The Emerging Role of Cytoskeletal Proteins as Reliable Biomarkers

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Cytoskeletal proteins are essential building blocks of cells. More than 100 cytoskeletal and cytoskeleton-associated proteins are known and for some, their function and regulation are understood in great detail. Apart from cell shape and support, they facilitate many processes such as intracellular signaling and transport, and cancer related processes such as proliferation, migration, and invasion. During the last decade, comparative proteomic studies have identified cytoskeletal proteins as in vitro markers for tumor progression and metastasis. Here, these results are summarized and a number of unrelated studies are highlighted, identifying the same cytoskeletal proteins as potential biomarkers. These findings might indicate that the abundance of these potential markers of tumor progression is associated with the biological outcome and are independent of the cancer origin. This correlates well with recently published results from the Cancer Genome Atlas, indicating that cancers show remarkable similarities in their analyzed molecular information, independent of their organ of origin. It is postulated that the quantification of cytoskeletal proteins in healthy tissues, tumors, in adjacent tissues, and in stroma, is a great source of molecular information, which might not only be used to classify tumors, but more importantly to predict patients' outcome or even best treatment choices.

1. Introduction

The cytoskeleton, a complex, dynamic fibrous network in the cellular cytoplasm, consists of three major components: microtubules (MT, diameter 20–25 nm), intermediate filaments (IF, diameter 10 nm), and microfilaments (MF, diameter 5–7 nm).[1–4] More than 100 proteins have been identified as either directly contributing to the structure or the regulation of the cytoskeleton. And although the cytoskeleton has been a topic of research for more than 70 years, new cytoskeletal elements are still discovered, such as the recently described γ-tubulin string mesh, which provides structure to the mitochondrial network and facilitates the movement and localization of mitochondria in cells.[5]

The visualization of the intact cytoskeleton was made possible in 1974, when Elias Lazarides and Klaus Weber used antibodies to demonstrate actin filaments by immunofluorescence microscopy.[6] While investigating IF of tumors, Mary Osborn provided the first experimental evidence, that assessing the abundance of IFs in tumors provides molecular information which might be useful in deciphering whether a tumor is malignant or not.[7] Since those early days, cytoskeletal proteins rarely take center stage in cancer research, probably because most are highly abundant and ubiquitously expressed; both attributes usually not necessarily associated with a sensitive and selective biomarker. Depending on the hypothesis and data analysis, differentially regulated cytoskeletal proteins might even be underreported, as researchers may focus on other key molecules in cancer instead, such as signaling molecules, kinases, or transcription factors.

In addition cytoskeletal proteins are often fibrous hydrophobic proteins and might be insoluble in aqueous buffers, which increases the risk of those proteins being discarded during sample preparation. Interestingly, most studies presented here have used methods such as western blot, immunohistochemistry, immunofluorescence, and 2D-DIGE, which either target the proteins of interest within the intact cells or tissue, or use high concentrations of SDS to solubilize proteins. Only a limited number of studies has identified differential expression of cytoskeletal proteins using aqueous cell lysis followed by LC-MS/MS.

To overcome this major challenge, extra care should be taken to improve accessibility of cytoskeletal and extracellular proteins for downstream analysis. Multiple improved protocols have been published, such as sequential extraction methods, using a combination of SDS buffer and urea.[8] In addition, for the targeted analysis of ECM by LC-MS/MS, improved protein and digestion methods have been developed, which will facilitate not only the detailed description of tissue-specific ECM composition but also the quantitative changes occurring during oncogenesis.[9]
Despite these challenges, the abundance of some cytoskeletal proteins are well accepted as indicators for tumor progression. For example, the loss of the cell adhesion molecule E-cadherin (epithelial marker) and the upregulation of the IF vimentin (mesenchymal marker) are markers of epithelial-mesenchymal transition (EMT) during cancer progression. Both proteins can be robustly detected and quantified using a range of proteomics technique such as ITRAQ LC-MS/MS, 2DE MALDI-TOF, IF, IHC, WB, probably facilitating their widespread acceptance as markers for EMT. Moreover, details emerge about the mechanism underlying their role in EMT, as increased vimentin expression enhances cell stiffness and has been correlated with increased production of extracellular matrix (ECM). These novel insights have strengthened the role of vimentin as a key regulator in this process and identified it as a promising new drug target.[10]

The stepwise progression of cancer from a healthy body cell to an aggressive cancer is a focus of intense research. The process is so universal, that single steps have been summarized as the hallmarks of cancer and more recently as the emerging and enabling hallmarks of cancer, for example, intrinsic events such as gene mutations, change in proliferation rate, and extrinsic events such as avoiding immune destruction. Cytoskeletal proteins facilitate the cancer cells’ ability to invade and metastasize. They not only provide the physical structure to be able to migrate and extravasate, but also regulate intracellular signaling, such as Rho GTPases and might also directly influence transcription.[13]

The phenotypical changes occurring in the tumor site are easily observed by standard histology procedures, where thin slices of the tumor are stained with hematoxylin and eosin before being analyzed under a microscope. During tumor growth, the cells usually lose their polarity and change their morphology. Clinical trials have investigated if cell shape alone can be a predictor of metastasis or patients’ outcome. Because these phenotypically shape changes are determined by the cytoskeletal proteome, one could anticipate a well-orchestrated change in cytoskeletal protein abundance during oncogenesis. In addition, the role of the ECM in tumor promotion has been clearly established in a number of cancers, for example ECM can be used to classify breast cancers.

Here, we summarize a number of comparative proteomic studies, in which patient samples or cell lines have been used to identify cytoskeletal biomarkers for tumor initiation and progression. The research implies that cytoskeletal proteins might be reliable biomarkers, which can help to describe and predict disease progress.

2. Microfilaments

Microfilaments (MFs) are composed of actin filaments which play an important role in maintaining cellular morphology, forming cytoplasmic cell protrusions, providing resistance against stress and participating in cell interaction with extracellular matrix.[12] All studies described in this review related to microfilaments are summarized in Table 1.

2.1. Actins

Actins are the most abundant eukaryotic protein group and have been estimated to make up to 1–5% of total cellular protein.
Table 1. Summary of proteomic studies identifying microfilaments as potential biomarkers.

| Protein family | Gene | Size [kDa] | Cancer | Study design | Study finding | Material(s) used | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|----------------|------|------------|--------|--------------|---------------|----------------|---------------------------------------------|------|----------------------------------------------------------------------------------|
| Actin          | ACTA1 | 42         | Breast | Normal vs tumor | Down in Tumor | TCGA data | Subnetwork detection, pathway analysis | [86] | Not diagnostic, tissue enriched                                                  |
|                | ACTA2 | 42         | Prostate | Normal vs tumor | Down in Tumor | FFPE | IHC | [87] | Expressed in all, unfavorable in renal cancer                                      |
|                | Ovarian |            |         | Benign vs malignant | Down in malignant compared to benign | | | | |
|                | Endometrial | | | Primary tumor vs metastasis | Down in metastasis | FFPE | MALDI-MSI, LC-MS/MS, IHC | [89] | |
|                | Basal cell carcinoma | | | Primary tumor vs metastasis | Down in metastasis | FFPE | IHC | [90] | |
|                | Lung | | | Primary tumor vs metastasis | Down in metastasis | FFPE | IHC, IF, WB | [91] | |
|                | Pancras | | | Primary tumor vs metastasis | Up in metastasis | FFPE | IHC | [92] | |
|                | Colon | | | Primary tumor vs metastasis | Up in metastasis | FFPE | IHC, IF | [93] | |
|                | ACTC1 | 42         | Brain  | Low grade glioma vs high grade glioma | Up in high grade | FFPE | IHC | [94] | Tissue enriched, unfavorable in head and neck cancer and in urothelial cancer |
|                | ACTB | 42         | Colon  | Highly metastatic vs low metastatic cell line | Up in highly metastatic cell line | Cell lines | IF, WB | [95] | Expressed in all, unfavorable in renal cancer and head and neck cancer |
|                | Gastric | | | Normal vs tumor | Up in tumor | Cell lines, frozen tissue, FFPE | WB, IHC | [96] | |
|                | ACTG | 42         | Salivary gland | Primary tumor vs metastasis | Down in metastasis | Cell lines | 2DE, WB | [97] | Expressed in all, favorable in colorectal cancer |
| Actin binding proteins | ACTN1 | 103        | Lung   | Normal vs tumor | Up in tumor | Frozen tissue | IHC | [98] | Expressed in all, unfavorable in renal, lung, head and neck, and urothelial cancer |
| α-actinins    | ACTN2 | 103        | Pancreas | Primary tumor vs metastasis | Up in metastasis | Frozen tissue, FFPE | IHC | [99] | Group enriched, not prognostic |
|                | ACTN3 | 103        | | | | | | |

(Continued)
| Protein family | Gene | Size [kDa] | Cancer | Study design | Study finding | Material(s) used | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|---------------|------|-----------|--------|--------------|---------------|----------------|------------------------------------------|------|---------------------------------------------------------------------------------|
| **ACTN4**     | 104  | Pancreas  | Normal vs primary tumor vs metastasis | Up in tumor | FFPE, cell lines | IHC, WB, FISH | [97] | Expressed in all, favorable in renal cancer, unfavorable in pancreatic and lung cancer |
| Bladder       |      | Up in tumor | Cell lines, FFPE | WB, IHC, IF | [98] | |
| Colon         |      | Up in tumor | FFPE, cell lines, mouse model | IF, IHC, WB | [99] | |
| Oral          |      | Up in tumor | Cell lines | IHC, WB | [100] | |
| Oesophagus    |      | Up in tumor | Frozen tissue, FFPE | 2DE, MALDI-TOF, IHC | [101] | |
| Ovarian       |      | Up in tumor | Frozen tissue, FFPE | 2DE, LC-MS/MS, WB | [102] | |
| **Myosin**    | 237  | Prostate  | Normal vs primary tumor vs metastasis | Up in metastasis | Cell lines | IHC | [104] | **MYO10**: Expressed in all, favorable in renal cancer, unfavorable in cervical cancer |
| Colon         |      | Up in metastasis | FFPE, TMA | MALDI-TOF, IHC, WB | [104] | |
| Breast        |      | Up in tumor | Frozen tissue, cell lines, mouse model | WB, IHC | [105] | |
| **Fascin**    | 54   | Breast    | HR-negative vs HR-positive tumors | Up in HR-negative | FFPE | IHC | [107] | **FSCN1**: Actin bundling protein, tissue enhanced, unfavorable in renal, lung, and head and neck cancer |
| Ovarian       |      | Up in tumor | Cell lines, FFPE | ICC, IHC | [107] | |
| Prostate      |      | Up in tumor | Cell lines, FFPE | WB, IHC, | [105] | |
| Colon         |      | Up in tumor | Cell lines, FFPE | IF, WB | [104] | |
| Pancreas      |      | Up in tumor | FFPE | IHC | [104] | |
| Gastric       |      | Up in tumor | FFPE | IHC | [104] | |

(Continued)
Table 1. Continued.

| Protein family | Gene | Size \( [\text{kDa}] \) | Cancer | Study design | Study finding | Material(s) used | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|----------------|------|----------------|--------|--------------|---------------|-----------------|----------------------------------------|-------|----------------------------------------------------------------------------------|
| Filamin        | FLNA | 280            | Breast | Normal vs primary tumor vs metastasis | Up in metastasis | Frozen tissue, FFPE | IHC | [43] | Expressed in all, unfavorable in renal, urothelial, and colorectal cancer |
| Lung           |      |                |        | Normal vs primary tumor vs metastasis | Down in metastasis | Cell lines, FFPE, TMAs | WB, IF, IHC | [45] |
| Prostate       |      |                |        | Primary tumor vs metastasis | Up in metastasis | FFPE | IHC | [106] |
| Melanoma       |      |                |        | FLNa-positive cells vs FLNa-negative tumor cells | Down in metastasis | FFPE, TMA, frozen tissue, cell lines | IHC, WB | [41] |
| Spectrin       | SPTA | 280            | Lymphoma | Primary tumor vs metastasis | Down in metastasis | FFPE, TMA | IHC | [46] | SPTA1: Tissue enriched, not prognostic |

IHC, Immunohistochemistry; FFPE, Formalin fixed paraffin embedded tissue; WB, Western blot; MALDI-MSI, Matrix assisted laser desorption/ionization mass spectrometry imaging; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; IF, Immunofluorescence; FISH, Fluorescence in situ hybridization; 2DE, 2D-gel electrophoresis, ICC, Immunocytochemistry; ITraq, Isobaric tag for relative and absolute quantitation; TMA, Tissue microarray.
In muscle cells actins are even more prominent with an estimate of 10% by weight of the total cell protein, enabling muscle contraction. Actins are highly conserved, and its monomers self-assemble, facilitating cellular functions such as contraction, migration, cell division, differentiation, and cell death. There have been six different isoforms identified in humans: α actin 1 (ACTA1), α actin 2 (ACTA2), cardiac muscle α actin (ACTC1), β actin (ACTB), γ actin (ACTG1), and γ enteric smooth muscle (ACTG2). α actin is primarily expressed in skeletal, cardiac, and smooth muscle cells and contributes to the process of muscular contraction. β actin facilitates cell migration and resides only in non-muscle cells, while γ actin exists in both muscle and non-muscle cells. Whereas actin plays an important role in maintaining normal cell function, altered expression of actin can lead to cancer, contributing changes in cellular function such as growth, invasion, migration, and stiffness. Actin was previously reviewed as upregulated in cancer; however, several studies indicate actin as a tumor suppressor.\textsuperscript{[13–16]}

α actin 2 (ACTA2) seems to be a soluble protein, easily detected and quantified using a range of techniques, such as MALDI-MSI, LC-MS/MS, IHC, and IF. When consulting the human protein atlas, α actin 2 expression was summarized as being selectively expressed in smooth muscle cells and myoepithelial cells and according to the literature is a marker for the mesodermal germ layer. When comparing primary tumors versus metastasis in skin cancer and endometrial cancer, α actin 2 is downregulated and in contrast upregulated in metastasis of lung, pancreatic, and colon cancer when compared to their primary tumors. These differences in regulation are inconsistent and maybe even contradictory. They cannot be easily explained and have caused considerable controversy within the biomarker field and might have even damaged the reputation of the biomarker field. However, when interpreting these results one should take into account, the possibly different expression levels and roles of α actin 2 in the tissue of origin, which all might not be well described or understood.

### 2.2. Actin Binding Proteins

The highly conserved structure of actin fibers is constantly remodeled by actin binding proteins such as fascin, filamin, spectrins, myosin, and α actinin. The assembly and disassembly of actin filaments is required for the development of 3D structures, such as filopodia (spike like protrusions), lamellipodia (sheet like protrusions), stress fibers (elastic contractile bundles), microvilli (finger-like surface protrusions), and invadopodia (invasive cell sheet), that could disrupt normal cellular function and correlates with cancer initiation and progression.\textsuperscript{[21]} Filamin is essential for cancer cell migration, as they are forming on the leading edge of cancer cells. Increased abundance of proteins which form or regulate the filopodia reflects the increased filopodia density observed during cell migration.

#### 2.2.1. α Actinins

α actinins are ubiquitously expressed cytoskeletal proteins which crosslink actin filaments to each other and to other subcellular structures. Four isoforms of ACTNs have been identified namely, the “muscle” ACTN2 and ACTN3 and the “non-muscle” ACTN1 and ACTN4.\textsuperscript{[17]} Actin binding proteins seem to be upregulated in tumors and metastasis from lung, pancreatic, bladder, colon, oral, oesophageal, and ovarian cancer as shown by a wide variety of techniques such as IHC, WB, IH, 2DE-MALDI, and LC-MS/MS. The role of ACTN4 in various cancer has been reviewed in detail in ref. [18]. Quick et al. have shown the differential role of ACTN1 and 4 in brain tumors.\textsuperscript{[19]} ACTN1 plays a role in the expansion of the astrocytoma cell population while ACTN4 is primarily involved in motility, lamellipodia formation, and cell adhesion.\textsuperscript{[19]} In our recent work, we have identified the cytoskeletal proteins annexin A2, annexin A1, and ACTN4 as potential biomarkers for metastasis in primary endometrial tumors, with annexin A2 being upregulated and ACTN4 and annexin A1 being downregulated in primary tumors which will metastasize.\textsuperscript{[20]}

#### 2.2.2. Myosin

Myosin constitutes a large and diverse family of actin binding proteins, which hydrolyze ATP to generate force and movement.\textsuperscript{[21]} This movement helps in muscle contraction and cell motility which is a prerequisite for migration of cancer cells. The human genome contains more than 39 myosin genes.\textsuperscript{[22]} The role of myosin in different cancers has been reviewed in detail in ref. [23] and the association between the expression of different classes of myosin and cancer has been reviewed in detail in ref. [24]. In addition the overexpression of myosin has been reported in various cancers, such as prostate,\textsuperscript{[25]} colorectal cancer,\textsuperscript{[26]} gastric,\textsuperscript{[27]} pancreatic,\textsuperscript{[28]} and breast cancer.\textsuperscript{[29]}

#### 2.2.3. Fascin

Fascin constitutes a distinct, unique, and evolutionally conserved family of actin binding proteins which plays an important role in the formation of membranes protrusions. This protrusions help in cellular motility and interaction with ECM or other cell types.\textsuperscript{[30]} Fascin has been shown to be upregulated in many cancers including colon,\textsuperscript{[31]} pancreatic,\textsuperscript{[32]} lung,\textsuperscript{[33]} stomach,\textsuperscript{[34]} ovary,\textsuperscript{[35]} and skin.\textsuperscript{[36]} In breast cancer, fascin expression correlates with high-grade tumors.\textsuperscript{[37]} Studies have shown the positive correlation of fascin with more aggressive tumors (reviewed in detail in ref. [38]).

#### 2.2.4. Filamin

Filamin is a family of actin binding proteins encoded by three different genes FLNA (filamin A), FLNB (filamin B), and FLNC (filamin C). Filamin plays an important role in the reorganization of the actin cytoskeleton. Filamin overexpression was found in a number of malignancies including prostate,\textsuperscript{[39]} colon,\textsuperscript{[40]} melanoma,\textsuperscript{[41]} and squamous cell carcinoma.\textsuperscript{[42]} In breast cancer, it has been shown that filamin overexpression is associated with increased cell motility and invasive potential.\textsuperscript{[43]} However, studies have also shown that filamin prevents tumor progression in breast cancer by regulating focal adhesion disassembly and suppressing the migration and invasion capability of the
Figure 1. Representative spatial ion intensity maps of potential discriminators: Tryptic peptide MALDI-MSI was performed on EC samples of patients diagnosed with \( (n = 5) \) or without \( (n = 5) \) lymph node metastasis. MALDI-MSI data acquired was used to generate the ion intensity maps using Scilab software. Randomly one patient from each group was chosen to show the spatial intensity of the significant \( m/z \) values. a) The annotated H&E stained sections are shown. The tumor region within the section was annotated by a pathologist (in black color). b) Representative MALDI-MSI images for 1542.831 \( m/z \pm 0.125 \) Da, tryptic peptide (GVDEVTIVNILTNR) belongs to annexin A2. c) Representative MALDI-MSI images for 1429.76 \( m/z \pm 0.125 \) Da, tryptic peptide (TINEVENQILTR) belongs to \( \alpha \) actinin 4. d) Representative MALDI-MSI images for 1099.591 \( m/z \pm 0.125 \) Da, tryptic peptide (NNAQRQQIK) belongs to annexin A1. Automatic hotspot removal and weak denoising was applied. Scale bars are 2 cm and intensity scale for each ion are included, intensity ranges from blue (lowest) to red highest. Corresponding ROC curves indicating the sensitivity and selectivity are included on the right (b–d). Reproduced with permission. [20] Copyright 2017, Elsevier.

This is a great example of isoform specific regulation. The different localizations of the isoforms are consistent with the predicted functions of the different isoforms, where full-length filamin in the cytosol/cytoskeleton increases cell mobility and the cleaved form of filamin can translocate to the nucleus and inhibit cell growth. [44, 45]

2.2.5. Spectrin

Spectrins are large, cytoskeletal, and heterodimeric actin binding proteins which play a crucial role in maintaining the stability and architecture of the cells. Mutations of spectrin have been shown to impair cell adhesion, cell spreading, and metastasis. [46]
3. Intermediate Filaments

Cytoskeletal IF proteins are strong and dynamic structural elements that provide mechanical support to the plasma membrane when it comes in contact with ECM.[47] In contrast to MFs and MTs, the family of IF proteins are widely divergent and can be formed from 40 different subunits. The IF protein family consists of six subtypes with cell type specific developmental stage dependent expression. Type I and type II IF includes acidic and basic keratins, expressed mainly in epithelial cells. Type III IF includes mesenchymal cell-specific vimentin, muscle cell-specific desmin, astrocyte specific glial fibrillary acidic protein (GFAP), and peripheral neuron specific peripherin.[48] Type IV IF proteins are typically expressed in neurons of central nervous system and include nestin, α internexin, and neurofilaments.[49] Type V includes nuclear specific lamins, Type VI includes embryonic neurons, nestin, and synemin.[50] All studies described in this review related to IFs are summarized in Table 2.

3.1. Acidic and Basic Keratins

Keratins also known as cytoteratins are the largest and most diverse class of the IF protein family. Keratins are mainly expressed in epithelial cells and are divided into two major subclasses: type I acidic and type II basic proteins, consisting of 28 and 26 proteins, respectively. Type I and type II keratins are the key structural components that form nails, hair, horns, claws, and wool.[47] They also play a significant role in protecting the epithelial cells from damage/stress by lining the epidermis of the skin. Type I and type II keratins bind to each other in a 1:1 ratio to form heterodimers. The role of keratin in various cancer has been reviewed in detail in ref. [51].

3.2. Vimentin

Vimentin is classified as type III IF protein and is most widely expressed in leukocytes, blood vessels, some epithelial cells, and mesenchymal cells and its diversity in health and disease has been reviewed recently in ref. [10]. Vimentin is highly conserved and ubiquitously expressed in cells of mesenchymal origin and its expression is induced in epithelial cells, which undergo EMT, both under physiological and pathological situations.[52] Unlike keratins, vimentin can form both homopolymers and heteropolymers with other type III or type IV IF proteins. Vimentin is the most abundant protein of type III IF protein family and plays a major role in maintaining cellular integrity and providing resistance against stress.[50] Vimentin expression seems to be upregulated in various cancers including prostate,[53] gastrointestinal,[53] lung,[54] and pancreatic cancer.[55] In vitro studies have indicated a role of vimentin in migration,[56] but the molecular mechanism(s) underlying the cell motility is not clear. In the majority of the cancers, vimentin is overexpressed and studies link its expression to the aggressiveness of the cancer.[50] Other studies have shown a positive correlation of vimentin expression with poor prognosis in various cancers.[57]

3.3. Desmin

Desmin is a muscle specific type III IF protein that connects sarcoma, Z disk, and nuclear membrane in sarcomeres.[58] Desmin plays an essential role in regulating sarcomere architecture, contracting muscle and stabilizing sarcomeres. Using 2DE-MS, MALDI-TOF, IF, and ELISA it has been shown to be upregulated in colon cancer when compared to normal tissue. In addition a study analyzing prostate cancer stroma has implicated a down-regulation of desmin in tumor stroma compared to normal fibromuscular stroma using IHC. This study highlights the importance of detailed annotation of primary samples for cancer cells, stromal components, and maybe also immune cell infiltration.

3.4. Peripherin

Peripherin is a type III IF protein expressed primarily in neurons of the nervous system. Like other type III IF proteins, peripherin can exist as homopolymer or can form heteropolymer with other type III IF proteins. The exact function of peripherin is yet to be known. Increased abundance of peripherin in tumor cells compared to normal cells, has been described as a hallmark of neural differentiation.[59]

3.5. Glial Fibrillary Acidic Protein

GFAB is tissue specifically expressed in the brain and encodes one of the major intermediate filament proteins of mature astrocytes. It is used as a marker for astrocytes during development and has been shown to be upregulated in high grade when compared to low grade glioblastoma.

3.6. Neurofilaments

 Neurofilaments (NF) are type IV IF proteins expressed mainly in neurons of the central and peripheral nervous system. The most important function of neurofilaments is to determine the radial growth of an axon. Based on the molecular weight, neurofilaments are divided into three major subunits also referred as NF triplet proteins: NF-L (light) ranging from 60–70 kDa, NF-M (medium) ranging from 130–170 kDa, and NF-H (heavy) ranging from 180–200 kDa. They have been implicated in the regulation of chemoresistance.

3.7. Lamins

Lamins are type V IF protein which are exclusively expressed in the cell nucleus. The most important function of lamins is to form fibrous network that support the nuclear membrane. Lamins are grouped into two types: A and B type lamins. A type lamins are encoded by single LMNA (Lamin A) gene and are usually absent during early stages of embryonic development but are typically expressed in all differentiated cells. B type lamins are encoded by two separate genes, LMNB1 (Lamin B1) and LMNB2 (Lamin B2). B type lamins are expressed in every cells and are crucial for maintaining the nuclear integrity, normal cell sur-
Table 2. Summary of proteomic studies identifying intermediate filaments as potential biomarkers.

| Protein family | Gene | Size [kDa] | Cancer | Study design | Study finding | Material(s) used | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|----------------|------|------------|--------|--------------|---------------|----------------|---------------------------------|------|--------------------------------------------------------------------------------|
| Type I (Acidic keratins) and Type II (Basic keratins) | CK | 40–47, 53–67 | Colon | Normal vs primary tumor vs metastasis | CK20+/CK7+ = High in advance stage CK20+/CK7- = early stage | FFPE | IHC | [107] | CK20: Tissue enhanced, unfavorable in renal and liver cancer CK7: Tissue enhanced, favorable in renal cancer, unfavorable in pancreatic, ovarian, and endometrial cancer |
| | | | | | | | | | |
| | | | | | | | | |
| Salivary gland | CK20, CK7 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| Type III | Vimentin | VIM | 54 | Prostate | Primary tumor vs metastasis | Up in metastasis | Cell lines | ITRAQ labeling, LC-MS/MS | [53] | Expressed in all, unfavorable in renal cancer, favorable in endometrial cancer |
| | | | | | | | | | |
| Gastric | | | | | | | | | |
| Oesophagus | | | | | | | | | |
| Liver | | | | | | | | | |
| Colon | | | | | | | | | |
| Protein family                  | Gene | Size [kDa] | Cancer                      | Study design | Study finding | Material(s) used                                      | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|--------------------------------|------|------------|-----------------------------|--------------|---------------|-------------------------------------------------------|---------------------------------------------|------|----------------------------------------------------------------------------------|
|                                |      |            |                             |              |               | Cell lines, serum, frozen tissue                      | 2D PAGE - MS, WB                            | [119]|                                                                              |
|                                |      |            |                             |              |               | Cell lines                                            | 2D DIGE, MALDI-TOF, WB                       | [55]|                                                                              |
|                                |      |            |                             |              |               | Cell lines                                            | IF                                          | [120]|                                                                              |
|                                |      |            |                             |              |               | Mouse model                                           | 2DE, MALDI-TOF, WB                          | [121]|                                                                              |
|                                |      |            |                             |              |               | Cell lines                                            | 2DE, WB                                    | [122]|                                                                              |
|                                |      |            |                             |              |               | Cell lines                                            | IF                                          | [123]|                                                                              |
|                                |      | 53         | Colon                       | Normal vs tumor | Up in tumor | Frozen tissue, FFPE                                   | 2DE-MS, IF                                 | [124]| Tissue enhanced, unfavorable in renal cancer                                    |
|                                |      |            |                             | Normal vs tumor | Up in tumor | Frozen tissue, FFPE, blood                            | 2DE, MALDI-TOF, WB, IHC, ELISA              | [125]|                                                                              |
|                                |      | 53         | Skin                        | Normal vs tumor | Down in tumor | FFPE                                                  | IHC                                        | [126]| Tissue enhanced, not prognostic                                                 |
|                                |      |            |                             | Normal vs tumor | Up in tumor | FFPE                                                  | IHC                                        | [127]| Tissue enriched, not prognostic                                                 |
|                                |      | 50         | Brain                       | Low grade glioma vs high grade glioma | Up in high grade | Serum, FFPE                                             | ELISA, IHC                                 | [128]|                                                                              |
|                                |      |            |                             | Low grade glioma vs high grade glioma | Up in high grade | Plasma, FFPE                                             | IHC                                        | [129]|                                                                              |
|                                |      |            |                             |               |               | Cell lines, frozen tissue                             | WB                                         | [130]| **NF-L, NF-M**: Tissue enriched, unfavorable in renal cancer. **NF-H**: Tissue enhanced, not prognostic, favorable in breast cancer |
|                                |      |            |                             |               |               | Cell lines, frozen tissue                             | IHC, TDE, WB                               | [131]|                                                                              |
|                                |      | 74         | Lung                        | Normal vs tumor | Down in tumor | Cell lines                                           | Protein microarray, WB, IHC                 | [65]| Lamin A: Expressed in all, not prognostic                                        |
|                                |      |            |                             | Normal vs tumor | Up in tumor | Serum, FFPE                                             | IHC, WB                                    | [61]|                                                                              |
|                                |      |            |                             | Normal vs tumor | Down in tumor | Cell lines                                           | IHC, WB                                    | [61]|                                                                              |
|                                |      |            |                             | Normal vs tumor | Down in tumor | Frozen tissue, FFPE                                   | IHC, WB                                    | [61]|                                                                              |

(Continued)
Table 2. Continued.

| Protein family | Gene | Size [kDa] | Cancer | Study design | Study finding | Material(s) used | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|----------------|------|------------|--------|--------------|---------------|----------------|-------------------------------------------|------|--------------------------------------------------------------------------------|
| Gastric        | Normal vs tumor | Down in tumor | Frozen tissue, FFPE | IHC, WB | [132] | | |
| Skin           | Normal vs tumor | Up in tumor | Frozen tissue | IF | [66] | | |
| B-type lamin   | LMNB1 | 67 | Different types of lung cancer | Up in SCLC compared to non-SCLC | Cell lines, frozen tissue | ICC, TDE | [133] | Lamin B1: Tissue enhanced, unfavorable in renal and liver cancer |
|                | LMNB2 | 70 | Gastrointestinal tract | Normal vs tumor | Down in tumor | Frozen tissue, FFPE | IHC | |
| Liver          | Normal vs tumor | Up in tumor | Plasma, frozen tissue | 2DE, MALDI-TOF | [67] | | |
| Prostate       | Normal vs tumor | Up in tumor | Frozen tissue | IF | [134] | | |
| Type IV        | Nestin | NES 177 | Pancreas | Normal vs tumor | Up in tumor | Cell lines, FFPE | IHC | [135] | Mixed, unfavorable in renal cancer |
|                | Prostate | Normal vs tumor | Up in tumor | Mouse model | IHC | [99] | |
| Breast         | Subtypes of breast cancer | Selective marker for basal epithelial breast tumor, not detected in other breast cancer subtypes | FFPE | IHC, IF, WB | [71] | | |
|                | Breast | Subtypes of breast cancer | Exclusively in Grade III and preferentially in basal-like and triple negative negative cancers | FFPE | IHC | [136] | |
| Malignant melanoma | Normal vs primary tumor vs metastasis | Up in metastasis | PPPE | IHC | [72] | | |
| Primary tumor vs metastasis | No significant difference between primary and metastasis | PPPE | IHC | [74] | | |
| Nestin expression in malignant melanoma and the relation with prognosis in patients | Poor prognosis in malignant melanoma | PPPE | IHC | [75] | | |
| Stage III vs Stage IV melanoma | Up in metastasis | Cell lines, frozen tissue, blood | WB | [137] | | | |

(Continued)
Table 2. Continued.

| Protein family | Gene | Size [kDa] | Cancer | Study design | Study finding | Material(s) used | Method(s) used to compare protein abundance | Ref. | Impact on clinical outcome according to the human protein atlas (www.proteinatlas.org) |
|----------------|------|------------|--------|--------------|---------------|-----------------|-------------------------------------------|------|----------------------------------------------------------------------------------|
| IF associate proteins (IFAPs) |      |            |        |              |               |                 |                                           |      |                                                                                   |
| Plectin        | PLEC | 531        |        |              |               |                 |                                           |      |                                                                                   |
| Head and neck  |      |            |        | Normal vs tumor | Up in tumor   | Cell lines, frozen tissue, FFPE | 2D-DIGE, WB, IHC | [138] | Expressed in all, unfavorable in renal, colorectal, and lung cancer               |
| Oesophagus     |      |            |        | Normal vs tumor | Up in tumor   | Frozen tissue | iTRAQ labeling, LC-MS/MS, IHC | [139] |                                                                                   |
| Pancreas       |      |            |        | Primary tumor vs metastasis | Up in metastasis | Cell lines, FFPE | IHC, WB | [140] |                                                                                   |
| Prostate       |      |            |        | Low metastasis cell lines vs highly metastasis cell lines | Up in metastasis | Cell lines | ITRAQ, LC-MALDI, WB | [53] |                                                                                   |
| Oral Bladder   |      |            |        | Primary tumor vs metastasis | Up in metastasis | FFPE | IHC, IF | [141] |                                                                                   |
| Muscle invasive bladder cancer cell lines vs superficial cell lines |      |            |        | Down in muscle invasive cell lines compared to superficial | | Cell lines | IF | [142] |                                                                                   |
| Liver          |      |            |        | Normal vs tumor | Down in tumor | Cell lines, frozen tissue, FFPE | IHC, IF | [143] |                                                                                   |
| Ankyrin        | ANK  | 206        |        | Primary tumor vs metastasis | Up in metastasis | Cell lines, FFPE | IHC | [144] | Tissue enriched, not prognostic                                                   |
| Desmoplakin    | DSP  | 331        |        | Normal vs tumor | Down in tumor | Cell lines, FFPE | Western blot, immunofluorescence, IHC | [145] | Favorable in renal cancer, unfavorable in urothelial cancer                        |
| Pancreas       |      |            |        | Normal vs tumor | Down in tumor | FFPE | IHC | [146] |                                                                                   |
| Colon          |      |            |        | Normal vs tumor | Down in tumor | Cell lines | WB | [147] |                                                                                   |
| Breast         |      |            |        | Normal vs tumor | Down in tumor | Cell lines, frozen tissue | WB | [148] |                                                                                   |
| Cervix         |      |            |        | Normal vs tumor | Down in tumor | Frozen tissue, FFPE | IHC | [149] |                                                                                   |
| Oral           |      |            |        | Primary tumor vs metastasis | Down in metastasis | Frozen tissue | IHC, WB | [79] |                                                                                   |
| Primary tumor vs metastasis |      |            |        | Down in metastasis | | FFPE | IHC | [80] |                                                                                   |
| Oral           |      |            |        | Normal vs tumor | Down in tumor | Cell lines, frozen tissue | IHC | [150] |                                                                                   |
| Oral           | FLG  | 435        |        | Normal vs tumor | Down in tumor | FFPE | IHC | [151] |                                                                                   |
| Oral           | SYNE | 1011, 796  |        | Normal vs tumor | Down in tumor | Cell lines, frozen tissue | IHC | [152] |                                                                                   |

IHC, Immunohistochemistry; FFPE, Formalin fixed paraffin embedded tissue; WB, Western blot; MALDI-MSI, Matrix assisted laser desorption/ionization mass spectrometry imaging; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; IF, Immunofluorescence; FISH, Fluorescence in situ hybridization; 2DE, 2D-gel electrophoresis; ICC, Immunocytochemistry; ITRAQ, Isobaric tag for relative and absolute quantitation; TMA, Tissue microarray.
vival, and development.\[60\] The discrepancy in the expression of A and B type lamins has been reviewed in detail in ref. [60]. Reduced expression of lamin in tumor cells compared to normal has been reported by some in refs. [61–63], whereas increased expression\[64–67\] has been reported by others. It is clear that lamins have multiple functions, which range from providing structure to the nucleus to being associated with transmembrane proteins and regulating signaling. Only further investigations into the molecular mechanisms of the observed changes will help us to understand the seemingly contradictory results.

3.8. Nestin

Nestin is a type VI IF protein which plays an important role in coordinating changes in cell dynamics by connecting the MFs, IFs, and MTs to each other. Nestin can form heterodimers by interaction with other IF proteins, but unlike other IF proteins, nestin does not form homopolymers.\[68\] Nestin is specifically expressed in nerve cells and has recently been identified as a marker for newly formed endothelial cells.\[4\] Increased nestin expression has been reported in various cancers including pancreatic,\[70\] liver, ovary, and brain\[77\] and easily monitored by multiple techniques, such as LC-MS/MS, 2D-DIGE, IHC, and IF. Studies have shown that increased levels of plectin with migration and invasion.\[78\] It seems to be consistently upregulated in tumors when compared to normal tissue, and in metastasis when compared to tumor tissue. Several studies have shown that the downregulation of desmoplakins is associated with invasive and metastatic behavior in tumor cells.\[79,80\]

5. Microtubules

Microtubules (MTs) are highly dynamic and the key components of the cytoskeleton. A single microtubule is comprised of 13 protofilaments, which are polymers of $\alpha$ and $\beta$ tubulin. MTs are involved in regulating intracellular transport, cell movement, and mitotic spindle formation.\[81\] The importance of microtubules during cell division makes them an important target for a chemically diverse group of tubulin binding anticancer drugs such as paclitaxel, which suppresses the dynamics of the mitotic spindle to cause mitotic arrest and cell death.\[82\] In contrast, altered microtubules expression have been associated with poor prognosis and chemotherapy resistance, tumor development, and cell survival in solid and hematological cancers.\[83\]

6. Conclusion and Future Directions

Cytoskeletal proteins represent a major network of proteins that impinge on motility, invasion, polarity, survival and growth of normal cells, and is often disrupted by tumor cells.\[5\] Emerging evidence on crosstalk between different components of the cytoskeleton in metastasis highlights the fact that the MF, IF, and MT cytoskeletons do not work in isolation but are inextricably linked together in tumor cell migration and metastasis.\[33\] A number of cytoskeletal proteins are already routinely analyzed by IHC in clinical diagnostic laboratories, such as CK5, 6, 7, and 20, p120 catenin, $\beta$-catenin, vimentin, desmin, SMMHC, and ICAM, mainly to classify tumor subtypes. This review highlights a number of cytoskeletal proteins, which are consistently regulated. Instead of random mutations leading to a regulation which favors migration, these regulations can be well orchestrated, for example by members of the miR-200 family, which plays a significant role in epithelial phenotype tumors.\[84\] The EMT gene signature has been identified in a number of tumors, as for example in the TCGA cohort. It would be interesting to compare the protein and gene signature from the tumor with adjacent tissue and healthy tissue. Structural and cytoskeletal phenotyping might not be able to explain the molecular mechanisms underlying tumor initiation and formation, nor identify driver mutations or necessarily drug targets, but it might give some understanding of the tumors’ ability to metastasize. The detailed monitoring of cytoskeletal proteins in tumors and adjacent tissues might help to better predict disease progression. According to the protein atlas,\[85\] not all genes associated with the cytoskeleton are expressed everywhere and we propose that the absolute quantification of cytoskeletal proteins could be used as a measure to understand the physiological role and regulation, as well as being a phenotypical readout of underlying biological processes, to predict tumor progression with great accuracy.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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