Identification and Characterisation of Pro-metastatic Targets, Pathways and Molecular Complexes Using a Toolbox of Proteomic Technologies

Identifikace a charakterizace prometastatických cílů, drah a molekulárních komplexů s využitím proteomických technologií

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Summary

Background: Cancer metastasis involves changes in signalling pathways, cell adhesion, migration and invasiveness. Modern proteomic, mass spectrometry based techniques enable discovery of new pro-metastatic proteins and their functional partners. Also, they might be involved in their functional characterisation and validation towards development of new diagnostic and therapeutic approaches. ***Aim:*** The aim of this communication is to describe current possibilities for proteomic techniques in the discovery and characterization of pro-metastatic targets. The NF-κB pathway is one of the players responsible for a number of pro-metastatic processes. The related proteins can be discovered using untargeted proteomic approaches by comparing proteomes with different metastatic potential. Stable isotope labelling based methods enable a parallel analysis of more tumour samples. The identified pro-metastatic proteins can be characterised in relationship to cell migration, invasiveness and proliferation and in terms of their involvement in molecular complexes *via* protein-protein interactions. Advantages of the metabolic labelling based methods can be taken in these studies, the same applies for characterisation of related surface proteins involved in cell adhesion, invasiveness and cell-to-cell communication. For clinical validation of pro-metastatic proteins in large sample cohorts, approaches of targeted proteomics based on selected reaction monitoring are becoming methods of choice. ***Conclusion:*** Current proteomics methods play an important role in the identification of novel pro-metastatic proteins, pathways and molecular complexes, in their functional characterisation and validation towards diagnostic and therapeutic application.

Key words

metastasis – proteomics – tumor markers – cell migration – membrane proteins – signal transduction – mass spectrometry – isotope labeling

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Souhrn

Východiska: Tvorba metastáz je spojena se změnami v signálních drahách, buněčné adhezi, migraci a invazivitě. Moderní proteomické přístupy na bázi hmotnostní spektrometrie umožňují vyhledávat prometastatické proteiny a jejich funkční partnery, uplatňují se při jejich funkční charakterizaci a validaci směrem k vývoji nových diagnostických a terapeutických přístupů. ***Cíl:*** Cílem článku je detailněji popsat a shrnout současné možnosti proteomických technik v identifikaci a charakterizaci proteinů zapojených do prometastatických procesů. Za regulaci řady prometastatických dějů je odpovědná například NF-κB dráha. Související proteiny lze vyhledávat pomocí necílených proteomických přístupů porovnávajících proteomy s různým metastatickým potenciálem. Paralelní analýzu většího množství nádorových vzorků přitom zjednodušují metody značení se stabilními izotopy. Identifikované prometastatické proteiny lze charakterizovat ve vztahu k buněčné migraci, invazivitě a proliferaci a v jejich zapojení do molekulárních komplexů pomocí protein-proteinových interakcí. Při tom lze využít technik metabolického značení, podobně jako při charakterizaci souvisejících povrchových proteinů buněk zapojených do buněčné adheze, invazivity a mezibuněčné komunikace. Při validaci prometastatických proteinů v rozsáhlých souborech klinických vzorků se uplatňují metodiky cílené proteomiky založené na monitorování vybraných reakcí. ***Závěr:*** Současné proteomické metody mají klíčový význam při identifikaci prometastatických proteinů, drah a molekulárních komplexů, při jejich funkční charakterizaci a validaci směrem k diagnostickému a terapeutickému využití.

Klíčová slova

metastázy – proteomika – tumorové markery – buněčná migrace – membránové proteiny – přenos signálů – hmotnostní spektrometrie – izotopové značení

Introduction

Cancer metastasis is a complex of processes in which cancer cells leave the primary tumour site and colonise other organs *via* body fluids circulation. This multistep process is responsible for the majority of patient deaths related to cancer. Despite its clinical importance, the metastatic cascade remains poorly understood at molecular, cellular and tissue levels. Pro-metastatic factors involve molecules of regulatory pathways such as NF-κB and are responsible for changes in cell adhesion, migration and/or invasiveness. As an example, we describe the mechanisms how NF-κB pathway members contribute to metastasis formation. Moreover, protein-protein interactions and cell surface protein composition are often altered in cells with the metastatic phenotype. Current proteomic technologies represent a powerful tool for identification of responsible pro-metastatic players in clinical material and are highly helpful during their functional characterisation and validation. Here we summarise the abilities of these methods in the investigation of cancer metastasis and show examples of their practical use.

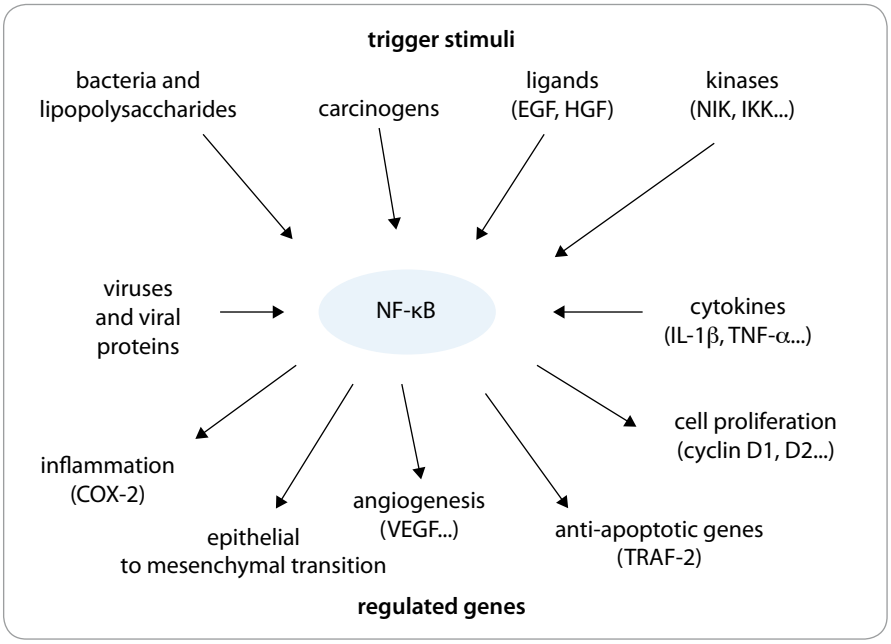

Fig. 1. Causes of activation of NF-κB pathway and the consequences. The stimuli which are able to induce aberrant activation of NF-κB in tumour cells are displayed in the upper part of the figure while genes and their products which expression is controlled by abnormally active NF-κB are shown below.

NF-κB as a Key Pro-metastatic Pathway

Nuclear factor-κB (NF-κB) is a family of sequence-specific transcription factors with five members (RELA, RELB, c-REL, NF-κB1 and NF-κB2) [1] which can associate with each other and form various homo- and heterodimers [2]. RELA, RELB and c-REL proteins are synthesised in a functional form capable of binding to DNA while NF-κB1 (p105) and NF-κB2 (p100) are synthesised as precursors [1]. Formation of the functional form p50 from NF-κB1 and of p52 from NF-κB2 requires proteolytic activation [3].

These transcription factors play important roles in the regulation of diverse biological processes in normal cells and typically regulate immune responses, cell proliferation and survival [4]. In normal cells there are three ways these factors can be activated and regulated which differ in their triggering stimuli and the participation of various kinases and regulatory proteins [5]. The first way is called canonical or classical and is triggered by various inflammatory stimuli [5]. The second is the alternative or noncanonical pathway, and is triggered by CD40 ligand, lymphotoxin-β or B-cell activating factor (BAFF) [5]. The third is the so-cal-

led atypical pathway and is usually evoked by genotoxic stress or replication [5].

In normal cells, activation of NF-κB is a tightly regulated event [1]. However, different molecular alterations in these three pathways [1] cause the dysregulation of NF-κB in tumour cells [3]. NF-κB is often constitutively activated in human carcinomas of breast, cervix, prostate, lung or colon [2]. Aberrant activation of NF-κB [2] may be due to divergent stimuli (Fig. 1) including overproduction of cytokines such as tumour necrosis factor-α (TNF-α) or interleukin-1β (IL-1β) [1]; mutations and/or overexpression of ligands such as epidermal growth factor (EGF) or hepatocyte growth factor (HGF) [3]; or abnormal activation of kinases like IKK, NIK [3] or casein kinase 2 [2]. Also external stimuli like radiation, bacteria and lipopolysaccharides (LPS), or viruses and viral proteins [1] can lead to constitutive activation of NF-κB [2]. Another way of activation is by various carcinogens [1].

A series of studies revealed that abnormally activated NF-κB regulates many genes involved in the promotion of cancer in tumour cells (Fig. 1) [1]. For example, it regulates production of prostaglandins *via* the COX2 gene and many other pro-inflammatory cytokines, which are overexpressed in many cancers [1]. Also, it regulates expression of angiogenic growth factors like vascular endothelial growth factor (VEGF) [1], expression of apoptosis inhibiting proteins like TRAF2 [1,2], expression of cyclin D1, D2 [2] and other proteins promoting cancer cell proliferation [1]. Last but not least, abnormally active NF-κB in tumour cell activates expression of genes which are involved in epithelial to mesenchymal transition (EMT) like vimentin, N-cadherin [6], matrix metalloproteinase-9 [7] and others, but also represses expression of some genes during EMT like E-cadherin [6]. EMT is a key event in tumour invasion and metastasis [6] and therefore abnormally active NF-κB also plays an important role in promoting tumour metastasis [6].

As mentioned above, cumulative evidence implicate abnormally active NF-κB in cancer development, progression and metastasis [2] making it an interesting therapeutic target in cancer [1]. Nevertheless, further studies are necessary for a complete understanding of its role in cancer development [8].

Identification of Novel Pro-metastatic Targets Using Proteomic Technologies

Searching for pro-metastatic targets on protein level, including those related to NF-κB pathway by proteomic techniques is mostly based on mass spectrometry (MS). High sensitivity, specificity and throughput of MS allow us to identify and quantitate proteins involved in metastatic pathways which could serve as valuable biomarkers. MS-based proteomics approaches are usually coupled with nanoflow liquid chromatography (LC) of peptides prepared by cleavage of complex protein mixtures with trypsin.

Quantitative proteomics can be categorised into two types: untargeted and targeted. Untargeted or "discovery" methods attempt to cover as many proteins as possible to select the most promising biomarker candidates by relative quantification between carefully selected or prepared sample groups. Targeted proteomics comes to the scene after the discovery phase to verify and validate proteins of interest from the previous stage before potential implementation in clinical practice. Nevertheless, there is still a long way ahead for a newly revealed biomarker to be used in routine clinical practice.

In a preliminary screen to identify potential target proteins that can serve as novel biomarkers of metastasis, there are several proteomic approaches that can provide very valuable data. The goal of these proteomic techniques is simultaneously identify and quantitate protein expression in response to a variety of changes that occur during metastasis development. Generally, the development of methods for accurate protein quantitation is nowadays one of the most challenging – and rapidly changing – areas of proteomics. The best choice from currently available methods for a particular project depends on multiple factors, i.e. the number of samples, the number of conditions being compared, the type of equipment available, or the expense and time required.

Relative quantitation with chemical labelling is perhaps the most widespread and frequently used method in current untargeted proteomic studies. It is based on the introduction of a differential mass tag in a form of small chemical label that allows the comparative quantitation of proteins in one sample to another. The label changes the mass of the protein/peptide and does not affect its analytical properties. The labelling can be done either chemically or metabolically, although the latter method is feasible only in the situation where the cells are metabolically active (cell lines). Metabolic labelling cannot be used on tissue samples or biofluids.

The iTRAQ technique (isobaric Tag for Relative and Absolute Quantitation), one of the chemical labelling techniques, was first described in 2004 [9] and since then has been used to analyse protein expression in variety of cell systems, tissue samples and biological fluids. The iTRAQ isobaric label consists of three parts: (i) a reporter group (variable mass of 114–117 Da or 113–121 Da), (ii) a balance group and (iii) an amino-reactive group that introduces a highly basic group at lysine side chains and at peptide N-termini. During tandem MS/MS mode, the reporter group is released as a singly charged ion of masses at m/z 113–121 in a quiet region of the MS/MS spectrum to allow relative quantification.

The method has been used to identify metastasis related markers in several cancer types. For example, Bouchal et al [10] focused their pilot study on potential candidates of metastasis in low grade breast cancer. A quantitative comparison based on iTRAQ labelling revealed 3 proteins with significantly increased and 3 with significantly decreased levels in metastatic primary tumours, and 13/6 proteins with increased/decreased level in lymph node metastasis compared to non-metastatic primary tumours. The group of Ho et al [11] employed iTRAQ-based proteomics to study a breast cancer metastatic model, which comprised four isogenic cell lines. The goal was to monitor pro-

tein expression changes of cancer cells acquiring increasing metastatic potential. From more than 1000 proteins detected, 197 proteins, including drug-targetable kinases, phosphatases, proteases and transcription factors, displayed differential expression when cancer cells became more metastatic. Another example of iTRAQ utilization not on clinical samples can be found in the work of Ghosh et al [12] who used an *in vitro* metastatic model where two isogenic colorectal cancer cell lines (primary and its lymph node metastatic variant) were compared. It led to the identification of 1,140 unique proteins, out of which 147 were found to be significantly altered in the metastatic cells. An application of iTRAQ on serum samples can be found in the study of Rehman et al [13] where they analysed pooled samples from 4 groups of patients with different progression of prostate cancer. Mapping the differentially expressed proteins onto the prostate cancer progression pathway revealed a dysregulated expression of individual proteins, pairs of proteins and ‚panels' of proteins to be associated with particular stages of disease development and progression. In another iTRAQ-based study by Yang et al [14] cathepsin S (which belongs to the group of extracellular proteases, which are attractive drug targets) was identified as a possible mediator of cancer cell migration and invasion, putatively *via* a network of proteins associated with cell migration, invasion, or metastasis.

The potential benefit to identify and quantify low abundance proteins in complex samples, coupled with the ability to multiplex up to eight samples in parallel [15,16] makes iTRAQ a very powerful method for quantitative discovery of new possible biomarkers, therapeutic and diagnostic targets.

Alternatively, the search for potential metastatic targets can be performed on *in vitro* models – cancer cell lines. For this approach, quantitative proteomics offers a very potent method called SILAC (Stable Isotope Labelling by Amino acids in Cell culture) which has been popularised by Mann and co-workers [17]. The principle lies in growing two cell cultures in isotopically distinct media, one containing "light" amino acids (normal) and a second containing "heavy" isotopically labelled (13C or 15N) amino acids. The labelled amino acids supplied in the medium are essential for the cell culture (Leu, Lys, Met or Arg) and are therefore incorporated into each newly synthesised protein in the cells. After isotope incorporation, the samples can be pooled and analysed by mass spectrometry. Because the "light" and "heavy" amino acids are chemically and biologically indistinguishable, the labelled peptides behave identically during preparative procedures process until entering a mass spectrometer where peptides with incorporated "heavy" amino acids are distinguished from their "light" amino acids containing counterparts by a characteristic mass shift. A well-designed combination of the labelled amino acids allows for quantitative differentiation up to three samples at a time [18]. There are also attempts to broaden the scope of SILAC-based proteomics from cell lines to clinical samples. In the pilot study [19] human tumour proteomes were quantified by combining a mixture of five SILAC-labelled cell lines with human carcinoma tissue. Moreover, the use of SILAC has been extended beyond simple tissue culture methods into live organisms including mice, and has been used in human tumour xenograft studies [20,21].

To summarise, methods of untargeted proteomics lead to discovery of proteins potentially involved in processes of interest such as cancer metastasis. However, to become true biomarkers, such proteins must undergo a long process of verification and functional characterisation before their clinical validation.

Mechanistic Studies on Protein Involvement in Pro-metastatic Mechanisms: Analysis of Cell Migration, Invasiveness and Interactome

Cancer metastasis is closely related to cell migration and invasion which are essential for tissue development and maintenance. Epithelial cells, which give rise to most tumours, are non-migrating under normal conditions. However, their migratory behaviour changes during tumour progression (for review see [22]). These cells increase their migration, which leads to higher invasion of the tumour to surrounding tissues and formation of metastasis. Cell migration is essential for development of metastasis and as such it becomes one of the promising targets of anti-metastatic therapy (reviewed by Iiizumi et al [23]). Many methods have been established to analyse cell migration and invasion. However, cell migration is a very complicated process; it has been shown that it depends not only on structural and molecular determinants of the tumour cells themselves, but also of the tissue environment (for review see [24]). Therefore, there are many aspects which have to be considered when designing experiments studying cell migration. Mainly *in vitro* assays are used, which simplify conditions in which the cells migrate. This simplification increases reproducibility and enables automation. On the other hand, such systems do not precisely reflect behaviour of the cells in the tumours. To improve this, surfaces can be covered by substrates which mimic the composition and structure of the ECM and thus *in vivo* conditions. Nowadays, there are two methods used for observation of the migratory behaviour of the cells in animal models: *in vivo* imaging and intravital microscopy (reviewed in [25]). In these methods, the cells are applied by different ways into the organism (intravenously, subcutaneously, intramuscularly or orthopically into certain organ) and subsequently visualised by different approaches (e.g. magnetic resonance, positron emission tomography or the most used luminescence/fluorescence microscopy, which use cells transfected with a plasmid expressing a detection marker such as GFP protein or luciferase). The way of application influences outcomes of whole experiments. For instance intravenous application leads to accumulation of the cells in the organs highly supplied with blood, while subcutaneously applied cells form primary tumours in the place of application. Both methods belong to *in vivo* methods. The difference is that meanwhile classical. *In vivo* imaging detects cells within the entire organism, intravital microscopy

detects it just in a certain organ or environment. A basic *in vitro* method is the scratch (wound healing) assay. In this method cells directionally migrate to heal the wound which has previously been created in a confluent monolayer of the cells. Its simplicity and adaptability to any plate format make it very popular. However, this method suffers from poor reproducibility and other drawbacks like cell destruction during the scratching process. These drawbacks can be overcome using so called Cell Exclusion Zone Assay in which a cell-free area is created by introducing a barrier on the plate before the cells are seeded into it and by its subsequent removal after the cells reach confluency [26]. Another group of methods, which are used mainly for a study of cell chemotaxis, includes transwell migration assays. Since its first introduction by Boyden in 1962 [27], many variations have been developed. In this system, cells migrate through a membrane with defined pore size, which separates the migration chamber into two compartments. The above mentioned methods study cell migration in 2D systems. However, tumour cells often migrate in 3D matrix *in vivo*, importance of extracellular matrix in cell migration has been recently nicely reviewed by Wolf and Friedl [28]. Therefore, it is useful to study the migratory behaviour of the cells in 3D substrates. In such systems, the investigated cells are embedded within a matrix and subsequently continuously visualised by either time-lapse videomicroscopy or confocal laser-scanning microscopy.

The vast majority of proteins does not occur separately, but interact with other proteins. These interactions are often necessary for regulation of their function and are thus crucial for many cellular processes. Finding the protein interaction network can give clues as to its cellular role. Methods to study protein-protein interactions can be divided into so called binary methods, which study direct interactions, and methods for study of protein complexes. A typical binary method is the yeast two-hybrid system. However, many cellular processes are characterised by the formation of intricate multiprotein complexes, in which proteins interact both directly and indirectly. The most common approach to study protein complexes includes affinity purification with subsequent protein identification using mass spectrometry. The affinity purification can be performed through either tag, which is fused with N- or C-terminus of the studied protein (pull down approach), or interaction with a specific antibody (immunoprecipitation). The biggest challenge of protein interaction studies is the problem of non-specific interactions. One of the promising approaches which allow distinguishing specific interactions from non-specific ones is an involvement of methods of quantitative proteomics. Quantitative proteomics encompass protein labelling using stable isotopes (SILAC) with their subsequent relative quantification using MS methods [29]. Overview of such an approach is provided in Fig. 2.

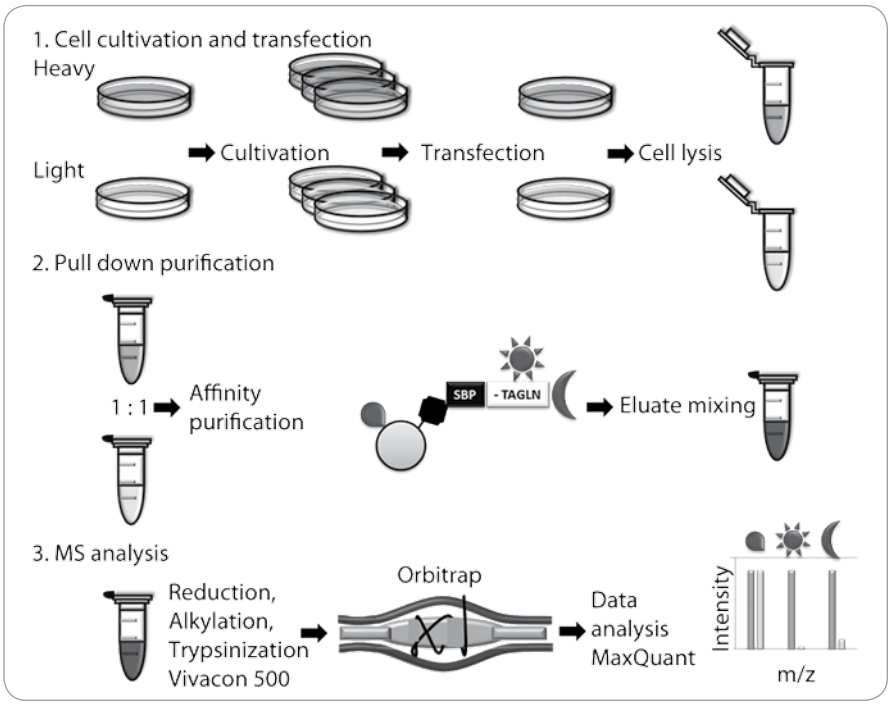

Fig. 2. Example of a proteomic experiment designed to identify protein-protein interaction partners via proteomics screening using SILAC labelling. The cells are typically cultivated in growth media containing either light (control cells) or heavy form of labelled amino acids. After at least five cell doublings which are necessary for complete incorporation of labelled amino acids into whole cell proteome, cells are transfected with either plasmid bearing studied gene (transgelin) or plasmid bearing control gene (GFP), both fused with SBP-tag in N-end of the gene. Thereafter, cells are harvested, lysed and protein concentration is determined. The same amounts of proteins from „heavy" and „light" lysates are used for pull down purification of tagged genes, which is done for each gene separately. Eluates from the purification are subsequently mixed. Mixed protein sample is subjected to reduction, alkylation and trypsin digest using filter aided sample preparation protocol (FASP). Proteins are identified and quantified via high resolution mass spectrometry measurement and data analysis in MaqQuant software. For distinguishing between specific and non-specific interaction it is important to determine a ratio of signals from „heavy" and „light" samples (H/L ratio). In case of non-specific interactions, this ratio is equal to 1, whereas it is significantly higher than 1 for specific interactions.

Cell Surface Proteins as Targets of Antimetastatic Therapy

Cancer metastasis is the result of a multistage process that involves changes in cell-to-cell and cell-to-matrix adhesion, which are mediated by cell surface proteins [30]. Changes in cell surface proteins of metastatic cancer cells can lead to altered binding to proteins of the extracellular matrix. The interacti-

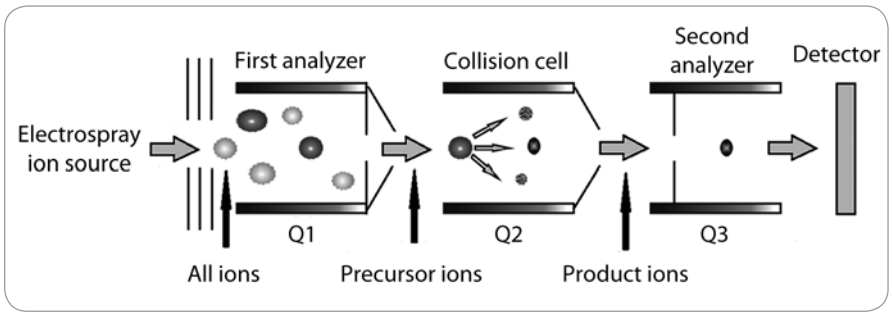

Fig. 3. Schema of mass spectrometry analysis in SRM mode (triple quadrupole (QqQ) mass spectrometer). Peptides separated by liquid chromatography enter the nanospray where the electric field (2,000–3,000 V) and thermal heat is applied over the analyte droplets. The droplets become charged, their volume decreases and as a consequence they split into smaller ones [51]. Charged peptides proceed into QqQ instrument. In SRM quantitative mode, first (Q1) and third quadrupole (Q3) serve as mass filters while the second one (Q2) works as a collision cell. Q1 selects precursor ions (peptides) which are subsequently fragmented in Q2 in a process called collision induced fragmentation (CID). Product ions (peptide fragments) enter Q3 where only the product ions used for quantification are selected and sent to detector [52].

ons between cell surface proteins and proteins of extracellular matrix trigger signalling cascades which regulate gene expression, cell migration, cell adhesion and cell survival mechanisms. These events lead to cancer cell spreading from primary tumour *via* degradation of interactions between cell surface proteins (mainly group of adhesion proteins) and the extracellular matrix. The extracellular matrix is degraded by proteases produced by invasive motile immortal cells. These cells finally form secondary tumours at new sites [31].

As the previous section suggests, cell surface proteins play important roles in cancer metastasis. They represent up to 50% of total plasma membrane weight. Cell surface proteins include proteins physically embedded in the lipid bilayer and proteins anchored to the plasma membrane [31]. Both groups may be sensitive to extracellular signals, may transport specific molecules, may be involved in cell adhesion, or be responsible for connecting the lipid bilayer to the cytoskeleton or the extracellular matrix [32]. Lectins, cadherins and selectins are the most important cell adhesion proteins playing a role in metastasis [31].

The study of plasma membrane proteins is complicated by their hydrophobicity [33]. Sample enrichment and fractionation are the available solutions to overcome this bottleneck. The most commonly used enrichment is based on selective labelling of plasma proteins, namely on extracellular domains [34]. The labelling molecule-linker may contain biotin group, hydroxysuccinimide group, cleavable S-S bond and a spacer arm to reduce steric hindrance. The linker reacts with ε-amino groups of lysine and with protein N-terminus [34,35]. Biotinylated proteins are then captured on streptavidin sepharose during affinity purification. The dissociation of streptavidin-biotin bond is hardly possible. Therefore the cleavage of linker must be performed by a suitable agent, typically in the elution step. Disulfide bond containing linkers (e.g. Sulfo-NHS-SS-Biotin) are frequently used and cleaved by mercaptoethanol. In photocleavable NHS-P-C--LC-biotin, UV light is used to elute purified proteins [36]. The elution step could also be improved using covalently modified avidin sepharose (e.g. nitroavidine) which tends to increase the Kd of the complex [36]. Protein precipitated from eluate should be free of other impurities which could inhibit subsequent trypsin digestion prior to MS measurement [37].

Roesli et al [38] compared the plasma membrane proteome of two closely related murine teratocarcinoma cell lines with different metastatic potential. Biotinylation was used for selective plasma membrane protein enrichment [36]. 998 proteins were identified using MS and their relative abundance was determined. The list of identified plasma membrane proteins originating from metastatic cell line involved well known proteins connected with metastatic spread, synaptojanin-2 (upregulated) or CEACAM-1 (downregulated). Moreover, this study indicated that proteins normally present inside the cell may be frequently expressed on cell surface of the cell with metastatic characteristics [38].

Selective plasma membrane protein enrichment connected with relative quantification could be used to quantitatively compare expression profiles of cell lines. Also, it could potentially help to find pro-metastatic plasma membrane proteins or drug targets in oncological therapy.

Validation of Pro-metastatic Proteins in Large Sample Cohorts Using Targeted Proteomics Towards Their Clinical Application

For targeted sensitive quantification of proteins in large sample sets, e.g. pro--metastatic targets, either ELISA, or mass spectrometry based method called selected reaction monitoring (SRM) could be used [39]. SRM is typically performed on triple quadrupole (QqQ) type of MS instrument (Fig. 3) [40], however its implementation in the workflows on high resolution instruments has been recently reported [41,42]. Sample preparation is coupled with addition of internal standards (isotopically labelled peptides) or a labelling step, respectively, if absolute or relative quantitation is performed [43,44]. In the case of very low abundant proteins, SRM combined with immunoenrichment can be used, namely at peptide level: SISCAPA (Stable-Isotope Standards and Capture by Anti--Peptide Antibodies) method is based on affinity enrichment of peptides characteristic for the investigated protein (proteotypic peptide) using antibodies. Alternatively, the most abundant proteins could be depleted, namely from plasma samples. The key term in SRM is "the transition" defined as a combination of precursor ion (peptide) isolated in first quadrupole (Q1) of mass spectrometer, and of product ion (peptide fragment) selected in third quadrupole (Q3)

(Fig. 3) [43]. For quantification of one protein, it is thus necessary to use at least two precursor ions, each of them is quantitated using at least two product ions [43,45]. To ensure robust, specific and sensitive SRM protein assay, the optimal combination of transitions as well as other analysis parameters have to be optimised for each measured protein.

The main advantage of SRM is the selectivity and ability to quantitate low abundant proteins. Kesishian et al developed quantitative multiplexed SRM assays for six proteins in human plasma that achieve limits of quantitation in the 1–10 ng/ml range. Abundant proteins were depleted and strong cation exchange (SCX) was used for fractionation at the peptide level [46]. Prostate serum antigen (PSA) is a well known clinically used marker for prostate cancer. SRM with immunoenrichment enabled PSA quantification in patient sera [47] as an alternative to currently used approaches. SRM methods could be also used for quantification of tissue biomarker isoforms. This approach allowed reliable quantification of 16 isoforms of mouse cytochrome P450 during a single analysis [48]. Moreover, SRM is applicable to paraffin blocks where it was used to find proteins related to different stages of lung adenocarcinoma. Analysis revealed that napsin-A and anterior gradient protein 2 homolog (AGR-2) levels correlated with stages and lymph node metastasis of lung adenocarcinoma. Increased expression of napsin-A in IA and IIIA stages correlated with better prognosis [49]. A new, technically different alternative to SRM in protein quantitation in a data independent mass spectrometry mode is called "SWATH". It is based on recording of consecutive high resolution fragment ion spectra of the precursor ions within a user defined precursor ion mass window [50]. This recent approach could make additional low abundant proteins available and might be used to measure quantitative digital fingerprints of clinical samples.

Conclusion

Investigating pro-metastatic mechanisms provides valuable information helpful in prognosis and therapy prediction for cancer metastasis. Both untargeted and targeted proteomic methods are powerful tools contributing to this general research aim. We hope that the application and further development in these technologies will provide new insights into this clinically important but not yet completely understood research area.

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