Actin binding proteins: Their ups and downs in metastatic life

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Abstract

In order to metastasise away from the primary tumour site and migrate into adjacent tissues, cancer cells will stimulate cellular motility through the regulation of their cytoskeletal structures. Through the coordinated polymerisation of actin filaments, these cells will control the geometry of distinct structures namely lamella, lamellipodia, filopodia and as well as the more recently characterised invadopodia. Because actin binding proteins play fundamental functions in regulating the dynamics of actin polymerisation, they have been at the forefront of cancer research. This review focuses on a subset of actin binding proteins involved in the regulation of these cellular structures and protrusions, and presents some general principles summarising how these proteins may remodel the structure of actin. The main body of this review aims to provide new insights into how the expression of these actin binding proteins is regulated during carcinogenesis and highlights new mechanisms that may be initiated by the metastatic cells to induce aberrant expression of such proteins.

1. Introduction

Cellular migration is an essential feature of life which is responsible for numerous physiological processes including accurate embryogenesis and wound healing. In some cases, however, the pathways regulating cell motility can also be used for aberrant purposes such as the dissemination of tumour cells away from their primary site of growth. Whilst formation of neoplasms is by itself an important concern for human health, the steps that lead to invasion of other tissues by the primary tumour cells, a term referred to as metastasis, is much more life threatening. Indeed this dissemination of cells, resulting in the formation of secondary tumours in other organs, accounts for more than 90% of the fatalities associated with cancer progression. Although we have made remarkable steps toward understanding some aspects of the metastasis process, much still remains to be learned. Some of the key questions which will need to be addressed in the future should focus on understanding the cellular mechanisms that favour 1) the actual migration of cancer cells out of the primary tumour and 2) how they can successfully enter, survive and then leave the blood and lymphatic circulations (intravasation and extravasation, respectively) in order to generate secondary tumours in other specific tissues and organs of the body.

In the majority of cases, the initial cellular events required to encourage metastasis are triggered by a switch from an epithelial cellular type to a less differentiated mesenchymal one, a process known as the epithelial mesenchymal transition (EMT) [1-3]. During this transition, cells will sever links with neighbouring cells. The loss of expression of the E-cadherin is seen as a hallmark towards such commitment[4, 5]. This down-regulation of E-cadherin is regulated by specific transcriptional repressors such as those of the Snail family[6, 7]. Another important step seen during carcinogenesis will result in changes in cellular migratory properties. Increased motility will encourage cells to move away from their initial niche and invade surrounding tissues. This migration can take place as a single cell (sometimes referred to as mesenchymal or amoeboid migration) or as a collective effort in cell sheets or clusters[8]. In both cases, the remodelling of the actin cytoskeleton is seen as a central step and significant alterations will take place at the cellular level. At the molecular levels, changes in the dynamics of actin polymerisation just under the plasma membrane will be the core process leading to these biological consequences. Pushing forces will be generated either directly or indirectly by the assembly of F-actin filaments and these forces will promote the formation of different protrusions at the leading edge, namely lamellipodia, filopodia, invadopodia and blebbing, all playing key roles in cellular migration, albeit under different circumstances[9].

Over the years, attention has been focused on identifying new cytoskeletal markers that demonstrate a good correlation between their expression and the degree of malignancy attained by tumour cells. Such candidate markers would have the potential to become invaluable tools to help comprehend better the stages involved in cancer biology, as well as providing powerful tools to improve both cancer prognosis and treatment. Different actin binding proteins have come to the fore and have been the focus of recent comprehensive reviews[10-12]. The work presented here focuses only on a subset of known actin binding proteins, namely the Arp2/3 (**A**ctin **r**elated **p**rotein 2 and 3 complex) and WASP/WAVE

(**W**iskott-**A**ldrich **S**yndrome **P**rotein / **WA**SP and **Ve**rprolin homologous protein) complexes, fascin and the tropomyosins all involved at different levels in the regulation of lamellipodium, filopodium, lamellum and possibly blebbing, and whose expression is aberrantly regulated during carcinogenesis. This review analyses the recent developments in the field aiming to propose mechanisms that may be utilised by the metastatic cells in order to control abnormal expression of these actin binding proteins. These new regulatory mechanisms, if proven to be determinant in carcinogenesis, may translate into potential new avenues of research and treatment in the future.

2. The actin cytoskeleton in tumour cell migration

The process of cellular migration is engineered as a cyclic procedure composed of 1) extension of cellular leading edge in the forms of membrane sheet-like, finger-like or bleb-like protrusion resulting from actin polymerisation in close proximity to the plasma membrane; 2) development of cell-extracellular contact points which may or may not be regulated by integrins and; 3) generation of forces by the actomyosin network to drive the morphological and architectural reorganisation that promotes cell movement. This review will focus mainly on specific actin binding proteins that promote extension of the leading edge and their involvement during cancer progression and metastasis, since other reviews summarising the global mechanisms of cell motility have recently been published [9, 13, 14].

Studies analysing the migratory behaviour of tumour cells have demonstrated the architectural organisation of different actin-rich structures and molecules within, depending on the environment in which they grow. For instance, an environment that promotes sufficient mechanical contacts and loosely organised extracellular matrix (ECM) will encourage an amoeboid-type migration where cells adopt a characteristic rounded shape. This style of migration, which relies on the continuous formation of dynamic cellular membrane protrusions, results in rapid locomotion and is typically seen in leukocyte cell lineages [15] but has also been observed in tumour cells[16]. Amoeboid motility does not require integrin nor other molecular interaction with the ECM[17] but relies on a continuous physical interaction and friction with the environment [18]. Furthermore, although cortical actin polymerisation plays a fundamental role in this type of 3 dimensional (3D) migration, providing a support to stabilise the newly formed bleb bulging forward [19], the filaments do not directly generate the protruding forces necessary to push the plasma membrane, as seen during mesenchymal motility. Equally important is the role of the Rho-ROCK signalling pathway that regulates the contractile cortical actomyosin network[20]. Indeed the generation of contraction forces by myosin II causes the membrane to delaminate from/or fracture the actin cortex resulting in the inflow of cytoplasm and increased pressure at the plasma membrane in the direction of movement, leading to the formation of membrane blebbing at the leading edge[16, 21]. Movements of actin and related binding proteins into the bleb, during the later stages of inflation or in the process of retraction result in the formation of a cage like structure[22]. As

the expansion of the bleb slows down, erzin appears to be one of the first proteins, studied so far, to be recruited, followed rapidly by actin[22]. The four actin binding proteins α-actinin, coronin, tropomyosin-4 and fimbrin are also observed to move rapidly into the newly form protrusion. In the final stage of the bleb retraction, recruitment of components of the contractility apparatus such as myosin regulatory light chain and tropomodulin occur, resulting in the assembly of discrete foci at the bleb rim[22]. Importantly, none of the factors known to promote actin nucleation, such as Arp2/3 or mDia have been observed in the newly formed bleb, highlighting some uncertainties as to how actin polymerisation is controlled and regulated.

Mesenchymal motility, which is typically seen during fibroblast migration, result in cells presenting a more elongated spindle-like shape, and orchestrating the modelling of different cellular organelles. Growth on 2 dimensional (2D) structures encourages cells to promote planar filamentous actin (F-actin) arrays known as filopodia/microvilli or sheet-like organisation identified as lamellipodia (Figure 1). Both of these structures rely on the controlled growth of the F-actin at their barbed end, leading to elongation of the filaments in the direction of the cell membrane. The overall morphology and molecular composition of these organelles determine their cellular importance. Lamellipodia are seen as the main driving force for locomotion and result from the large agglomeration of short branched filaments at the leading edge. The still increasing number of actin binding partners involved coordinate the nucleation of actin filaments (formins, Arp2/3/WASP complexes), their severing and depolymerisation from the pointed end (gelsolin, ADF (**A**ctin **D**epolymerisation **F**actor)-cofilin) and the control of capping of the F-actin filaments (VASP (**VA**sodilator **S**timulated **P**hosphoprotein), capping proteins, Arp2/3 complex) [9, 10]. Contractile forces generated by myosin II activity are also required for stable lamellipodia extension and organisation, acting at the back of the lamellipodium and facilitating both actin filament disassembly at this location [23] as well as generating sufficient tensions to encourage focal adhesion maturation and stabilisation through the interactions of contact points with the substratum. Filopodia, on the other hand, are believed to act as sensory and guidance organelles, probing the external environment for cues. Reflecting the idea of a “probing stick or antenna”, they are rod-like extensions made of 10-30 tight bundles of long actin filaments[24]. Both formins and fascin have been shown to be major contributors in actin polymerisation in filopodia whilst both cdc42 and other Rho GTPase proteins are important to initiate the formation of filopodia [12, 24].

However in a thick 3D ECM, and therefore a more *in vivo* environment, mesenchymal migration is dependent upon some significant proteolytic digestion of the ECM [25], a characteristic seen when cells develop a ventral membrane protruding a highly dynamic actin-rich structure with ECM degradation activity. These structures are known as invadopodia. These protrusions can be observed on the lateral side of invading cells, as well as at the front and also at the base and branching sites of invading structures[25, 26]. In a high proportion of cases, it seems that formation of invadopodia is a prerequisite for cellular invasion and has been observed for numerous cancer cell lines that are capable of invading *in vitro* assay systems or in animal xenograft models [27]. Although a clear case for the

importance of invadopodia in invasion *in vivo* is still ill defined, circumstantial evidence has highlighted the importance of invadopodia associated proteins in metastasis promotion [28]. For instance, using a xenograft model and through knockdown of N-WASP (**N**eural-**W**iskott-**A**ldrich **S**yndrome Protein), Gligorijevic et al [29] were able to demonstrate that inhibiting formation of invadopodium *in vitro* correlated with a loss of invasion, intravasation and lung metastasis. Others have suggested that lamellipodia and invadopodia, two independent structures when studied in 2D conditions, may indeed merge into one invasive structure, located at the cellular leading edge and be capable of multiple rounds of protrusion and retraction, when cells are cultured in a 3D matrix and in conditions *in vivo* [30].

The molecular mechanisms underlying the formation of the structures involved in mesenchymal migration are still being characterised, but common factors have now been demonstrated to be involved in both sets of protrusions. Actin polymerisation is driven by the Arp2/3 complex and its nucleation-promoting factor N-WASP or WASP have been shown to be essential components of both invadopodia and lamellipodia/filopodia[31-33]. Recently, the well characterised actin bundling factor fascin, which is known to play a key role in promoting the protrusion of filopodia, has also been characterised as an essential component of invadopodia formation[34]. Finally, the tropomyosin family of proteins is thought to play important parts in both the amoeboid and mesenchymal-type migrations having been observed in both blebbing protrusions[22] and regulating lamellipodia and filopodia[35], respectively. Each of these components will now be discussed, in terms of their biological functions towards actin polymerisation, reviewing also their aberrant expression during carcinogenesis and highlighting possible molecular mechanisms that the cancer cells will deploy to achieve such changes.

a) Arp2/3 and WASP/WAVE family

The Arp2/3 complex consisting of 7 subunits (proteins ARPC1-5 and Arp2 and Arp3) polymerizes new actin filaments from the sides of existing filaments, forming 70^{o} side-branched networks. Because of their similarity in structure to the monomeric actin molecules, it is thought that the Arp2 and Arp3 proteins cooperate to form an active dimer for nucleation of the newly branching filament[36]. At the molecular level, it is thought that all seven subunits of the Arp2/3 complex play key roles in the binding of the complex to the actin mother filaments, but only the Arp2 and Arp3 subunits contribute to the initiation of the new daughter filament[37]. Regulation of the activity of the Arp2/3 complex to bind actin filaments is controlled by cortactin, through its interaction with the Arp3 subunit [38] or the WASP superfamily of proteins. This large family which is still in the process of being characterised, is currently composed of the WASPs (WASP and N-WASP) and SCAR/WAVEs partners (**S**uppressor of **C**yclic **A**MP **R**eceptor mutation and **WA**SP and **V**erprolin homologous protein), is defined by a conserved C-terminal VCA domain. This domain is crucial for binding to the Arp2/3 complex and to the globular form of actin (G-actin), thereby recruiting all components to encourage new nucleation[37, 39]. The VCA domain is seen as the main regulatory element of WASP binding to the Arp2/3 complex and is tightly controlled by intra-molecular interactions that mask it away and prevent its interaction with other binding

partners. This direct auto-inhibition is the main regulator of the Arp2/3 promoting activity and is therefore recognised by a plethora of pathways including Rho family GTPases, phosphoinositide lipids, Src Homology SH3 domain containing proteins, kinases and phosphatases[40]. The N-terminal element of WASP family proteins is also seen as an important regulator of the biochemical activities of the VCA domains and is thought to be responsible for cellular localisation, as well to control the association with ligands. Expressions of WASP and WAVE proteins and that of the Arp2/3 complex have been shown to be altered during oncogenesis[41] and such aberrant regulation result in important changes in the overall architecture of the actin cytoskeleton, principally the lamellipodium.

Another cellular protrusion in the form of finger-like sensory and exploratory extensions which push the plasma membrane outward is the filopodia. This structure is primarily composed of parallel bundles of actin filaments (Figure 1). Their formation is regulated by a growing number of proteins including the Arp2/3 complexes [42]. Whilst it was originally perceived that the Arp2/3 complex was not required for filopodia formation, because of the absence of such branched structures in the thin finger-like structure, new experiments suggest that the complex may have important roles in the initiation of such protrusions since individual filaments of the filopodium emanate from the branching point on other filaments found in the lamellipodium[43, 44]. The actin bundling protein fascin has unequivocally been demonstrated to be a key regulator of filopodia stability.

b) Fascin

The 55-kD monomeric globular protein fascin has been shown to cross-links actin filaments *in vitro* into unipolar and tightly packed bundles [45] through 2 actin binding sites, located at the N- and C-terminal ends of the protein in what is thought to be 2 different β-trefoil domains [46, 47]. Humans express three forms of fascins, fascin-1 and fascin-2 showing the highest degree of homology, whereas fascin-3 has only a very low homology with the other two isoforms[48]. Fascin-1 (termed from now on as fascin) is found ubiquitously expressed by mesenchymal tissues and in the nervous system, whereas fascin-2 and fascin-3 are much more precisely expressed in retinal photoreceptors and in testis, respectively [49]. The role of fascin in the formation of filopodia has been a rapidly expanding field and has generated wide-ranging interests due to its involvement in cancer progression (see below). Recent investigations have shed new light onto the mechanism for its regulation. The Rac and Rho proteins have been shown to act upstream of fascin through the PAK1 pathway or the p-Lin-11/Isl-1/Mec-3 kinases, respectively [50, 51], but the main body of work highlighting post-translational modification of fascin activities has been demonstrated through the regulation of Protein Kinase C (PKC). Specific phosphorylation of serine 39 within the N-terminal actin-binding domain by this kinase results in the loss of actin bundling by fascin[52, 53], offering a possible mechanism to control fascin involvement in both physiological and disease states. The spatial localisation of fascin at the leading edge of crawling cells is important for the assembly of filopodia and the actin bundles generated through its bundling action allow the binding of the myosin motors II and V[54]. Recent work suggests that F-actin filaments bundled by fascin may be important for the regulation of Myosin X motor processivity in filopodia formation [55].

Whilst both the Arp2/3 complex, its regulator and fascin play essential functions in the control of actin polymerisation and organisation, resulting in leading edge extension at the front of a migratory cell, other similarly important mechanisms are also required to promote cellular motility. Thus F-actin filaments need to be anchored to the extracellular environment via the formation of focal complexes and adhesions [13] and this change along with the remodelling of the actomyosin network generates tensile forces. It is the generation of such tensile forces by the myosin family of proteins that drives some of the morphological and architectural reorganisations that promote cell movement. The actomyosin contractile network represents a structural complex which is spatially posterior to the lamellipodium[56] and is referred to as the lamellum. The biological mechanisms responsible for the segregation of these two cellular subdomains are not clearly understood. The tropomyosin family of proteins may be one of the factors responsible for such spatial discrimination since they regulate the recruitment of myosin motors to the actin filaments[57].

c) Tropomyosin

The tropomyosins (tpms) are thought to be mainly absent from the dynamic Arp2/3 containing compartment[58], although such concepts have been recently challenged following the observations of tropomyosin isoforms in both the lamellipodia and filopodia of spreading normal and transformed cells[35]. Originating from four distinct genes, there are today more than 40 tpms isoforms that have been discovered so far. More than 10 of these tpms isoforms are expressed from TPM1(a-TM) and TPM2 (b-TM) genes alone in vertebrate and are classified further into high molecular (HMW) and low molecular weights (LMW) tpms. Tpms are rod-shaped coiled-coil dimers actin-binding proteins that bind along the length of the actin filaments and have been implicated in the assembly and stabilisation of actin filaments[59]. Some recent advances in the field indicate that the isoforms Tm1, Tm2/3, and Tm5NM1/2 are required for assembly of stress fibres in cultured osteosarcoma cells, stabilising the actin filaments at distinct regions[60]. Tpms have also been shown to prevent ADF-cofilin or gelsolin interaction with F-actin *in vitro*, regulating also their localisation in the process, although such properties seem to be isoform-specific[61, 62]. For instance, the tropomyosin isoform Tm5NM1 promotes inactivation of ADF-cofilin and leads to its displacement from the cell periphery while another isoform, TmBr3 stimulates the association of ADF-cofilin with actin filaments, therefore promoting its localisation at the leading edge[63]. Interestingly, such properties also reflect the ability of these tropomyosin isoforms to recruit myosin II motors to the actin filaments with Tm5NM1 having a positive control over the binding of myosin II to F-actin filament whereas TmBr3 regulates its inactivity[63]. These diverse regulatory functions correlate with differential changes in cell size and shape, along with alterations in lamellipodial formation, increased cellular migration, and reduced stress fibers[63].

All in all, the cellular pathways promoting cellular motility are diverse and complex and, not surprisingly, we find that the paths to carcinogenesis are similarly varied and multiple, involving the aberrant expression of many different targets. In an effort to correlate protein expression to possible mechanisms involved in their regulation, and the biological consequences of their interactions in cellular migration, this reviews brings together some of the recent findings that have shed new light on such processes, tackling in the first instance how the levels of these specific actin binding proteins are changes in the cancer cell (Table 1).

3. Regulation of specific actin binding proteins in cancer progression

a) Arp2/3 and WASP/WAVE family

The proteins of the Arp2/3 complex play essential orchestrating functions in actin organisation. Their binding to already formed actin filaments, forming 70^{o} side-branched networks, is crucial for the modelling of the lamellipodia. Reports have shown that the metastasis process correlates with changes in the expression pattern of components of the Arp2/3 complex, although some uncertainty remains as to the degree of correlation as discussed below.

Cancer progression of gastric cells appears to result in the robust and synchronous reduction in the expression of Arp2/3 proteins, with the reduction of at least four mRNAs of the seven subunits in more than 78% of the cases[64]. Among all components analysed, the Arp2, ARPC2 and ARPC3 mRNAs, as determined by reverse transcriptase polymerase chain reaction, were found to be the most prominently reduced in cancer samples compared to their control counterparts. However, some of the gastric cancer cells and tissues had been obtained from primary tumours and no information was provided regarding their metastatic abilities. Furthermore, the experiments measured only the mRNA contents for the different components of the complex without assessing how the correlating protein levels were affected. More recent work investigating the aberrant levels of Arp2/3 proteins in pancreatic, colorectal and breasts carcinomas highlighted the elevation of the complex's proteins with the rise in invasiveness and metastatic abilities. Increased levels of both Arp2 and Arp3 proteins, measured by immunohistochemical staining correlated with the rise in atypical properties of the colorectal neoplasms[65]. This aberrant change in expression is not exclusive to the Arp2/3 proteins as other components of the complex have also been shown to be affected during carcinogenesis. Expression pattern of the ARPC2 subunit has been reported to be increased in breast cancer cell lines[66]. The latter study provides further support for the role of ARPC2 as a sole promoter of cellular invasion, since knockdown of its expression using siRNA was sufficient to attenuate SK-BR3 breast cancer cells incursion into Matrigel. Independently other components of the Arp2/3 complex have been shown to be equally important in the pathogenesis of the head and neck squamous cell carcinoma, when the expression of the ARPC5 subunit at both the mRNA level and protein level were significantly up-regulated in malignant invasive cells and tissues compared to the control counterparts[67]. These

observations were further substantiated using human head and neck squamous cell carcinoma lines, that showed that high levels of ARPC5 expression resulted in both higher rates of cellular migration, cellular invasion, and to a certain extent cellular proliferation. When ARPC5 levels were specifically down-regulated in these cells, using siRNA, all these properties were significantly diminished when compared to the mock transfected control cells. Both results demonstrated the direct influence of ARPC5 on these pathways.

It is unclear why such contradictory patterns of expression have been observed between the different studies. Studies using immunohistochemical analysis as only way to assess the levels of proteins may themselves have short-coming, as they do not offer a satisfying quantitative measurements of the real concentration and can further be influenced by the localisation of the proteins. Furthermore, besides the potential explanation that the different results are related to the different origins of the samples and tissues, there is also the more pertinent possibility that levels of the Arp2/3 proteins may be up-regulated at a specific time and/or indeed be critically required for the enhancement of invasive properties. Direct demonstrations using knockdown experiments above highlighted the importance of the expression of both ARPC5 and ARPC2 in enhancement of both cellular migration and invasion. It is therefore reasonable to suggest that invasive cells could gain an important selective advantage by aberrantly and timely up-regulating the expression of such proteins, whilst other tumour cells which fail to modulate such control will remain in their original environment.

This suggestion is further supported by studies that aim to link directly the Arp2/3 complex and cell invasiveness. The subunits ARPC3 and ARPC5 have been shown to be expressed at high levels in the expression profiling of invasive subpopulations of MTLn3-derived or Polyoma Middle T oncogene (PyMT)–derived mammary tumor cells selected *in vivo*[68, 69]. In both instances, cells that demonstrated an ability to invade into adjacent tissues had up-regulated the expression of the Arp2/3 complex components as well as molecules such as cofillin, to coordinate the activation of motility pathways.

Moreover some degree of correlation has now been reported between the increased expression of Arp2 proteins, Arp3 proteins , cortactin and fascin and the tumour depth of invasion in gastric carcinoma[70], or between components of the Arp2/3 complex and their activators, when significant up-regulation of WAVE2 and Arp2 are reported in breast cancer samples[71, 72] and in colorectal carcinomas [73]. Altogether, it is therefore tempting to speculate that although the expression of single components of the Arp2/3 complex may play an important role to promote cell migration away from the primary tumour, they may not in their own right be sufficient, and that concomitantly other actin binding proteins may also need to be specifically targeted.

The importance of WASP and WAVE in the regulation of cancer invasion has been the topic of excellent recent reviews[12, 41, 74] and will only be briefly discussed here. WASP/WAVE family of proteins play key functions in the regulation of the activity of the Arp2/3 complex, acting via the VCA region, to act as a scaffold to promote interactions between the complex and actin[37]. As a result, they are necessary for the cell protrusive activity that is associated with cell migration and invasion. Whilst WASP proteins have been shown to be directly responsible for the formation of invadopodium in carcinoma cells[29], WAVE components appear to regulate formation of lamellipodia and membrane ruffles as well as that of

filopodia[75-77]. Reports have highlighted their possible implication with metastasis, as their expression encourages cells to migrate away from primary tumours. Thus recent work has shown that N-WASP activity is required for invadopodia *in vivo* and promotes some of the initial invasive steps of metastasis[29]. Not surprisingly protein levels of N-WASP have been shown to be increased in esophageal squamous cell carcinoma. This increase has been correlated with lymph node metastasis and pathological staging[78]. Similarly, WAVE proteins have also been found to be elevated in different cancer tissues. Thus a correlation between elevated levels of WAVE3 and advances in breast cancer progression has been highlighted[79]. Furthermore when WAVE3 is down-regulated using siRNA in MDAMB231 cells, the resultant cells show an inhibition in cell motility and invasion, suggesting that WAVE3 may be a significant element in tumour cell migration[80]. Similar observations were made following immunohistochemical staining for WAVE3 in prostate tumour sections or prostatic cancer PC-3 and DU-145 cell lines[81]. Once again, reducing WAVE3 to the more basal levels of non-tumorigenic cells led to a much reduced invasiveness, as quantified through cell penetration of the basement membrane, without affecting growth or matrix adhesion. In parallel WAVE2 transcription (mRNA and proteins) was reported to be at high levels in node-positive cases as well as in moderately and poorly differentiated breast tumours and it correlated with a poor prognosis[82].

The story is, however, not as clear cut as first thought, since more recent reports have now also indicated that low expressions of N-WASP or WAVE can also reflect a poor outcome. For instance N-WASP has been reported to act as a tumour suppressor gene both *in vitro* and *in vivo* using human breast cancer cell lines and tissues[83]. Both protein and mRNA levels, determined by immunohistochemical staining/Western blots analysis and quantitative PCR, respectively, on freshly collected breast tissues, revealed that cancer tissues presented much lower levels of expression of N-WASP than their control counterparts and that ectopic overexpression of N-WASP could significantly reduce motility and invasiveness of MDAMB231 cells *in vitro*, as well as reduced tumour growth in animals. However the ability of N-WASP overexpressing MDAMB231 cells to form secondary tumours and metastasis was never tested in this work, providing no further details as to the potential invasive properties of this protein. The same group reported that WAVE1 and WAVE3 transcripts were not increased in node-positive cases, as well as in moderately and poorly differentiated breast tumours[82].

b) Fascin

Numerous reports link fascin to cancer progression. Importantly, fascin expression has now been reported to be associated with invasion of epithelial tumour cells and clinically aggressive tumours (see references herein and recent reviews[48, 84]). Fascin expression, revealed by immunohistochemical staining, suggests that this protein is increased in dendritic cells and tumour epithelia in thymomas and thymic carcinomas [85], as well as in endometrioid carcinoma [86], pancreatic adenocarcinoma [87] and hepatocellular carcinoma [88]. In the latter work, cortactin expression was also up-regulated along with that of fascin. The increased expression of fascin during cancer pathogenesis is not merely coincidental since when expression of fascin is induced using plasmid transfection in pancreatic tumour cells[89]

or oral squamous cell carcinoma[90] motility and invasion of the transfected cells are increased. This up-regulation of fascin was mirrored by important increases in F-actin-based structures like filopodia and lamellipodia. The inverse experiment, where levels of fascin are down-regulated, also provides evidences of it playing a key role in invasion. Thus when fascin levels are depleted in melanoma CHL1 cells or MDAMB231 breast adenocarcinoma cells by siRNA the resultant cells showed a reduction in invadopodia[34]. The mechanisms responsible for the promotion of invasion by fascin are still not fully understood but essential elements have recently been provided. It appears that the actin bundling properties of fascin are key for formation of invadopodia since expressing a form of the protein that has lost its actin bundling activities, following knockdown of the endogenous protein, failed to restore such migratory characteristics in CHL-1 or A375MM cells[34].

c) Tropomyosin

Because of their important role in actin organisation and anchorage-independent growth, tropomyosins (tpms) have been classified as tumour suppressors [91]. Reduced levels of both tpm1 and tpm2 have been reported in tumorigenic cells thus highlighting their roles as core components of cell transformation[92, 93]. More recent work also indicates that reduction or loss of tropomyosin correlate with tumours that invade and/or metastasise in breast, prostate, bladder and colon cancer, possibly through its regulatory function on the assembly of stress fibers[94-96]. Thus for example a marked reduction in tpm1 was found in metastatic breast MDAMB231 and colon SW620 cancer cell lines[96]. Moreover when tpm 1 was overexpressed in MDAMB231 cells, the level of stress fibers formation was increased and this correlated with a reduction in actin ruffles at the leading edges and a loss of cell motility[96]. Similarly, Tm5NM1, a low molecular weight isoform from the TPM3 gene, inhibits both the mesenchymal to amoeboid and amoeboid to mesenchymal cell transitions, as a result of stabilisation of actin filaments and inhibition of cell migration in a 2D culture system[97]. Logically when Tm5NM1 was reduced the resultant cells were seen to increase significantly directional persistence, presumably through a greater formation of focal complexes[98]. From this information, it is probable that progression to metastasis by certain primary tumours may require the down-regulation of specific tpms but reports providing such information are, to the best of my knowledge, not currently available. In fact most of the information linking tropomyosins proteins to carcinogenesis presents them as potential promoters of cellular invasions. Thus tpm3 has been shown to be highly expressed in malignant breast tumour cells found in lymph nodes[99]. Furthermore, chromosomal translocation of the tpm3 gene to other DNA regions has also been reported to lead to carcinogenesis. Thus examples of the fusion of the tpm3 gene to the anaplastic lymphoma kinase ALK gene, resulting in the chimera TPM3-ALK has been linked to inflammatory myofibroblastic tumours [100] and anaplastic large cell lymphoma[101], whereas fusion of the tpm3 gene to the T*RK* kinase gene leads to human thyroid papillary carcinoma[102]. In all these cases, a direct role for tpm3 as an oncogene has always been in question since it could potentially act indirectly by promoting dimerisation/ multimerisation which would be sufficient to lead to activation of the associated kinase protein. A recent report however, demonstrates the presence of elevated levels of both tpm3 mRNA and protein in human hepatocellular carcinoma when compared to the adjacent non-

tumour liver tissue[103]. A significant correlation was also seen between elevated tpm3 levels and poor recurrence-free survival [103]. Much remains to be learned about the role of tpm3 during tumorigenesis, since it is currently unclear if tpm3 is solely responsible for all of the observations reported or happens to be a coincidental partner expressed during cancer progression.

All in all, the roles of the different actin binding proteins listed here in carcinogenesis have been studied for many years, but uncertainties remain as to how they are involved and their biological consequences. The challenge now is to comprehend, at the cellular level, how different mechanisms may be diverted towards a single goal. Thus studies that concentrated on the expression of a solitary protein may therefore have been blinded from others changes that had taken place. A more globalistic approach, that has been embraced over the last few years to monitor changes in cancer cell progression, will in turn provide a much greater understanding of the different regulatory events responsible for the occurrence of metastasis. The ramifications of such comprehension may lead us to identify overlapping regulatory pathways that may be affected by cancer cells to alter the expression of specific proteins.

4. Possible mechanisms high-jacked by cancer cells to regulate the expression of actin binding proteins

A plethora of work has now highlighted the differential expression of actin binding proteins during carcinogenesis and acquirement of the metastatic state. It is, however, unclear as to how these protein levels are controlled both in terms of their global cytoplasmic expression and specific subcellular localisation. Indeed comparative genomic versus proteomic studies have indicated that mRNA expression is not always a good predictor of the changes in protein levels in eukaryotes[104]. Therefore different mechanisms acting post-transcriptionally may have been established to control gene expression and to regulate the levels of cellular proteins and these will be discussed in regards to the specific actin binding proteins that have been linked to cancer progression.

MicroRNAs (miRNAs or miR) are a class of naturally occurring small (20-25 nucleotides) non-coding RNA molecules that have been shown to have critical roles in the regulation of gene expression, resulting in important control of biological and metabolic processes such as cell growth, differentiation, cell maintenance and cancer[105, 106]. The precursor miRNAs are initially transcribed by RNA polymerase II and further processed by RNAse III Dorsha and DGCR8. They are then exported by exportin 5 to the cytoplasm, where they will be converted into an active form by Dicer. Their post-transcriptional functions are exerted through the complementary binding of 3'-UTR (untranslated region) of target mRNAs, resulting in either their degradations or their blocks in translation[107]. Numerous lines of evidences indicate that miRNAs also play vital functions in tumorigenesis and their expressions is aberrantly regulated in several types of human cancers[106]. Certain miRNAs such as miR-373 and miR-520c have been classified as metastasis-promoting factors[108, 109] while others play a role in inhibiting tumour invasion and metastasis. Indeed numerous studies have reported that miR-145, miR-143 and miR-133a/b play a tumour-suppressive role in various cancers and are

consequently down-regulated in the miRNA expression signatures of various human malignancies [110-112113, 114]. Their regulation has highlighted their importance in controlling specifically the levels of different actin binding proteins and hence they are discussed here (Table 2).

a) Arp2/3 and WASP/WAVE family

Studies of the Arp2/3 complex and its binding partners have significantly improved our understanding of the mechanisms that are in place in cells to modulate their actin polymerisation activities. Work presented so far in this review, also reveals that protein levels of the Arp2/3 complex are seen to be elevated during tumorigenesis in the great majority of cancers studied, but much remains to be discovered to explain how such increases are attained. A few new perspectives have now been put forward. Using head and neck squamous cell carcinoma cells and a genome wide gene expression analysis, Kinoshita *et al.* have now demonstrated that mRNA for the subunit ARPC5 is a target for miR-133a [67]. Whilst ARPC5 is seen as a marker of invasion and is significantly overexpressed in head and neck squamous cell carcinoma, silencing its expression by siRNA led to reduced migration and invasiveness. Interestingly, enhanced expression of miR-133a specifically down-regulated levels of ARPC5 and also reverted the invasion and motility of the cells to a more wild type phenotype. Recent data has revealed that miR-133a functions as a tumour suppressor and its down-regulation plays a critical part during the progression of different tumours of the bladder or esophageal squamous cell carcinoma[115]. It is therefore tempting to speculate that such increase in tumorigenesis may in fact be linked to the enhanced expression of ARPC5 thereby causing an increase in invasiveness.

Other miRNAs have also been reported to down-regulate specifically the expression of other subunits of the Arp2/3 complex, albeit not in tumour cells. ARPC3 has been identified as a target for miR-29a and miR-29b in primary hippocampal neurons and mouse N2A cells[116], whereas miR-129-3p specifically reduces the level of Arp2 in human retinal pigment epithelial cells[117]. Although there is currently no information linking miR-29a and miR-29b in the progression of cancer, levels of miR-129-3p are affected by DNA hypermethylation in primary gastric cancers, resulting in reduced expression and correlating to poor clinic-pathological features[118]. Direct connections have yet to be made between expression of miR-129-3p and Arp2 levels in cancer cells and tissues, but these connections highlight another possible regulatory mechanism that cells could initiate en route to full carcinogenesis. More work in this field is therefore required to establish new links between these observations and the importance of Arp2/3 in metastasis.

One needs to be mindful that posttranscriptional regulation of the expression of certain subunits of the Arp2/3 complex is not necessarily the only method to achieve such elevated levels. Other mechanisms are also being put forward to explain this phenomenon. Possible mechanisms for the overexpression of subunits of the Arp2/3 complex may also be linked to amplification of specific DNA target genes. Indeed, fluorescent *in situ* hybridisation of pancreatic cancer cell lines and primary tumours has revealed an increase in copy number of an amplicon core region containing the ARPC1A gene, this demonstrates a significant correlation between amplification and elevated levels of expression of ARPC1A[119].

Although much remains to be discovered regarding the regulatory mechanisms that govern the expression of the family of WASP proteins in tumorigenesis, some recent findings are starting to shed light on this process. As we have seen, correlation between expression levels of WAVE3 and breast cancer progression have been highlighted, indicating that this protein may act as a key inducer of metastasis. A link between its expression and that of specific miRNAs, miR-31 and miR-200 have now also been recently documented[120, 121]. Thus an inverse correlation between expression of WAVE3 and that of either miR-31a or miR-200 has been reported with WAVE3 levels increasing as cells underwent EMT while both miR-31 and miR-200 levels were found to be reduced. These observations were seen to be more than mere coincidence since miR-31 and miR-200 were shown to target specifically a portion of the 3'-UTR of WAVE3 mRNA, and this resulted in a significant reduction of both its mRNA and protein, whereas its targeting had no effect on either WAVE1 or WAVE2 mRNAs. This initial descriptive observation will need to be characterised further to identify whether such regulation helps to trigger the progression of a tumour in to a more malignant state.

b) Fascin

As previously discussed, clear evidence has now demonstrated that elevated level of fascin is associated with poor prognosis and corresponds to changes in various tumours to more aggressive phenotypes (Table 1). Interestingly, increases in expression may be tightly linked to the process of metastasis, at least in human colon carcinomas, since levels of fascin appear to return to more basal levels once cells reach their destination where migration ceases and proliferation is enhanced[122]. Recent work has therefore been aimed at understanding how the elevated expression of fascin is regulated in such a precise and clockwork-like manner when it is required most, since experiments have shown that enhanced expression of fascin is solely capable of inducing cellular migration *in vitro* using colonic non-invasive carcinoma lines[123]. The regulatory mechanisms that could explain such observation are still currently lacking, although activation of pathways involving Insulin Growth Factor -1 (IGF-1) or Tumour Necrosis Factor alpha (TNF-α) have been shown to up-regulate specifically the expression of fascin in breast and bile duct carcinomas [124, 125], however the direct mechanisms need to be identified in greater depths. Alternatively recent reports over the last few years have established a link between fascin expression and that of specific miRNAs, mainly miR-133a/b, miR-143 and miR-145 in prostate, bladder, esophageal squamous and in breast cancer cells [111-113, 126, 127]. Interestingly, most of these miRNAs have also now been shown to be specifically regulated in carcinogenesis[110-113114] (see earlier comments). Indeed the miR-145 cluster is located in 5q33, a region of the genome that has been shown to be frequently altered in cancers cells through chromosomal deletions, epigenetic changes and aberrant transcription. Experiments using the breast cancer cell line MDAMB231have further directly implicated the role of miR-145, since overexpression of miR-145 was shown to reduce dramatically the levels of fascin protein and coincidentally led to much reduced cellular invasion capabilities[128]. The inverse experiment also demonstrated that when miR-145 levels were lowered, using specific anti miR-145 oligonucleotides, invasive abilities were enhanced in less invasive breast tumour T47D cell lines[128]. These properties of miR-145 do not appear

to be breast specific since similar work link it to fascin expression and invasion in the DU145 or PC3 prostate cancer cell models[111] or bladder cancer cell lines[113]. The list of newly characterised miRNAs that can specifically target fascin is likely to rise in the future as recent reports demonstrate that miR-143 and miR-133a can similarly reduce its levels [113, 126]. It remains to be elucidated however how exactly this process occurs and the biological relevance of such observations. For example is this suppressive effect directly due to the reduction in stability of fascin mRNA [111-113] or linked to the translation regulatory mechanisms that prevent the recruitments of its mRNA to the ribosomes?

c) Tropomyosins

Recently, independent experiments aiming to identify new target genes for miR-145, using comprehensive gene expression analysis, have implicated miR-145 as a regulator of tpm3 levels, down regulating its expression in esophageal squamous cancer cells and prostate cancer cells[111, 112]. Similarly, tpm3 (as well as tpm2) were identified as target genes controlled by miR-133a in head and neck squamous cell carcinoma[67]. In all these cases however, such observations were not the direct focus of the investigations. Because these miRNAs are known tumour suppressors, down-regulation of the tpm 3 isoforms could possibly be a mechanism of such suppression. Establishing the true oncogenic nature of tpm3 and whether a direct connection occurs between its expression and that of specific miR-133a and miR-145 should certainly provide a focus of future work.

The link between miRNAs and tropomyosins also appears to involve other members of their respective families. Indeed gene repressing regulatory functions for miR-21 have also been shown towards tmp1 in MCF-7 and MDAMB231 cells[129, 130]. These breast cancer cell lines express high levels of miR-21 and coincidentally low levels of tpm1, one of the potential factors that could explain some of their malignant properties. When levels of miR-21were reduced in both of these cell lines they expressed high levels of tpm1, and sole expression of myc-tagged tpm1, by transfection of an expression vector, in the MDAMB231 cells was sufficient to reduce invasive capacity[129]. Interestingly, such regulations of tpm1 levels was shown to be exerted at the translational level, presumably through inhibition of the recruitments of tpm1mRNA to the ribosomes since its mRNA levels were unchanged[130]. Links have therefore now been initiated between tropomyosin isoforms and their regulation post-transcriptionally through specific miRNAs. This avenue of research is in its infancy, since such observations are currently mainly coincidental but they may prove to be biologically relevant and possibly key in the route to metastasis.

d) Regulation of expression of specific actin binding proteins through localisation of their mRNAs at the leading edges of migratory cells

Work in different organisms has now clearly demonstrated the importance of mRNA localisation of target proteins to the protein's sites of function and their translation *in situ*. Thus localisation of β-Actin mRNA near the cellular leading edge promotes cell motility[131] since its delocalisation leads to a random distribution of the protein, as well as casual spatial

arrangement of the barbed end filaments and their nucleation sites[132]. Recent work has also indicated that localising actin mRNA at the leading edge is not a sole requirement since mRNAs encoding core components of both the lamellipodium and focal adhesions may also be selectively targeted to this region. Indeed, through the use of both fluorescent *in situ* hybridisation and tyramide-signal amplification, mRNAs for the seven members of the Arp2/3 complex were found to be localised to protrusions in fibroblast cells[133]. As for the displacement of the β-actin mRNAs to non-physiological subcellular regions, recent work has shown that preventing the proper localisation of the Arp2 mRNA to the leading edge leads to narrow cellular protrusions and loss of directionality[134].

Similarly localisation of α-actinin mRNAs to the leading edge has been shown to be critical for the proper regulation of the assembly of focal adhesion sites and migration[135]. Experiments looking at mRNA localisation in highly invasive MDAMB231 cells have shown that α-actinin mRNA levels are remarkably low at the leading edge, presumably correlating with low amount of proteins, this low level results in small and largely un-matured focal adhesions [135]. Moreover when the localisation of α-actinin mRNAs at actin-rich cellular protrusion was elevated, through the increased expression of the **Z**ipcode **B**inding **P**rotein 1 (ZBP1, discussed further below), increased size and greater levels of mature focal adhesions were produced, suggesting a link between mRNA spatial organisation of α-actinin and assembly of focal adhesions.

It is therefore conceivable that proteins whose function is to target specific mRNAs to precise regions in cells could therefore "indirectly" regulate the direct functions of such proteins. One such factor is ZBP1, a primarily cytoplasmic 68 kDa protein, which contains several recognizable regions, including two RNA-recognition motifs, four hnRNP K homology domains, as well as potential nuclear localization and export signals [136]. ZBP1 binds to specific mRNAs through the recognition of cis-acting elements usually found in the 3'-UTR[136] (and reviewed in[137]). mRNAs for components of the Arp2/3 complex and α–actinin have been shown to bind to ZBP1 proteins in MTLn3 breast cancer cells in microarray experiments[138]. High expression of ZBP1 results in the localisation of their mRNAs at the cell leading edge and as a result is directly implicated in decreased turnover of focal adhesions[135]. This in turn leads to loss of the metastatic potential of a cell line derived from breast tumours[68]. Conversely, when ZBP1 expression is repressed, this change not only increases cell migration, but also promotes the proliferation of metastatic cells[138]. Coincidentally, or possibly strikingly, it appears that the same cells that boost the levels of the Arp2/3 complex proteins are also the ones that reduce the amount of ZBP1 on their route to metastasis[68, 69]. All together these reports indicate that the steps when ZBP1 is down-regulated and the expression of specific actin binding proteins is simultaneously increased may be critical in metastatic progression. This step could control both structural regulation of the leading edge and cell polarity along with assembly of focal adhesions and their stability by spatially regulating the translation of both the mRNAs discussed as well as others potentially relevant to motility. To support this theory, a significant decrease in transcription activity of the ZBP1 gene has been observed in cells from metastatic tissues through methylation of its promoter region. Such changes have resulted in a dramatically silenced

expression of ZBP1 in highly invasive cells (MTLn3 and MDAMB231) compared to non-invasive cells[138]. The route to metastasis is a twisted, not necessarily unique path, that will be achieved through a series of regulatory events. It is therefore conceivable to speculate here that different mechanisms, working synergistically, will be at play to accomplish a common goal. Regulating the expression of specific actin binding proteins such as those of the Arp2/3 complex, along with that of ZBP1 may be key for such progression and future work may focus on analysing their levels in cancerous tissues and samples.

Whilst there is little doubt that transport of specific mRNAs to the leading edge will play essential roles in regulating the expression of factors involved in lamellipodia, filopodia and invadopodia, it is still uncertain as to how these targeted mRNAs will be retained at the correct subcellular site. Although the role of ZBP1 or other carrier is critical for the transport of the actin binding proteins, they do not by themselves interact directly with structures that would allow their accumulation and anchoring at specific sites. Although no factors have so far been reported for the mRNAs of either components of the Arp2/3 complex or α-actinin, β-actin mRNA has been shown to be anchored onto actin filament by the eukaryotic Elongation Factor 1 alpha (eEF1A) [139].

eEF1A, whose primary function is the delivery of amino-acyl tRNAs to the elongating chain of the newly synthesised protein of the ribosome has also been reported to have numerous other non-canonical functions; one of these functions is the remodelling of the actin cytoskeleton which occurs throughout eukaryotes[139-141]. Both mammalian isoforms, eEF1A1 and eEF1A2 have been reported to be important in carcinogenesis although not necessarily for the same reason[142, 143]. eEF1A2 has been shown to promote the formation of filopodia through the generation of phosphatidylinositol-4,5 biphosphate in both the cytosolic and membrane bound cellular compartments [144], a regulatory mechanism that appears to play an important role in acinar development and mammary neoplasia [145]. A direct connection between tumorigenesis and eEF1A1 remains elusive, but could be linked to its ability to interact with the actin cytoskeleton[142], through regulation of the Sphingosine kinase 1[146] or intracellular alkalinization-induced tumour cell growth[147]. It appears, however, that increased expression of eEF1A can lead to transformed phenotypes[148]. More recently, the levels of expression of eEF1A1 and its role during cancer progression has led to further uncertainty. Some observations demonstrate increased eEF1A1 levels as single cells acquire metastatic properties in primary mammary tumours [149] but eEF1A1 levels were subsequently found to be significantly decreased in an invasive subpopulation of Polyoma Middle T oncogene (PyMT)-derived mammary tumours, to a level similar to that of ZBP1[69]. Further work on the eEF1A protein is therefore required to shed more lights onto the potential oncogenic properties of this factor and whether its ability to spatially organise mRNAs for actin and for potential other target proteins that have hitherto not yet been identified plays some role in the metastatic process.

5. Concluding remarks

The route to carcinogenesis is a lengthy and time-consuming process, as is the road that scientists have been following in order to make sense of it all. In a mass of tumour cells,

some cells will regulate concurrently the expression of many different proteins, this is a key step required to acquire more invasive properties, thereby allowing cells to infiltrate adjacent tissues. Indeed, the penetration of the basal membrane, and the surrounding structures of this physical barrier is seen as one of the most significant characteristics of malignancy. Yet it is also one of the most challenging aspects of the cancer pathology to recapitulate *in vitro* since cell invasion requires dynamic interaction between the tumour cells, especially when considering collective migration, host cells from neighbouring and distant tissues to be invaded and the basal membrane matrix itself. Recent advances, using elegant *ex vivo* or *in vivo* techniques, such as the rat peritoneal basal membrane [33] or chick chorioallantoic membrane [150] invasion assays, respectively, have generated new avenues of research that will provide further insights in to the different steps of invasion and will allow identification of more of the important players in this process. A subset of actin binding proteins, some of which have been presented in this work, have now advanced as hallmarks for carcinogenesis and some possible mechanisms for their regulations have also been reviewed. More in depth studies on the importance of miRNAs and on localised regulation of protein expression will be needed to provide a more complete picture of the mechanisms that facilitate cellular invasion. In turn, the potentially newly-characterised factors involved in this process may prove to be targets that will allow us to understand their full potential in tumour progression.

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Legend

Figure 1: Actin organisation in migrating cancer cell

A) Staining for F-actin using Phalloidin-Alexa488 in a migrating Rama 37 malignant cell expressing high levels of S100A4. In this image, the structures of lamellipodium/lamellum and filopodium are clearly visible at the leading edge of the cell. **B** and **C** present models for the lamellipodium/lamellum and filopodium and the respective molecular organisation within, focusing on the proteins presented in this review. **B**) A simplified model for lamellipodium/lamellum formation. In the lamellipodium, free barbed ends of actin filaments recruit the Arp2/3 complex via activation by WASP/WAVE complex and cortactin. The Arp2/3 complex nucleates a new actin filament from the side of existing filaments and remains at the branching point. In the lamellum, actin filaments are bound to tropomyosins, preventing interactions with other actin binding proteins. **C**) A simplified model for filopodia formation. Individual filaments of the filopodium emerge from the branching point on other filaments, through actin polymerisation promoted by the Arp2/3 complex. Further addition of actin monomers at the barbed end of actin filaments is nucleated by the formin family, whereas fascin regulates filopodia stability through its bundling activities.

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List of abbreviations

ADF	Actin depolymerising factor
Arp2/3 complex	Actin related protein 2 and 3 complex
ARPC	Actin related protein complex subunit
ECM	Extracellular matrix
eEF1A	Eukaryotic Elongation Factor 1 alpha
EMT	epithelial mesenchymal transition
F-actin	Filamentous actin
G-actin	Globular actin
HMW	High molecular weight
LMW	Low molecular weights
miRNA or miR	MicroRNAs
PKC	Protein kinase C
siRNA	Small interference RNA
Tpms	Tropomyosins
UTR	Untranslated region
VASP	Vasodilator stimulated phosphoprotein
WASP	Wiskott-Aldrich Syndrome Protein
WAVE	WASP and Verprolin homologous protein
ZBP1	Zipcode Binding protein 1
2D	Two dimensional
3D	Three dimensional

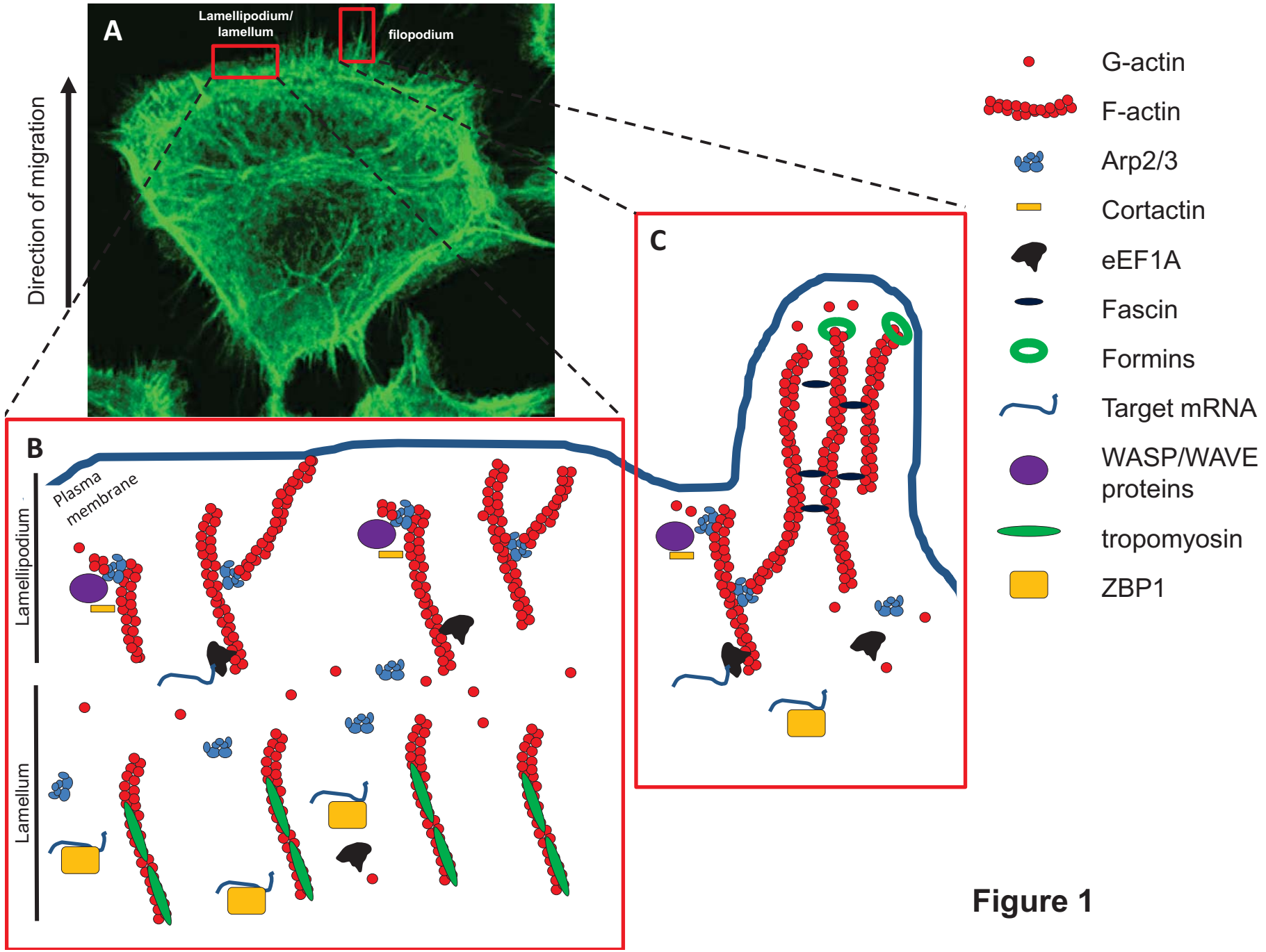
A
Lamellipodium/
lamellum
filopodium
Direction of migration
B
Plasma
membrane
Lamellipodium
Lamellum
C
G-actin
F-actin
Arp2/3
Cortactin
eEF1A
Fascin
Formins
Target mRNA
WASP/WAVE
proteins
tropomyosin
ZBP1

Figure 1

Table 1: Regulations of actin binding proteins in cancer tissue samples and cell lines

Actin binding protein affected	Level of regulation and tumour origins	References
Arp2/3		
Arp2	**Down** in gastric carcinoma	64
	Up in gastric carcinoma	70
	Up in colorectal carcinoma	65,73
	Up in breast carcinoma	71,72
Arp3	**Up** in colorectal neoplasms	65
	Up in gastric carcinoma	70
ARPC1	**Up** in pancreatic carcinoma	119
ARPC2	**Down** in gastric carcinoma	64
	Up in breast carcinoma	66
ARPC3	**Down** in gastric carcinoma	64
	Up in breast cancer cell lines	*68*
	Up in PyMT tumor cells	*69*
ARPC5	**Up** in breast cancer cell lines	*68*
	Up in PyMT tumor cells	*69*
	Up in head and neck squamous cell carcinoma	67
WASP/WAVE		
N-WASP	**Down** in breast carcinoma	83
	Up in esophageal squamous cell carcinoma	78
WAVE1	**No changes** in breast carcinoma	82
WAVE 2	**Up** in breast carcinoma	82
WAVE3	**No changes** in breast carcinoma	82
	Up in breast carcinoma	79
	Up in prostate carcinoma	81
Fascin	**Up** in thymomas and thymic carcinomas	85
	Up in endometrioid carcinoma,	86
	Up in pancreatic adenocarcinoma	87
	Up in hepatocellular carcinoma	88

Tropomyosin		
Tpm1	**Down** in breast cancer cell line **Down** in colon cancer cell line	[96] [96]
Tpm3	**Up** in breast cancer **Up** in hepatocellular carcinoma	[99] [103]
ALK-TPM3	**Up** in inflammatory myofibroblastic tumours **Up** in anaplastic large cell lymphoma	[100] [101]
TRK-TPM3	**Up** in thyroid papillary carcinoma	[102]

Table 2: miRNAs dependant mechanisms regulating the levels of actin binding proteins in different cancer samples and cell lines

Actin binding protein affected	Possible miRNA mechanisms	Tumour origin	References
Arp2/3			
ARPC5	miR-133a	Head and neck squamous cell carcinoma	67
WASP/WAVE			
WAVE3	miR31	Breast cancer cell lines	121
		Prostate cancer cell line	121
	miR-200	Breast cancer cell line	120
		Prostate cancer cell lines	120
Tropomyosin			
Tpm1	miR-21	Breast cancer cell lines	129,130
Tpm2	miR-133a	Head and neck squamous cell carcinoma	67
Tpm3	miR-133a	Head and neck squamous cell carcinoma	67
	miR-145	Prostate cancer cell lines	111
		Esophageal squamous cancer carcinoma	112
Fascin	miR-133a	Bladder cancer cell lines	113
	miR-143	Esophageal squamous cell carcinoma	126
	miR-145	Breast cancer cell lines.	128
		Prostate cancer cell lines	111

		Bladder cancer cells lines Esophageal squamous cancer cell lines	[113] [126]