Review

Proteomic definitions of basement membrane composition in health and disease

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Abstract

Basement membranes are formed from condensed networks of extracellular matrix (ECM) proteins. These structures underlie all epithelial, mesothelial and endothelial sheets and provide an essential structural scaffold. Candidate-based investigations have established that predominant components of basement membranes are laminins, collagen type IV, nidogens and heparan sulphate proteoglycans. More recently, global proteomic approaches have been applied to investigate ECM and these analyses confirm tissue-specific ECM proteomes with a high degree of complexity. The proteomes consist of structural as well as regulatory ECM proteins such as proteases and growth factors. This review is focused on the proteomic analysis of basement membranes and illustrates how this approach can be used to build our understanding of ECM regulation in health and disease.
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Introduction

Basement membranes (BMs) are essential extracellular matrix (ECM) structures within multicellular organisms. In most cases BMs form barriers that separate epithelial, mesothelial or endothelial cells from the underlying stroma. It is considered that laminin networks form the basis of all BMs [1,2] and that type IV collagen networks provide structural strength and stability of BMs [3]. There are 16 different laminin αβγ trimers [4] and three different type IV collagen ααα trimers that are known to occur in nature [5] and these trimers form networks of sheet-like structures. Laminin and type IV collagen networks have differential expression throughout mammalian tissues [6,7], most likely conferring different physical properties to BMs. Indeed BMs fulfil varied and sophisticated roles in normal tissues, in addition to forming barriers, they direct cell differentiation, morphology and survival [8,9]. As a result, BMs comprise an extensive repertoire of ECM components. Other well-characterised BM components include: heparan sulphate proteoglycans (perlecan, agrin), nidogens, type XVIII collagen and type XV collagen. However, this list is not exhaustive and the tissue-, developmental stage- and disease-specific expression of all putative BM proteins has yet to be determined.

BMs must be carefully regulated in health and their disruption often results in disease. Altered BM homeostasis is seen with an accumulation of ectopic ECM within BMs in fibrosis [10–12], excessive BM glycosylation in diabetes mellitus [13,14] and BM degradation associated with cancer metastasis [15–17]. Additionally, mutations in genes encoding BM components can cause hereditary diseases affecting multiple organs including nephropathy, sensorineural hearing loss, epidermolysis bullosa, ocular abnormalities and neuromuscular deficit [18–20]. It is therefore of clinical importance to characterise the spatio-temporal expression of BM proteins and the plethora of potential posttranslational modifications that occur in health and

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disease. This undertaking is a major challenge, however, with the development of ECM proteomics, new insights into the composition of ECM have been achieved [21] and the 'omic' approach complements more traditional candidate-based approaches.

Rationale for mass spectrometry-based proteomic investigation of BMs

Gene expression analysis techniques, such as microarray and RNA sequencing, enable global quantification of BM components at the transcript level. These analyses allow the collection of vast amounts of information, and are often used as surrogates for protein abundance. However, although powerful, these techniques frequently misrepresent the abundance of proteins within biological systems due to post-transcriptional regulation of protein abundance [22]. This phenomenon is particularly evident for ECM proteins, which may have low turnover, and indeed many studies have shown poor correlation between ECM-transcript and ECM-protein level [23–25].

Mass spectrometry (MS) is a highly sensitive analytical technique that facilitates the detection and quantification of proteins, including those that are of low abundance, in a global manner. Moreover, unlike gene expression analysis, MS-based techniques enable researchers to probe the plethora of potential post-translational protein modifications. The analytical instrument, a mass spectrometer, generates charged gaseous ions, measures the mass-to-charge ratio (m/z) of these ions and records the number of ions at each m/z value. MS analysis of whole proteins is known as "top down" proteomics. Since MS of whole proteins is less sensitive than MS of peptides [26] and biological samples are complex and frequently contain thousands of proteins, the m/z of an intact protein in a complex mixture by itself is insufficient for unique identification. In order to detect, and ideally quantify, all of the proteins present within a biological sample, proteins are digested to peptides referred to as "bottom up" proteomics. This is frequently performed using enzymatic trypsin digestion. The benefit of trypsin digestion is that predictable tryptic peptides are produced. Peptides have defined (either lysine or arginine) C-terminal protonated amino acids following trypsin digestion and this provides an advantage in peptide sequencing and subsequent database matching. However, the m/z of tryptic peptides in complex mixtures is also non-unique leading to ambiguity. To perform global shotgun proteomic experiments tandem MS (MS/MS) is required, whereby peptide ions are selected, fragmented and detected. Information from both the precursor peptide (MS1) and the peptide fragments (MS2) enables the confident identification of peptides that in turn may be unique to, and therefore specific for, a given protein.

Peptide ions in the gaseous phase are most frequently generated by electrospray ionisation (ESI) [27]. These ions are then detected by mass analysers, which operate by measuring the trajectories of ions in an electric field or by trapping ions for further manipulation. Examples of mass analysers include: quadrupole mass filters, time of flight instruments, Fourier transform ion cyclotron analysers and the orbitrap. These analysers can be stand alone or assembled in series to take advantage of their various strengths.

Strategies to increase proteome coverage from a given sample, involve separating or fractionating the sample prior to analysis. This is achieved with high performance liquid chromatography (HPLC) [28], which is a frequently used modality, and prior to HPLC proteins can be separated by SDS-PAGE [29], off-gel electrophoresis (OGE) [30] and run as separate fractions. Although these techniques all increase total proteome coverage, they do not specifically increase ECM proteome coverage. For instance the introduction of OGE into a MS pipeline for ECM proteomics by Naba et al. led to a sixfold increase in proteins identified, but only a threefold increase in ECM proteins [31]. However, without this fractionation step growth factors were not identified within ECM enriched samples. In contrast, as discussed in this review approaches to isolate the ECM sub-proteome are essential for ECM/BM proteomic experiments.

To identify peptides, tandem MS (MS/MS), employs two analysis steps in series. Firstly, peptide ions within a narrow m/z range are selected by either data dependent or data independent acquisition. Data dependent acquisition (DDA) is the most commonly applied for global proteomics. Using DDA the most intense peptides are selected from an initial MS scan (MS1). The exact mass of this precursor peptide ion is determined and it is then fragmented along its backbone, usually by collision induced dissociation (CID), electron transfer dissociation (ETD) or higher energy C-trap dissociation (HCD). These fragment ions are then detected, giving rise to characteristic spectra derived from a single precursor, which can be used to deduce the sequence of the precursor peptide. DDA is sequential, so the next most intense ion is selected and so forth. Limitations in acquisition speed mean that certain lower intensity peptides may not be selected and therefore undetected. In contrast, MS2 spectra are acquired from all precursor peptide ions in data independent acquisition (DAI) mode. One example of DIA is sequential window acquisition of all theoretical mass spectra (SWATH-MS). Theoretically, SWATH-MS allows all peptides to be analysed, and to identify peptides from the complete MS2 produced by mixed species of fragment ions. This

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