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Filopodia and adhesion in cancer cell motility

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Slender bundled actin containing plasma membrane protrusions, called filopodia, are important for many essential cellular processes like cell adhesion, migration, angiogenesis and the formation of cell-cell contacts. In migrating cells, filopodia are the pioneers at the leading edge which probe the environment for cues. Integrins are cell surface adhesion receptors critically implicated in cell migration and they are transported actively to filopodia tips by an unconventional myosin, myosin-X. Integrin mediated adhesion stabilizes filopodia and promotes cell migration even though integrins are not essential for filopodia initiation. Myosin-X binds also PtdIns(3,4,5)P_3 and this regulates its activation and localization to filopodia. Filopodia stimulate cell migration in many cell types and increased filopodia density has been described in cancer. Furthermore, several proteins implicated in filopodia formation, like fascin, are also relevant for cancer progression. To investigate this further, we performed a meta-analysis of the expression profiles of 10 filopodia-linked genes in human breast cancer. These data implicated that several different filopodia-inducing genes may contribute in a collective manner to cancer progression and the high metastasis rates associated with basal-type breast carcinomas.

Introduction

Integrins are heterodimeric cell surface adhesion receptors which link the cellular cytoskeleton and signaling machinery to molecules of the extra-cellular matrix (ECM). They are a family of 24 heterodimers formed of non-covalently associated α- and β-subunits.[1] Integrins are expressed at high levels on the surface of all cell types expect erythrocytes and they are required for many physiological processes during development as well as in the maintenance of tissue homeostasis.[2] Since integrins provide cells with a connection to the ECM, integrin mediated cell adhesion is important for migration.[3,4] In addition, integrins are involved in the matrix induced assembly of large signaling platforms called focal adhesions and many signaling molecules activated by integrins are implicated in the regulation of cell motility and survival.[5] Due to their important role in these processes, altered expression of integrins has been shown to correlate with poor prognosis in human cancer.[6] While focal adhesions are widely acknowledged as signaling platforms regulated by integrins, these receptors are found also in other types of plasma membrane structures like fibrillar adhesions and filopodia.[7,8]

Filopodia are plasma membrane protrusions which have been described as "finger-like." They are formed of tightly bundled parallel actin filaments of 10 or more.[9,10] The actin filaments in the filopodia are organized in a parallel manner with their barbed ends facing toward the plasma membrane. Filament bundling is mediated by small crosslinking proteins like fascin.[10,11] The polarized nature of the actin filaments allows motor proteins to actively transport cargoes to the slender protrusions.[12] The tips of the filopodia are dense and have been described to contain many proteins, including integrins.[13] At present it is not known whether the filopodia tips also function as platforms for integrin outside-in signaling. However, this is an intriguing possibility and may underlie the important role of filopodia in cell migration, which is the topic of this review.

The classical view has been that cells use filopodia to probe the environment for cues[14] and that they function in the leading edge as pioneers. Therefore, the role of filopodia in migration is well established in many physiologically important processes like wound healing, angiogenesis, chemotaxis, embryonic development and adhesion.[11,13,15] Interestingly, integrins have been implicated all of these processes as well.

Filopodia, Integrins and Myosin-X

Integrin α- and β-subunits have short cytoplasmic domains that have been shown to interact with a multitude of proteins.[16,17] The β-tail contains two conserved NPxY-motifs known to bind proteins that contain a "band 4.1, ezrin, radixin, moesin" FERM domain. Many of these interactions are critically important in regulating integrin signaling and function in focal adhesions.[18] Interestingly, myosin-X, a motor protein involved in the regulation of filopodia,[19] also contains an integrin binding FERM-domain and it has been shown to transport integrins to the filopodia tips[13] (**Fig. 1**).

Myosins constitute a family of actin-binding motor proteins that have been associated with cell motility, vesicle trafficking and formation of actin protrusions.[20] Especially myosin-X is a strong promoter of filopodia formation.[21] Myosin-X belongs to the class of unconventional myosins and in addition to the actin motor domain it possesses three IQ motifs, a coiled-coil domain that may mediate dimerization, a PEST sequence, three PH domains, a myosin tail homology 4 domain (MyTH4) involved

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Figure 1 (See opposite page). Actin cytoskeleton, myosin-X and β1-integrins in filopodia formation. (1) β1-integrins are endocytosed from the plasma-membrane and recycled back to cell surface via tubulating actin-dependent recycling endosomes. (2) Monomeric PIP_3-unbound myosin-X is in a closed conformation. PIP_3-unbound myosin-X is transported via microtubule tracks to the plasma-membrane in small GTPase Rab7 positive vesicles. (3) Dimerized PIP_3-bound myosin-X promotes actin fiber convergence during filopodia initiation. (4) Lateral movement of β1-integrins along the leading edge could be a myosin-X driven process. (5) β1-integrins are transported to filopodia tips in a myosin-X dependent manner. Myosin-X and β1-integrins serve as a link between the cellular cytoskeleton and the extracellular matrix.

in microtubule binding and a band 4.1/ezrin/radixin/moesin (FERM) domain.[22]

Integrins are actively transported to the filopodia tips by myosin-X.[13] However, there remains some controversy whether integrin binding is required for the ability of myosin-X to regulate filopodia formation. The motor-domain of myosin-X alone is sufficient to induce the initiation of filopodia formation[23] and dorsal filopodia induced by the overexpression of myosin-X in COS-7 cells appear to be unattached to the matrix.[19] Thus, it has been proposed that myosin-X would induce filopodia in an adhesion-independent manner. In line with this, a mutant construct of Myosin-X which lacks the integrin binding FERM-domain retained the ability to induce dorsal filopodia.[19] On the other hand, filopodia induced by wild-type myosin-X are longer, more stable and depend on integrin mediated adhesion to elicit these characteristics.[13] Consistent with this stabilization, filopodia engaged in integrin mediated adhesion have been shown to induce nucleation of lamellipodia in a Rac1-dependent manner.[24] Thus it is feasible to conclude that integrins are not required for myosin-X induced filopodia initiation but contribute to the stability and most likely functionality of these protrusions, at least in migrating cells.

Mechanisms of Cell Migration

Mesenchymal cell migration relies on coordinated function of actin filament structures. Leading edge protrusions of motile cells are formed by sheet-like lamellipodia and rod-like filopodia. Lamellipodium is a meshwork of branched actin filaments assembled by the Arp2/3 complex whereas the actin filaments in filopodia are parallel and tightly bundled.[25] Filopodia originate from the lamellipodial actin meshwork[26] (**Fig. 1**). The driving force of both actin structures is the barbed-end (plus end) elongation of the actin filaments by actin polymerization toward the plasma membrane—a process that pushes the cell edge forward and is the key step in cell migration.

Actin polymerization at the leading edge also facilitates rapid movement of active integrins at the cell front (**Fig. 1**). Interestingly, these integrins have been shown to be active yet unengaged. Such a pool of integrins is ideally primed for probing the microenvironment and could function to stabilize lamellipodia embedded filopodia in migrating cells.[8] Integrins are constantly trafficked in cells and localized traffic in the protrusive cell front has been shown to contribute to motility.[27,28] In endothelial cells these trafficking integrins have been shown to be in an active conformation.[29] Furthermore, the rapid recycling of endocytosed integrins in migrating cells may be actin dependent.[30] Therefore it is possible that targeting of primed active integrins to nascent filopodia could also involve integrin traffic (**Fig. 1**). However, this remains to be investigated.

Phosphoinositides and Filopodia

Followed by a directional cue, cells polarize and form a defined front by activating Rac and phosphoinositol PI3-kinase (PI3K) at the leading edge.[31] The leading edge is characterized by an enrichment of a gradient of PI3K products: PI 3,4,5-triphosphate [PtdIns(3,4,5)P_3] and PI 3,4-bisphosphate [PI(3,4)P_2].[32] Also PI 4,5-bisphosphate, PI(4,5)P_2, has been shown to strictly localize to the leading edge in neutrophil-like cells.[33] These membrane-anchored lipids serve as docking sites for many pleckstrin homology domain-containing proteins, which selectively bind PIP_3, PI(3,4)P_2 or PI(4,5)P_2.[34] Thus, by recruiting a vast number of proteins to the leading edge PIs support cell motility toward directional cues (**Fig. 2**) and therefore inhibition of for example the PtdIns(3,4,5)P_3 metabolism leads to reduced cell motility.[35] One of the key events promoting directional actin polymerization is the formation of the lamellipodia and the preceding recruitment of WASP-family proteins (WAVE1, WAVE2, WAVE3, N-WASP and WASP) to the leading edge by PtdIns(3,4,5)P_3 and PI(4,5)P_2.[36] WASP and WAVE proteins share the verprolin-cofilin-acidic-domains (VCA-region) which binds to actin monomers and to the actin nucleation promoter Arp2/3-complex facilitating actin polymerization toward the front of the cell[37] (**Fig. 2**). WASP and N-WASP are kept in an inhibited conformation by binding to the WASP interacting protein (WIP).[38] WASP and N-WASP are activated by Cdc42 and PI(4,5)P_2 binding which opens the auto-inhibited conformation of the WIP-N-WASP/WASP-complex.[37] The open-conformation allows the binding of the SH3 containing regulator proteins to WASP and N-WASP which in turn contributes to the Arp2/3-complex activation.[38] Thus PI(4,5)P_2 functions as a critical activator of Arp2/3 mediated actin polymerization.

Filopodia originate from the lamellipodial actin meshwork. The two models of filopodia formation, convergent elongation and tip nucleation are reviewed in this issue and are therefore only discussed here briefly. The barbed ends of actin filaments are associated with elongation factors (such as formins) and are protected against actin filament capping proteins (with the help of ENA/VASP) to support constant elongation of the filaments. The growing actin filaments become parallel and clustered by actin crosslinking proteins (e.g., Fascin) (reviewed in ref. 9). Intriguing new evidence on filopodia formation shows that filopodia-like structures are also able to self-assemble without the lamellipodial core.[39] In order to form filopodia-like structures in vitro negatively charged PI(4,5)P_2 membranes and membrane-tubulating I-BAR (Inverted Bin-Amphiphysin-Rvs) proteins were needed to create membrane curvature and to recruit actin nucleation promoting factors N-WASP and Arp2/3 to the site of initial filopodia assembly.[39] A similar

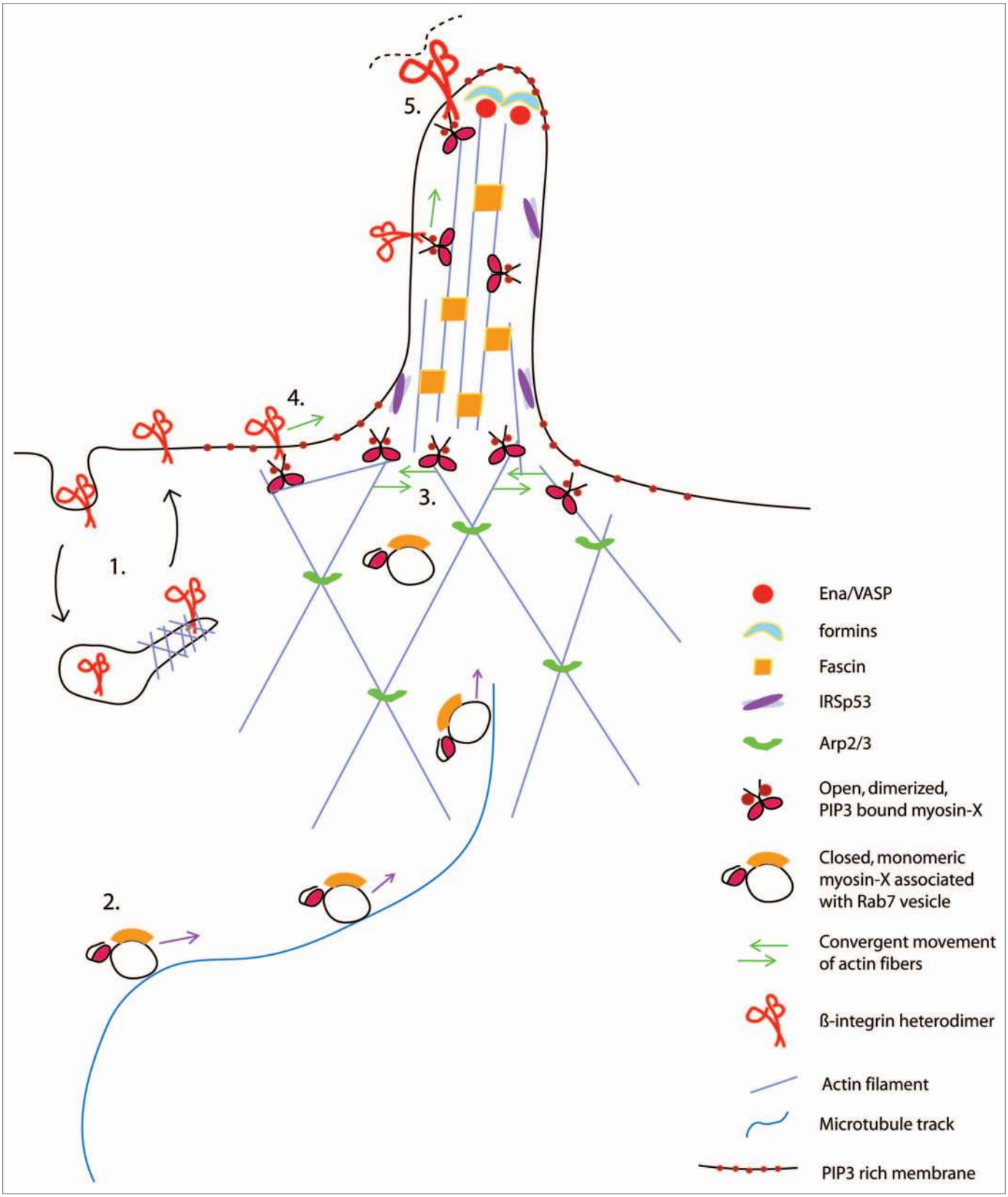

Figure 1. For figure legend, see page 422.

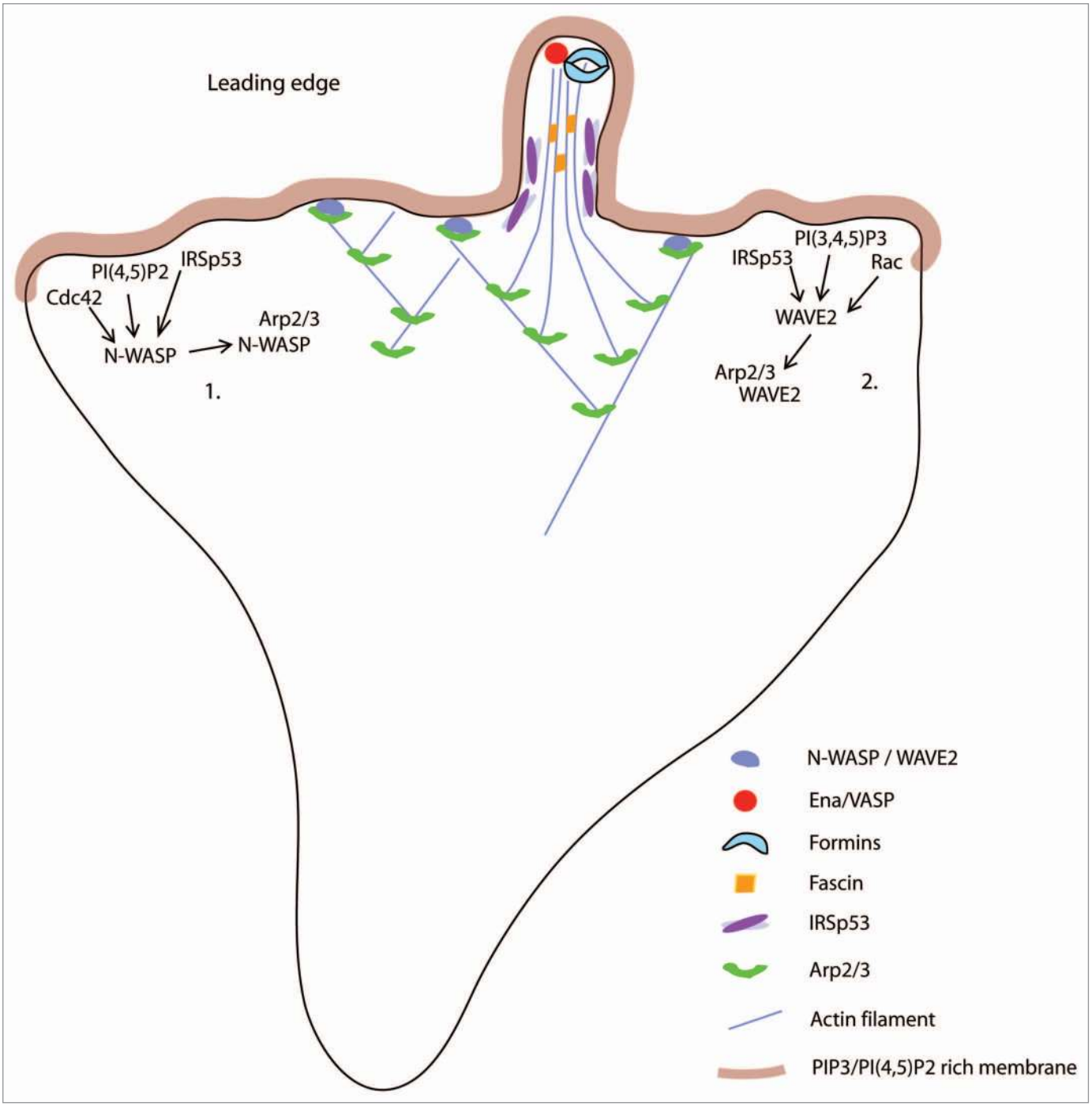

Figure 2. Phosphoinositides and filopodia. (1) N-WASP is activated by PI(4,5)P_2 and GTP-Cdc42 binding. The resulting open-conformation of N-WASP with an exposed VCA-domain interacts with and activates the Arp2/3-complex and increases the rate of actin polymerization. The binding of the SH3-domain of IRSp53 to N-WASP can also result in activation of N-WASP. (2) WAVE2 is localized to the leading edge by binding to PI(3,4,5)P_3 and IRSp53. GTP-Rac and IRSp53 both enhance WAVE2 mediated Arp2/3 activation.

mechanism has been suggested for IRSp53 to induce filopodia. IRSp53 (insulin receptor phosphotyrosine 53 kDA substrate) is a strong filopodia inducer and is composed of I-BAR, Cdc42 binding and SH3 domains.[40,41] IRSp53 can interact, curve and tubulate PI(4,5)P_2 rich membranes with the help of the I-BAR domain[42] (**Fig. 2**). The SH3 domain of the IRSp53 recruits regulators of filopodia formation (e.g., Ena/VASP, N-WASP, mDia and Eps8) to the site of membrane curvature via its SH3 domain.[41]

Eukaryotic elongation factor 1α (EF1A) family proteins recruit amino-acylated tRNA to ribosomes during the elongation phase of protein synthesis. Another biological function of the EF1A2 is to stimulate the formation of filopodia in an Akt- and PI3K-dependent manner.[43] In a more recent study, EF1A2

expression increased the plasma membrane levels of $PI(4,5)P_2$ and stimulated filopodia formation in a Cdc42-dependent manner.[44] This further supports the role of $PI(4,5)P_2$ in filopodia formation.

Although many of the filopodia tip complex proteins are unidentified, there are clearly proteins which are implicated in the formation of both lamellipodial and filopodial protrusions (e.g., IRSp53, WAVE2, Arp2/3).[39,45] This implies there might be similarities in the formation of these two different actin structures. However, filopodia can be formed in the absence of WAVE2 and Arp2/3, supporting the role of formins and Ena/VASP in filopodia formation.[46] The transition from lamellipodial actin structures to filopodia could be a very dynamic process or filopodia could have more than one way to be induced. Filopodia tips can serve as initial adhesion sites and as a nucleation core to form lamellipodia in order for cells to spread efficiently. Active Rac, Cdc42 and functional β1-integrin adhesions have been shown to be needed in this process.[24,47]

Phosphoinositides Regulate Myosin-X

As discussed above, myosin-X has the ability to move along the actin filaments.[21] The movement is directed toward the plus-end of the filament and the proposed function of myosin-X is to transport cargo to the filopodia tip. The tail of myosin-X associates with various cargo proteins such as β-integrins, Mena/VASP, VE-cadherin and netrin[13,48-50] and the transport of integrins to the filopodia tip supports filopodia elongation and cell adhesion.[13] In addition, transport of Mena/VASP to filopodia tips supports elongation by allowing them to compete with actin capping proteins.[48]

Myosin-X is primarily found at the filopodia tips and to a lesser extent in the cytoplasm.[51] Recently, Plantard et al. showed that the translocation of myosin-X to filopodia tips and the induction of filopodia formation was dependent on $PI(3,4,5)P_3$ binding via the PH-domain of myosin-X.[52] Disruption of the $PI(3,4,5)P_3$ binding of myosin-X induced a reversible cytoplasmic localization of Myosin-X in Rab7-positive endosomal vesicles (**Fig. 1**). Swapping of the myosin-X-PH2-domain with PH-domains from Btk, PLCσ1 or TAPP1 (known to specifically bind $PI(3,4,5)P_3$, $PI(4,5)P_2$ and $PI(3,4)P_2$, respectively) indicated that binding of $PI(3,4,5)P_3$ and $PI(4,5)P_2$ promoted myosin-X localization to filopodia tips. In contrast, insertion of the $PI(3,4)P_2$ binding PH-domain of TAPP1 did not rescue the myosin-X localization.[52] The myosin-X and Rab7 positive vesicles were found to move to close proximity of the plasma membrane along microtubule tracks. Thus, the trafficking of myosin-X to the sites of filopodia initiation could be guided by these vesicles. Rab7 did not colocalize with myosin-X at the filopodia tips indicating that there is probably a transition to actin-bound myosin-X before filopodia induction.[52] Interestingly, $PI(3,4,5)P_3$ has been shown to be enriched at the filopodia tips[53] and Rab7 and myosin-X are already known to function together in another actin-dependent process, namely phagocytosis.[54]

The $PI(3,4,5)P_3$-dependent regulation of the function of myosin-X was further studied on structural level by Umeki et al. They show that the PH-domain and the FERM domain are binding to the myosin-X head in an intramolecular manner.[55] This intramolecular binding keeps the myosin-X in an auto-inhibited and folded conformation and blocks the dimer formation of myosin-X. $PI(3,4,5)P_3$ binding to the PH-domain opens up the conformation and allows the dimer formation of myosin-X. The dimer formation is a key step for the ability of myosin-X to induce filopodia.[23] These data together indicate that on the endosomes myosin-X is monomeric whereas the myosin-X at the filopodia tip is dimerized (**Fig. 1**). This further indicates that $PI(3,4,5)P_3$ function as an activator of filopodia formation via myosin-X, similarly to the way $PI(4,5)P_2$ supports N-WASP function in the formation lamellipodia.[38]

Clinical Relevance of Filopodia in Cancer

Controlled cell proliferation, morphology and polarity are all critical factors contributing to maintenance of tissue homeostasis. Cell migration is important for several aspects of cancer progression including metastasis and cancer angiogenesis.[56-58] Here we will discuss the existing literature regarding the cancer relevance of proteins which have been linked to filopodia formation or function.

Fascin. Increased filopodia formation has been shown to promote migration.[13] In addition, abundant filopodia have been described as a characteristic of invasive carcinoma cells. During the progression of colorectal carcinogenesis the activation of the Wnt/β-catenin signaling pathway[59] results in the upregulation of Fascin mRNA and increased the expression of Fascin and filopodia at the invasive front.[60] Fascin is a filopodial actin bundling protein which is evolutionarily conserved. It regulates filopodia formation in cells[10] and Fascin1 expression stimulates cell migration in vitro.[61,62] Among the filopodia regulating proteins, fascin has the strongest implications in cancer progression and metastasis to date. In correlation with the characteristics of a good biomarker, fascin is usually expressed at low levels in normal epithelium, but is upregulated in several types of carcinomas.[63,64] Thus the clinical relevance of fascin expression has been studied rather extensively. These studies have been described recently in a nice review in reference 63, and therefore only some points will be discussed here.

The function of fascin has been studied particulary in Esophageal Squamous Cell Carcinoma (ESCC) and in colon adenomas and adenocarcinomas.[65,66] In these cancer types, high expression of fascin associated with an increased risk of invasion.[66-68] In non-small cell lung cancer and breast cancer, increased fascin expression correlates with poor prognosis.[69,70] In addition, fascin is included in a gene expression signature which positively correlates with the occurrence of lung metastasis of breast cancer.[71]

Upregulation of fascin increases motility in both normal and cancer cells. Increased motility is most likely linked with the ability of fascin to bundle actin protrusions and generate structures like filopodia. In addition, invasive carcinoma cells express a specific form of actin-based protrusions called invadopodia. These share many of the same features as filopodia and recently fascin

was shown to regulate these proteolytic invasive structures.[72] Interestingly, Li et al. suggest that invadopodia represent invasive filopodia but also display dynamics of actin comets.[72] In addition, a group of tumor-suppressive micro-RNAs (miRNAs) have been shown to target the 3' UTR of *FSCN1* (the gene encoding fascin1) and to suppress cell invasion and proliferation in a fascin dependent manner.[73]

Fascin can be linked to cancer progression in another way as well. Fascin is expressed in breast-carcinomas exhibiting the basal-like phenotype. These basal-like tumors are defined by their gene expression and more aggressive phenotype.[74,75] They are triple-negative (negative for ER, estrogen and progesterone receptors) and the basal-like phenotype has been associated with upregulation of EMT markers, such as vimentin and N-cadherin as well as downregulation of epithelial markers (E-cadherin). The relationship between fascin and EMT-markers has been studied both in primary tumors as well as in tumor cell lines. Immunohistochemical stainings of hepatocellular carcinomas (HCC) revealed, that high fascin expression at the invasive front of tumors correlated with low E-cadherin.[76] Similar results were obtained in breast cancer cell lines upon fascin1 overexpression in vitro.[77] Furthermore, ectopic expression of fascin1 associates with elevated expression of the *SNAIL2* gene.[76] However, it is not entirely clear if fascin promotes EMT or whether increased expression of fascin coinsides with invasion and metastasis through other mechanims. In fact, in colon cancer, expression of fascin in the primary tumor correlates with cancer spread but fascin expression is not detected in the metastasis themselves.[60]

Formins and Rif. Formins are a group of 15 Rho GTPase effectors which all contain a conserved actin-polymerizing formin homology 2 domain.[78] Formins are involved in important cellular processes like adhesion, migration, cytokinesis and cell polarity.[79] They induce the formation of unbranched actin filaments by progressive barbed-end nucleation and elongation.[78] Therefore, they have been suggested to trigger filopodia formation. The most studied formin with respect to filopodia is Dia2. Overexpression of Dia2 induces filopodia and loss of Dia2 inhibits filopodia formation in melanoma cells.[80] In addition, compensatory upregulation of Dia2 in cells lacking Dia1 correlates with increased filopodia formation.[81] A member of the Rho family GTPase, Rif (encoded by RHOF), is also a potent stimulator of filopodial protrusions.[82] Its ability to induce filopodia has been shown to be independent of the small GTPase Cdc42 but interestingly dependent on Dia2.[82,83]

Formins are widely expressed in several widely used invasive cancer cell lines like MDA-MB-231 and MDA-MB-435 breast cancer cells and HT1080 fibrosarcoma cells.[84] In addition, formins are upregulated in several types of cancers.[57] For example, *FMNL1* has been found to be overexpressed especially in T cell lymphomas since high expression of *FMNL1* associated with activation of Akt as well as lymphoid malignances.[85] *FMNL2*, on the other hand, has been found to be highly expressed in colorectal cancer and especially in those tumors with increased metastatic potential.[86] Recently, a RNAi screen targeting all formins demonstrated that loss of Dia2, FMN1, FMN2, FMNL1 and FMNL2 inhibited cancer cell invasion into Matrigel-matrix.[84] Even though this study did not analyze the effect of these genes on filopodia formation, it is possible that these data are linked to filopodia-mediated motility and invasion, too.

Other proteins implicated in filopodia. Pro-angiogenic signals, such as VEGF, trigger filopodia formation in the tip cells during angiogenic sprouting.[15] Since angiogenesis is critical to enable tumor growth beyond the one cubic centimeter, mechanisms regulating filopodia formation in endothelial cells are relevant for cancer.[87] Recently, vascular endothelial cadherin (VE-cadherin) has been shown to associate with the FERM domain of myosin-X and to be transported along filopodia to nascent endothelial cell-cell contacts.[50]

Wiskott-Aldrich syndrome protein/WASP-family proteins are scaffolds that convert the signals from the small GTPases such as Cdc42 and Rac to the actin-related proteins 2 and 3 (Arp2/3) (**Fig. 2**). Based on the convergent elongation model of filopodia initiation, Arp2/3 induces the filopodia formation by promoting the branching of the actin filaments.[88,89] The members of WASP/WAVE protein family, as well as Arp2/3-complex have been connected to cancer progression. Arp2 and WAVE2 seem to have synergistic effects and their function has been linked to EGF-sensitivity. Coexpression of Arp2 and WAVE2 are predictive of a poor outcome in breast, colorectal and lung carcinomas.[90-92] Furthermore, WAVE2-Arp2/3 signaling has been proposed to be enhanced in some breast cancers and the coexpression of these proteins correlates with the overexpression of HER2.[93] In addition, increased expression of either gene positively correlates with increased size of the tumor and venous invasion and a shortened mean survival time in hepatocellular carcinoma.[94,95] These proteins also have a function in the progression of ESCC by promoting lymph node metastasis.[96]

ENA/VASP family of proteins takes part in actin filament elongation by recruiting the actin nucleating factors such as Arp2/3 and profilin at the sites of active actin assembly.[97] ENA/VASP-family proteins typically localize to focal adhesions, the leading edge and tips of filopodia[98] and they display anti-capping activity which enables continued actin filament elongation. Myosin-X has been shown to transport VASP to the filopodia tip.[48] Mena, a member of Ena/VASP, is upregulated in the invasive and metastatic populations of breast cancer cells[99] and it can be alternatively spliced to an invasion promoting isoform named Mena (INV). The observation that Mena (INV) sensitizes cells to EGF and increases the matrix degradation in tumor cells, links Mena expression to increased incidence of distant metastasis.[100,101]

Gene Expression Profiling of Filopodia-Associated Genes in Breast Cancer

Enriched numbers of filopodia have been connected to increased invasiveness, aggressivity and decreased survival rate in various types of cancer. Above we have discussed several filopodial proteins and their relevance to cell migration and cancer. Out of interest, we decided to take these proteins and hyaluronan synthase 3, which has also been shown to regulate filopodia[102] and investigate whether their gene expression would correlate with

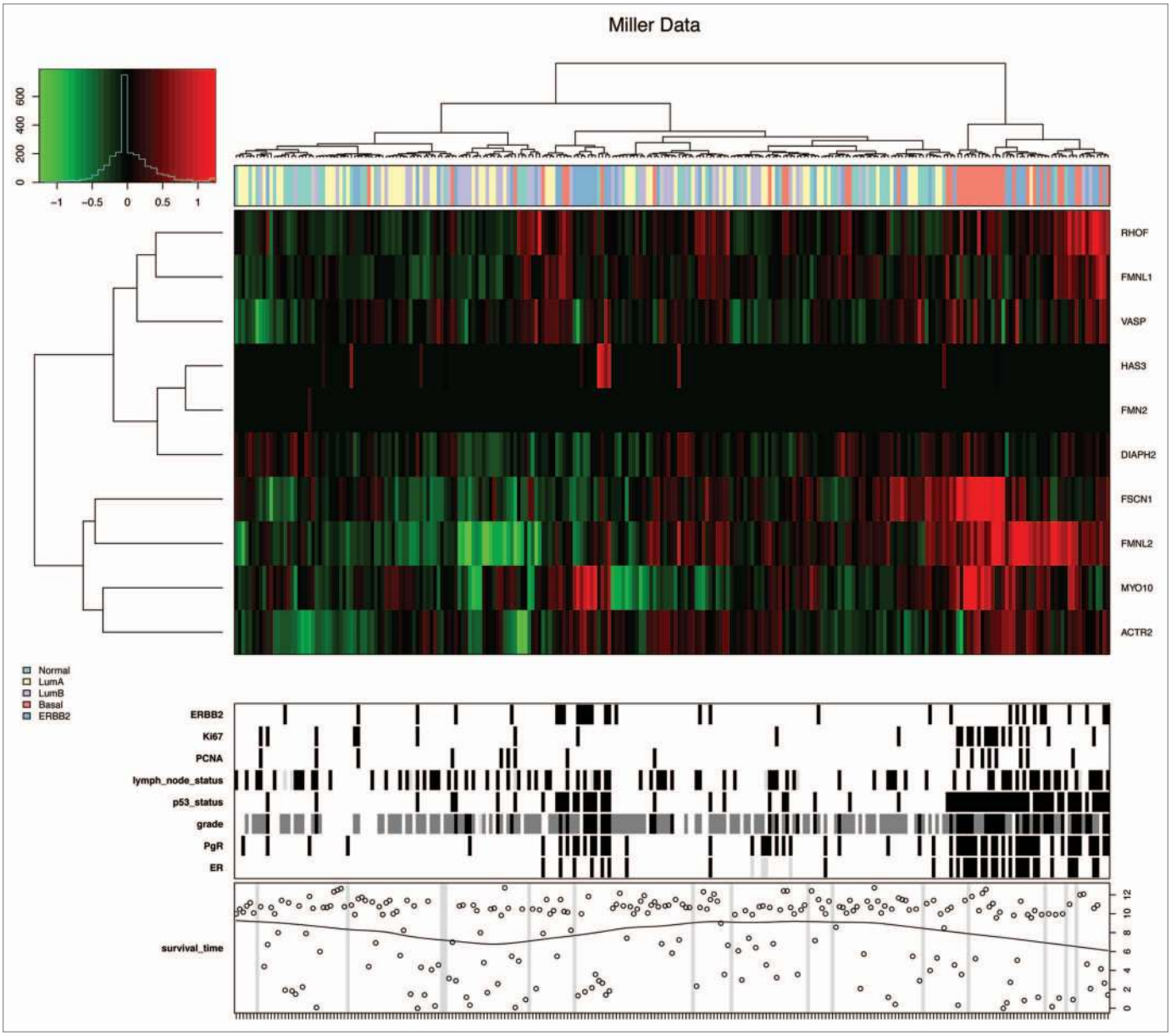

Figure 3. Filopodia-associated genes are upregulated in breast carcinomas with a poor prognosis. In silico transcriptomics analysis of a set of filopodia related genes discussed in this review. Unsupervised hierarchical clustering of the expression levels of 10 filopodia related genes in 251 breast tumors. Each cell in the cluster (middle part) shows the log2 expression ratio for the particular gene in separate tumor samples divided by the median expression of that gene in all samples. Red indicates expression above the median; green, below the median. Upper part: color-coded tumor-type classification (see the legend) of each sample. Lower part: clinicopathological parameters related to each sample. Black bars indicate high ERBB2 expression, high K_i-67 expression, high PCNA expression, lymph node positivity, presence of p53 mutation and PrR and ER negativity. Tumor grade is indicated with white (grade 1), gray (grade 2) or black (grade 3) bars in the row adjacent to the label grade. Patient survival is blotted in the bottom part. Numbers on the right indicate survival in years. The survival time panel displays for each sample the documented survival time. In addition a curve is fitted through all the available survival dots. The genes included encode for the following proteins: RHOF (Rif), FMNL1, VASP, HAS3 (hyaluronan synthase 3), FMN2, DIAPH2 (Dia2), FCSN1 (fascin1), FMNL2, MYO10 (Myosin-X) and ACTR2 (Arp2).

clinicopathological profiles in breast cancer. We applied meta-analysis of these genes in a previously published breast cancer gene expression analysis.[103] Transcript profiles of 251 primary breast tumors were assessed in comparison with clinocopathological variables: Tp53 mutation, K_i-67, PCNA, ERBB2, estrogen receptor (ER), progesterone receptor (PrR) and lymph node status; tumor grade; and patient survival (**Fig. 3**). In addition, tumors were divided into previously defined cancer subtypes: normal, luminal A, luminal B, basal type and ERBB2 positive.[104] By using unsupervised hierarchical clustering of gene expression data of these filopodia genes, a subgroup of the basal-type tumors (indicated in red in the colored bars at the top, **Fig. 3**) associated with the aggressive clinopathological characteristics formed a separate cluster from the rest (**Fig. 3**). Even though

this is merely a bioinformatic exercise with a sample set of filopodia related genes, these data implicate that several different filopodia inducing genes may contribute in a collective manner to cancer progression and the high metastasis rates associated with basal-type breast carcinomas. However, this remains to be investigated further.

Concluding Remarks

Invasion of cancer cells into the surrounding tissue is a prerequisite for cancer spread. To date, numerous cellular components like proteases, receptors, kinases and components of the cytoskeleton have been attributed to increased invasion in vitro. In many cases these data have been followed up with analysis of clinical samples demonstrating a positive correlation between such factors and poor prognosis of the patient. In this review we have described some of the proteins involved in the formation of filopodia and their potential roles in cancer. It is evident from the emerging literature that many filopodia-inducing proteins are also implicated in some cancer types. However, the picture is far from complete and many important questions remain. These include at least the following: What is the exact role of filopodia in cancer cell invasion? Are the clinically relevant lipid kinases and phosphatases (Like PI3K and PTEN) linked to filopodia formation? The current literature discussed above suggests filopodia may be important for migration out from the primary tumor, for degradation of the basal lamina or for intravasation. However, it is possible that filopodia induction is required only very transiently, and is later shut down during the formation of metastasis.[60] At present, there are no clear links between for example PTEN and filopodia, however since the activity of myosin-X is critically regulated by the binding of $PI(3,4,5)P_3$ to the protein and treatment of cells with a PI3K inhibitor inhibits myosin-X induced filopodia formation,[52] it could be speculated that the clinical relevance of lipid phosphatases and kinases could be linked to filopodia formation as well.

Taken together, big advances have been made on the molecular level in improving our understanding of the regulation of filopodia formation in cells. Several proteins are now known to contribute to the formation of these actin-based structures. Furthermore, phosphoinositide-phosphates are emerging as important regulators of filopodia, at least in the case of myosin-X induced protrusions. Hopefully, in the future these in vitro findings can be taken further to increase our understanding of the molecular mechanism of cancer dissemination in vivo.

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