance with data from another group characterizing UCB-MSCs [44]. It was also found to be among the top-expressed candidates in gene expression data from human UC vein-MSCs and BM-MSCs [24]. Vimentin is the most ubiquituos intermediate filament protein and the first to be expressed during cell differentiation. All primitive cell types including BM and cord blood derived nonhematopoietic (mesenchymal, stromal) progenitor cells express vimentin [44, 45]. In most non-MSCs it is replaced by other intermediate filament proteins during differentiation. A variety of MSC types including fibroblasts, endothelial cells, *etc*. stably express vimentin after differentiation, which is therefore a key protein to identify progenitor cells of mesodermal origin. Its precise role in stem cells, however, remains mostly unknown today, but present data suggests a prominent function in their developmental dynamics. In MSCs, vimentin is one of the most prominent phosphoproteins and its phosphorylation is significantly enhanced during cell division, at which time the filaments are reorganized [46]. Eriksson *et al.* [47] have shown that this can lead to an influence on its phosphorylation-dependent assembly-disassembly equilibrium, which in turn can have regulatory effects on important signal transduction pathways, many of which play roles in cellular proliferation,

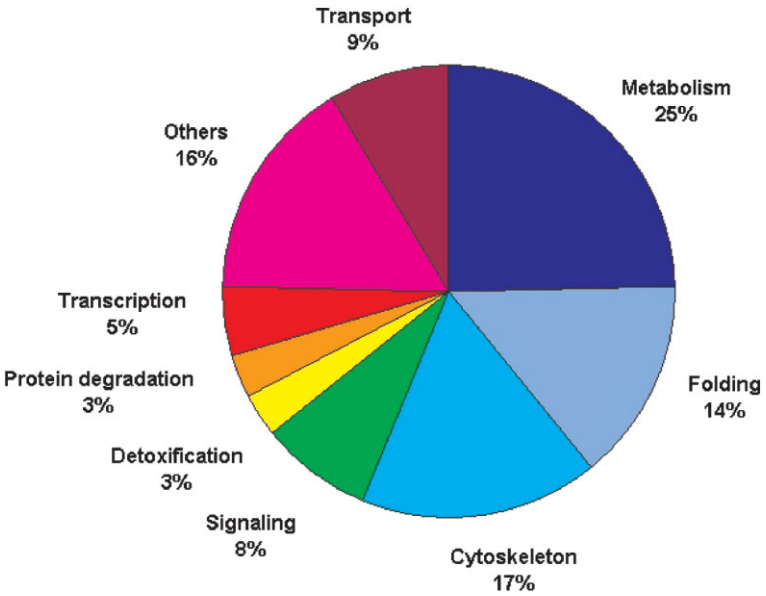

Figure 4. Functional distribution of identified proteins of UCB-MSC. The chart shows the relative distribution of the identified proteins sorted into functional categories. The group "others" featured proteins with known roles in cell cycle regulation, apoptosis, calcium homeostasis as well as hypothetical proteins.

differentiation, and apoptosis. With respect to proliferation, Takai *et al.* [48] have found vimentin phosphorylation to be a function of mitosis activation mediated by the protein kinase C (PKC) whereby PKC acted as a "mitotic vimentin kinase" to coregulate the cell cycle. In the UCB-MSCs, ten different vimentin spots have been detected which may be the results of different phosphorylation states of the protein. As MSCs in culture are highly proliferating cells, an active phosphorylation of vimentin is not unexpected.

We also demonstrated the expression of the neuropolypeptide h3 (nph3) in UCB-MSCs, which has to our knowledge not been described in MSCs before. It is known to play a role in differentiated human cells of neuroectodermal origin [49] where it is thought to be associated with brain disease [50]. In mammalian cells, nph3 is a component of the important feedback inhibition in G-protein coupled signal transduction [51] and plays a role in coordinating of the regulation of the NF-kappa B pathway [52]. Most interestingly, however, nph3 is a participant in the cell's repertoire for controlling the essential Ras/Raf-1/MEK/ERK signaling module. It does so by interfering with MEK phosphorylation and activation of Raf-1, thereby suppressing the pathway as well as AP-1 dependent transcription [53, 54]. By this way, it is able to forward mitogenic and differentiation signals to the cell's nucleus [55]. Besides in neural and lymphocyte tissue, nph3 has been assumed recently to perform such functions in multipotent stem cells from the adult brain [26]. It is therefore tempting to assume that it exerts a distinct physiological role also in the differentiation and development of MSCs from cord blood.

Gelsolin is another of the detected proteins, which is involved in differentiation processes. Gelsolin is an actin-binding protein that nucleates actin polymerization at low calcium concentrations but causes severing of actin filaments at high concentrations. It also binds to phosphatidylinositol bisphosphate, linking actin organization and signal transduction. Upon stem cell differentiation, gelsolin expression is downregulated whereas α-actin is activated [19]. This regulation may also influence the cell shape and migratory capacity, with a potential impact on the homing features of stem cells [56].

Also, the protein prohibitin has been found which plays an important role in progenitor cells [57]. The two prohibitin proteins, Phb1p and Phb2p (BAP37), have been ascribed various functions, including cell cycle regulation, mitosis and proliferation, apoptosis, assembly of mitochondrial respiratory chain enzymes, and aging. They participate in the control of the G_1/S phase and interact with the retinoblastoma protein pRB counting as potential tumor suppressors. Being coexpressed during development and in adult mammalian tissues, their expression levels are indicative of a role in mitochondrial metabolism, but are not compatible with roles in the regulation of cellular proliferation or apoptosis. Senescent cells in contrast have been shown to downregulate prohibitin expression [58].

Interestingly, we have also found proteins involved in stroma interactions during hematopoiesis: collagen type VI was shown to be strong adhesive substrate for hematopoiesis [59]. Also, tropomyosin and α-tubulin are regarded as important interactors in stroma-dependent hematopoiesis [60].

There is a massive debate on whether stem cells up- or downregulate tissue specific proteins upon differentiation. Embryonic stem cells seem to use the strategy to express genes representing various differentiation pathways. Upon differentiation, they select only a few for continuous expression [61]. Thus, the presence of proteins may be indicative for lack of differentiation or, *vice versa*, the absence of proteins may be typical for differentiated cells. In this context, we have detected proteins known to be indicators of differentiated cell types although their exact role in UCB-MSC is not yet known. Proteins like h-caldesmon, α-actinin, transgelin, tropomyosin, and vinculin are commonly found in myocytes and have been identified in the present preparation of UCB-MSCs. Thin filament associated myofibrillar proteins, like the detected tropomyosin, are the first proteins to be expressed upon cardiomyogenic differentiation [62]. Other proteins are indicative for hepatic precursor cells and their progeny: we have identified four spots of the enzyme pyruvate kinase (PK) as well as two spots of albumin. The discovery of the latter characterizing *in vitro* differentiated hepatocyte-like cells derived from UCB-MSCs has recently been confirmed [63], and PK has been found in other MSCs [64, 65]. Both proteins are thought to be associated with the emergence of immature hepatocytes from human mesenchymal tissue [63, 66]. Their detection could therefore be an initial indicator for the ability of UCB-MSCs to commit to a hepatic lineage *in vitro*. The above findings of various proteins expressed in UCB-MSCs could be an indication that these cells, although still considered to be multipotent and undifferentiated, do already exhibit leadoff molecular signs of prospective lineage commitments on their proteomic level. Our approach to investigate UCB-MSCs with proteomic methods in the present as well as previous studies on neural stem cells [26, 27] thus contributes to the understanding of how stem cells can be defined in terms of their protein expression. Dynamics in protein expression might be linked to functional stem cell capacities such as self-renewal, commitment, or death pathway

actions [67]. The proteome database presented in this study has promptly identified 57% of all differentially regulated proteins in human BM-MSCs under TGF-β stimulation [19] and 43% of the differential proteome in small *versus* large human marrow stromal cells [20]. It can therefore be of immediate help in the identification of functional expression profiles in human stem cells of mesenchymal origin.

4 Concluding remarks

In the present study, we established the first proteome database for human UCB-MSCs. It serves as a starting point for building-up a comprehensive database of the proteome of this type of cells. Using PMF, PS, and gel-matching, we could annotate a total of 205 protein spots representing 145 different proteins altogether and 60 of their isoforms or post-translational modifications.

We have identified several proteins, which are known to be associated with pathways involved in developmental processes such as differentiation and proliferation. This may indicate a functional role of these proteins in UCB-MSCs. Moreover, the expression of the protein nph3 has not been described in MSCs before, highlighting its putative role in the cellular signaling of UCB-MSCs directed towards mitosis and differentiation. Hence, the presented proteome map is a useful inventory which will facilitate the identification of changes in signal transduction components activated or suppressed in UCB-MSCs during proliferation, differentiation, or under other experimental conditions. Additionally, we could identify proteins which are hitherto known as indicators for differentiated mesodermal as well as entodermal phenotypes, such as myocytes or hepatocytes.

The analytical methodology for the separation and identification of large numbers of proteins employed in the present study is an effective tool in stem cell research. In the future, the UCB-MSC database can be further expanded step by step *via* the inclusion of additional, specifically isolated cellular and subcellular fractions as well as enriched low-abundance gene products. Our data show that protein identification from gel-matching as verified by physical mass spectrometric methods exhibits a high degree of reliability, if matched with gels derived from intraspecies probes and, even more so, for sample material with identical germ layer origin. Since no systematic study of the protein expression pattern of human stem cells of mesodermal origin is presently published, we also matched our results with an interspecies proteomic database for rodent stem cells of neuroectodermal origin. The hereby achieved degree of reliability was significantly lower. This may allow the conclusion that gel-matching is rather unreliable for cross-species protein identification, whereby the phylogenetic proximity of two species could be a relevant factor.

In summary, this database includes a considerable and diverse repertoire of proteins expressed by UCB-MSCs. It can thus serve as a reference database for future stem cell proteomic experiments, support the exploration of UCB-MSC intrinsic biological properties, and aid the development of their therapeutic applications.

Note added in proof: During editorial processing of this manuscript, Zenzmaier, C., Gesslbauer, B., Grobuschek, N., Jandrositz, A., *et al.*, published a proteome profile of human stem cells derived from umbilical cord blood (*Biochem. Biophys. Res. Commun.* 2005, *328*, 968–972).

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