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WASP and WAVE proteins: Vital intrinsic regulators of cell motility and their role in cancer (Review)

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Abstract. Cell migration is critical during the metastatic spread of cancer cells. Metastases, rather than primary tumours, are responsible for most cancer-related deaths. Invasive cancer cells acquire a migratory phenotype which is associated with an increased expression of several genes involved in cell motility. Actin, which is the most abundant protein in most eukaryotic cells, is necessary for whole cell locomotion. Reorganisation of actin filaments is regulated by a highly integrated signalling cascade governed by 'molecular switches' which belong to the Rho GTPase family. WASP family proteins are downstream molecules which form a link between the GTPases and the actin cytoskeleton. The WASP family includes 5 members and is structurally divided into 2 groups: Wiskott-Aldrich Syndrome proteins (WASPs) and WASP verprolin homologous proteins (WAVEs). Current evidence suggests that WAVEs are crucial for cell motility and metastasis. This is a review on the possible role of WAVEs in cancer and the clinical associations found in human cancer.

Contents

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1. Introduction

Cancer metastasis and cell migration. Migration of cancer cells is critical for metastasis, from the first stage to the final one where metastatic cancer cells settle in a secondary organ. The ability to migrate and invade tissues allows cancer cells to proliferate within tissues (growth), expand to adjacent tissues (invasion), and travel to distant organs (metastasis) (1). Metastases, rather than primary tumours, are responsible for most cancer deaths (2). Tumour invasion is a complex process involving several strategies, including mesenchymal and amoeboid movements, by which cells move through tissues (3). Cell migration through the extracellular matrix (ECM) begins with the formation of cell protrusions that contain filamentous actin (F-actin) and recognise the external environment; this is a common feature of invasion. Thus, it may be possible to control the invasive and metastatic phenotypes of cancer cells by manipulating the proteins contributing to such protrusions.

It was demonstrated that invasive cancer cells acquire a migratory phenotype associated with an increased expression of several genes involved in cell motility (4). Migration through tissues and endothelial barriers are a complex series of events involving a highly regulated cycle of cell protrusion, retraction, adhesion, detachment, and major dynamic rearrangements of the actin cytoskeleton. Actin, which is the most abundant protein with a concentration higher than 100 μM in most eukaryotic cells, is found in the highest density at the cell periphery. The shape of a cell is determined by actin filaments which are necessary for whole-cell locomotion. Cancer cells move within tissues during invasion and metastasis by their own motility. Control of cancer cell migration is an important problem in tumour treatment. Also, actin filaments in response to a positive stimulus, form many cell surface projections. Various signalling molecules that regulate multiple processes are involved in cell migration (5). The actin cytoskeleton and its regulatory proteins are crucial for cell migration in most cells. The actin cytoskeleton is dynamically remodeled during cell migration and this reorganisation produces the force necessary for cell migration (6). Actin-related protein (Arp) 2/3 complex-dependent actin polymerisation and its regulation are of particular interest. Reorganisation of actin filaments is

regulated by a highly integrated signalling cascade governed by 'molecular switches' belonging to the Rho family. Among the members of the Rho family, RhoA, Cdc42 and Rac play a key role by which the signalling inputs converge thereby initiating output pathways leading to site-directed actin polymerisation (7). This family is associated with the metastatic phenotype through regulation of cellular motility and invasiveness (8). Rac and Cdc42 stimulate formation of protrusions at the leading edges of cells while RhoA induces retraction at the tail ends of cells. Also important, are downstream molecules which are directly involved in actin reorganisation and form links between GTPases and the actin cytoskeleton. They belong to a group known as the Wiskott-Aldrich Syndrome protein (WASP) family proteins (9).

WASP family proteins are the final downstream effector linking the GTPases to the Arp complex, which are then activated to carry out actin polymerisation. The term WASP is derived from the clinical condition called the 'Wiskott-Aldrich Syndrome' which is characterised by manifestations in the immune system due to malfunctions of the actin cytoskeleton. The syndrome is linked to the mutation of the Wiskott-Aldrich Syndrome protein gene.

Wiskott-Aldrich Syndrome (WAS) and WAS protein (WASP) family proteins. Wiskott-Aldrich Syndrome (WAS) is an X-linked primary immunodeficiency originally described as a clinical triad of immunodeficiency, thrombocytopenia, and eczema (10). The presentation of this syndrome varies among patients. Some express the full triad of clinical manifestations while others have a milder phenotype and survive to adulthood. Phenotypic expression varies over time in a given patient (11). Patients with autoimmune and inflammatory manifestations are usually in a high-risk group with poor outcome.

The WASP gene. The WASP gene was mapped to the region Xp11.22-Xp11.3 after extensive studies of DNA from large WAS families with multiple affected members (12). By means of positional cloning and based on these mapping data, Derry *et al* (13) isolated the WASP gene and observed mutations in lymphoblastoid cell lines from patients with WAS-XLT. The WASP gene encodes a 502-amino-acid intracellular protein (WASP) expressed exclusively in haematopoietic cells. Patients with WAS and WASP-deficient mice have defective migratory behaviour of macrophages and dendritic cells. WASP-deficient dendritic cells exhibit similar abnormalities of cytoarchitecture, chemotaxis, and quality of migration (14). Defects of migration, anchorage, and localisation were also present in other cell lineages, including T and B lymphocytes, neutrophils, and haematopoietic stem cells (HSCs) (15).

Additional members of the WASP family. After the identification of WAS and WASP (13), an isoform was later isolated from the brain and called neural WASP (N-WASP). Although its discovery came later it is widely expressed. A protein related to WASP was identified in a genetic screen in *Dictyostelium* and named Scar, as disruption of the Scar gene suppresses abnormalities caused by loss of one of the cARs (cAMP receptors), namely cAR-2 (16). In the same year, a mammalian homologue of Scar was isolated independently by two groups (17,18). One group retained the name Scar and the other named the protein WAVE (WASP family verprolin homologous protein); the names are used interchangeably (WAVEs/Scar Proteins).

To date, five mammalian WASP family proteins have been identified: WASP, N-WASP, and WAVE1, 2, and 3. These proteins share two main regions of homology: a central segment rich in proline residues and a carboxy-terminal module comprising three characteristic domains called the VCA domain. While the V domain is a monomeric actin binding site, the CA domain binds the Arp2/3 complex. The Arp2/3 complex is activated when bound by the VCA domain and this complex then catalyzes actin polymerisation. N-WASP was first purified from bovine brain and is expressed ubiquitously, particularly enriched in the brain (19). N-WASP shares 50% homology with WASP. N-WASP induces filopodium formation downstream of Cdc42 activation (20). This then induces a conformational change that releases auto-inhibition (21). Activated N-WASP then binds to the Arp2/3 complex and induces rapid actin polymerisation.

2. Structural homology and divergence of the WASP family proteins

All members of the WASP family share a similar modular organisation with a conserved C-terminal 'output' domain and a much larger and divergent N-terminal region involved in targeting and regulating the activity of the output domain. This domain initiates the growth of new actin filaments by bringing together actin monomers and the actin-nucleating Arp2/3 complex. This is achieved through a VCA module consisting of a verprolin homology domain [V domain, also called WASP homology (WH) 2 domain] that binds to an actin monomer, a C-terminal acidic (A) region that associates with the Arp2/3 complex and intermediates conserved sequence (C), named the Cofilin domain. The C region acts in concert with the V and A regions, driving the conformational changes of Arp2/3 that are necessary to stimulate nucleation. Comparative analysis of the catalytic properties of the VCA module of WASP, N-WASP and WAVE revealed that the isolated domains displayed the unique kinetics of actin assembly. The VCA domain of N-WASP has a distinctly higher rate of nucleation than the VCA of both WASP and WAVE domains which are distinctly shorter and have less charge.

The amino-terminal half of the molecule defines two functional groups on the basis of the presence of either WASP homology 1/Ena VASP homology 1 (WH1/EVH1) domain (found in WASP and N-WASP) or WAVE homology domain suppressor of cAMP receptor (SCAR) homology domain (WHD/SHD), specific to WAVE isoforms. This is discussed in detail in the following sections.

Functional role of the N-terminal domain of the WASP family proteins. The overall conservation among the WASP and WAVE family members within the catalytic VCA domain contrasts the divergence of their N-terminal portion, which includes >85% of the entire amino acid sequence. WASP and N-WASP display a common modular organisation, including the WH1 domain [also called enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) homology 1], a basic region (B),

-binding domain (GBD) [also called CRIB (Cdc42 nteractive binding domain)] and a proline-rich region.

The WH1 domain includes the first 150 amino acids (22). Twenty-eight of the 35 identified missense mutations that cause Wiskott-Aldrich Syndrome lie within this domain and therein lies its functional importance (23). This provides a mechanism for explaining some of the manifestations of Wiskott-Aldrich Syndrome because amino acid exchanges in the WH1 domain resulting from mutations in the WASP gene lead to a disruption of interaction with WIP family members, resulting in protein malfunction.

The B, GBD, and proline-rich regions are implicated in regulating WASP and N-WASP functions, either by mediating auto-inhibitory interactions that block the VCA domain output activity, as in the case of the B and GBD regions, or by reversing this inhibition (24). The GBD region is also known as the CRIB region (25). The unlocking of the auto-inhibitory interactions occurs by binding to upstream activators. While activated Cdc42 containing GTP interacts with GBD, PtdIns(4,5)P2 interacts with the B domain. A plethora of Src-homology (SH)3-containing proteins (26,27) associate with the proline-rich residues. Moreover, the B and proline-rich regions are implicated in F-actin (28) and profilin (29,30) binding, respectively.

The N-terminal region of WAVE proteins is much less characterised. In contrast to WASP and N-WASP, WAVE proteins do not contain CRIB or the WH1 domains. They lack a surface for direct linking to Rho GTPases. Consistent with this, WAVE proteins instead possess an N-terminal conserved region, known as the WAVE homology domain (WHD) (18,24). This region displays a significant sequence similarity to WH1 of WASP and N-WASP, implying that it might fold in an analogous fashion. However, the functional roles of WASP/N-WASP and WAVE members are distinct. The WHD domain does not bind to WIP but mediates direct binding to Abl-interacting protein 1 (Abi1) (31), a scaffolding molecule originally identified as a mediator for the non-receptor tyrosine kinase Abl (32), involved in Ras to Rac signalling (33). This interaction is crucial for the assembly of a WAVE-based macromolecular complex, which mediates a direct association with activated Rac and positively regulates *in vitro* WAVE2-Arp2/3-dependent actin polymerisation (34,35). Also similar to WASP and N-WASP, WAVE proteins contain a B and proline-rich region. The latter mediates the association with insulin receptor substrate (IRS) p53 and is implicated in physically linking WAVE2 and Rac (36).

3. Signal integration by WASP family proteins: Mechanism for activation of the Arp2/3 complex

The WASP/N-WASP model of signal integration. As previously reported (21,31), WASP and N-WASP exist as auto-inhibited monomers *in vitro* and, possibly *in vivo*, as suggested by the observation that ectopic expression of full-length N-WASP has no effect on actin dynamics (20). To transform to an active state, N-WASP requires a positive external stimulus and is based on several protein-protein and protein-lipid interactions. In the N-WASP closed conformation, the N-terminal B region and the GBD are packed over hydrophobic residues of the C domain in the VCA module, essential for activation of Arp2/3 (31,32,37). Two possible mechanisms are proposed for how the WASP molecule is kept inactive *in vivo*. One theory proposes that both the B and CRIB regions act as a cooperative unit that directly binds to the VCA-Arp2/3 complex imposing structural constraints that prevent catalytic activation of Arp2/3 ('direct inhibition model') (31,32). The second theory proposes that CRIB is sufficient in itself to sequester the VCA domain in a dormant state ('sequestration model') (35).

Cooperative binding of PtdIns(4,5)P2 and Cdc42 to the B and CRIB regions, respectively, is required for operational molecules. This causes a conformational change resulting in the release of the C terminus, enabling its interaction with, and activation of Arp2/3. This cooperative activation mechanism demonstrates how combinations of simple binding domains are used to integrate and amplify coincident signals. Reinforcing this view, WASP and N-WASP regulation are exerted through two additional signal-dependent mechanisms: (i) by binding to several SH3-domain-containing signalling proteins and (ii) by phosphorylation (38,39). For example, the SH2 and 3 adaptor Grb2, a weak activator by itself, cooperates with Cdc42 to elicit full WASP and N-WASP activity, similar to the actions of Nck and PtdIns(4,5)P2 (26). By contrast, WASP-interacting SH3 protein (WISH) can elicit maximal activation of N-WASP in a Cdc42-independent manner. The biochemical effects of the association of other SH3-containing signalling molecules, such as Src, Fyn, p85 and phospholipase Cγ, with WASP and N-WASP are yet to be described. Several endocytic proteins [endophilin A, intersectin and syndapin (or pacsin1)] might modulate N-WASP activity, suggesting its involvement in trafficking or internalisation processes. These studies support a role for WASP and N-WASP as convergent 'nodes' of different signalling pathways for the execution of actin-dependent processes. A final level of integration and regulation is exerted by phosphorylation. WASP and N-WASP are phosphorylated on both serine and tyrosine residues (34,39). Constitutive phosphorylation by caseine kinase 2 of a serine located within the VCA domain increases the nucleation-promoting activity of WASP. Src family kinases (also focal adhesion kinase) mediate signal-dependent phosphorylation of both WASP and N-WASP on Y291 and Y253, respectively. This post-translational modification synergises with PtdIns(4,5)P2, leading to maximal WASP activation. Also, the observation that tyrosine phosphorylation, in addition to the subsequent dephosphorylation, occurs only in the presence of activated Cdc42 suggests a mechanism for regulation of initiation, duration and amplitude of N-WASP activation (40).

The WAVE model of signal integration: Similarities and differences to the WASP/N-WASP model. The WAVE proteins lack the CRIB domain (as in WASP and N-WASP), hence no direct link between them and Rho GTPases is possible for actin reorganisation. Nevertheless, WAVE proteins function downstream of Rac in mediating membrane protrusion, as indicated by the fact that WAVE1 dominant negative mutants abolish Rac-dependent ruffling and neurite extension (41). As mentioned before, another distinct difference between the WAVE family members and N-WASP/WASP is that WAVEs are inherently active under normal circumstances, suggesting that a specific and separate mode of regulation exists (26). Also, WAVE assembles into multimolecular complexes to

function *in vivo* (43). Once activated, these complexes serve as a link from the signal to the Rac molecules, regulating the nucleation activity of the WAVEs. This ensures that it is spatially restricted to the leading edge of the cell, where protrusion is initiated. Both positive and negative modes of regulation of the AVE complexes have been reported. As mentioned earlier, IRSp53 acts as a link binding WAVE2 to activated Rac. This is unique to WAVE2 and is not observed in WAVE1 and 3.

The 'trans-inhibitory theory' proposes an alternative mechanism for control of WAVE proteins (44). This suggests that WAVE1 is kept dormant by its association to four other proteins Nap1 (an Nck-associated protein), PIR121 or Sra-1 (identified as Rac effectors) (45), and HSPC300 (a small 9-kDa protein). In *in vitro* studies, this complex could not stimulate actin polymerisation. However, addition of activated Rac removed this inhibition by inducing the disassembly of the inhibitory Nap1-PIR121 sub-complex from the WAVE1-HSPC300 unit. Though this theory provides an attractive explanation, it does not account for the mechanisms through which the activity of the complex is restricted to specific sites within cells destined for the formation of membrane extensions. Another detailed study on the individual components of the Abi1-Nap1-PIR121 concluded that Abi1 is an essential component which directly interacts with the WHD domain of WAVE2. Both WAVE1/2-Abi1-Nap1-PIR121 reconstituted *in vitro* were as active as the WAVE1/2-Abi1 subcomplexes, respectively, at stimulating actin nucleation. Also, the addition of activated Rac either *in vitro* or *in vivo* did not disrupt the complex (31,33). Studies of *Drosophila* and mammalian cell lines confirmed that WAVE proteins form stable complexes that are not disrupted following Rac activation or during cell migration. Studies in which each of the molecules i.e., WAVE, Nap1, PIR121/Sra1 and Abi1 were knocked down in *Drosophila*, S2 Schneider cells (46,47) and mammalian cells showed that this manipulation resulted in complete loss of Rac-induced actin remodelling and lamellipodia formation. Another function of this complex inferred from this observation is that individual ablation of Abi1 or any other component of the complex lead to the degradation of WAVE molecules. This indicates that proper assembly of the complex is required for WAVE stabilisation.

4. Functions of WAVE family proteins in cancer cell migration

Two major physiological roles are described for WASP family proteins with respect to migration and invasion of cancer cells in 3-D matrices. These are the regulations of mesenchymal cell migration and the degradation of extracellular matrix (ECM). The WASP family proteins regulate these processes through the formation of various actin-based protrusive membrane structures down-stream of Rac and Cdc42 activation.

WAVE 1 and formation of dorsal ruffles, podosomes and invadopodia. Characteristic protrusive membrane structures are involved in ECM degradation in some invasive cells. These structures have been variably named as dorsal ruffles, podosomes, and invadopodia (48). Formation of these unique structures is also regulated by WASP family proteins.

Dorsal ruffles. On stimulation by growth factors, a dramatic change in dorsal membrane structures was observed in fibroblasts as well as epithelial cells. These protrusive membrane structures are known as dorsal ruffles. However, not much is known about their physiological functions. A study which analysed the functions of WAVE1-deficient cells showed that dorsal ruffles are involved in ECM degradation. Stimulation of mouse embryonic fibroblasts (MEF) by PDGF results in formation and induction of dorsal ruffles. Also, accumulation of the ECM-degrading enzyme, MMP-2, occurs at the dorsal ruffles. However, this formation of dorsal ruffles and lamellipodial protrusions are dependent on Rac. WAVE2 has no similar functions in dorsal ruffle formation as does WAVE1. A WAVE1 deficiency decreases dorsal ruffle formation and subsequent MMP secretion. While normal chemotactic migration toward PDGF is seen in WAVE1-deficient MEF, chemotactic invasion into the ECM toward PDGF is decreased. Dorsal ruffles are formed only when cells are cultured on 2-D substrates. It is unknown what form they take in 3-D matrices. The relationship between dorsal ruffle structure and chemotactic invasion remains to be determined. However, it is clear that WAVE1-mediated protrusive membrane structures are crucial for migration within ECM.

Podosomes and invadopodia. Invadopodium formation occurs in highly invasive cancer cells, thus is implicated in tumour cell metastasis. Podosomes have a remarkable similarity to invadopodia in molecular composition.

Podosomes are dynamic actin-rich adhesion structures that form cell-substrate adhesion sites. These differ from focal adhesions usually observed in adhesive cells. Podosomes are formed by monocyte-derived cells, such as macrophages, some nonhaematopoietic cells and by transformed fibroblasts (49). In oncogene transformed cells these are named invadopodia (50). EGF stimulates invadopodium formation in carcinoma cells, indicating that invadopodium formation is regulated by chemotactic and invasive signals (51). Podosomes and invadopodia are involved in ECM degradation. Alhough the distinction between these structures is unclear, it is known that invadopodia persist longer than podosomes and many believe that these could be the precursors of invadopodia. However, podosomes are observed only in limited cell types like osteoclasts and macrophages. WASP is involved in the formation of these structures (52). Podosomes consist of filamentous actin-rich cores and surround ring structures containing adhesive proteins such as vinculin and talin. When Src-transformed cells are cultured on fibronectin, an ECM protein, spot-like degradation of this protein is observed which colocalise with podosomes. N-WASP is recruited to podosome structures and is essential for podosome formation. Expression of a dominant-negative form of N-WASP, which cannot induce Arp2/3 complex-dependent actin polymerisation, proved inhibition of podosome formation and therefore subsequent degradation of ECM. Recent studies using RNAi methods for gene silencing also demonstrated the role of N-WASP in invadopodium formation in metastatic carcinoma cells (53).

WAVE2 and its role in mesenchymal migration. WAVE2 is concentrated at the tips of membrane protrusions formed in the direction of cell locomotion when a cell migrates (54,55).

ent to the leading edge is mediated by binding basic in WAVE2 to PIP3[i]. Downstream of Rac, WAVE2 induces Arp2/3-mediated actin polymerisation, causing lamellipodium formation (37), which is crucial for mesenchymal cell migration. Lamellipodia are thin, flattened structures that consist of branched actin networks (50). WAVE proteins activate the Arp2/3 complex, which then bind to the pre-existing actin filaments and induce new side-branched actin polymerisation. Of all the WAVE proteins, it is predominantly in the fibroblasts where WAVE2 is localised to the tips of lamellipodial protrusions. Studies show that in WAVE2-deficient fibroblasts, lamellipodium formation is severely inhibited. Also in WAVE2-deficient cells, defective lamellipodium formation leads to decreased cell migration and invasion toward chemoattractants. Thus, WAVE2 is important for directional movement in fibroblasts (54). WAVE2 also plays a role in the migration of B16F10 melanoma cells, which undergo mesenchymal migration in 3-D matrices (56). Yamazaki *et al* showed that repression of WAVE2 expression by RNAi methods, reduces cancer cell migration and metastasis into the lungs by B16F10 melanoma cells via regulation of membrane protrusions (57). Thus, WAVE2 is essential for mesenchymal migration, and inhibition of the WAVE2 function can inhibit migration and invasion of cancer cells.

Role of WAVE3 in lamellipodium formation. The function of WAVE3 discovered last among the WASP family. Knockdown of WAVE3 expression by RNAi techniques prevented the PDGF-induced lamellipodium formation and cell migration, while increasing actin stress fibers linked to focal adhesion, which are generally associated with static cells. This study showed that knockdown of WAVE3 expression also inhibited the expression levels of MMP-1, -3, and -9. The WAVE3-mediated MMP production is independent of both WAVE1 and 2, whose expression levels were not affected by loss of WAVE3. Endogenous WAVE3 was concentrated in the lamellipodia at the leading edge of migrating MDA-MB-231 cells. Down-regulation of WAVE3 impaired motility and invasiveness of these cells. Treatment of cells with LY294002, an inhibitor of phosphatidylinositol 3-kinase (PI3K), also abrogated the PDGF-induced lamellipodium formation and cell migration, suggesting that PI3K may be required for WAVE3 activity. These findings confirm that WAVE3 regulates actin cytoskeleton, cell motility, and invasion through the p38 mitogen-activated protein kinase (p38 MAPK) pathway and MMP production, which act as downstream effectors of WAVE3 in cell motility and invasion (58).

5. Genomic organisation and expression of the human WAVE gene family

The 3 human WAVE genes are fully characterised (18,19,59). WAVE1 is primarily expressed in the brain and lung, although a detectable PCR product is also observed in the placenta, liver, and pancreas. These results extend previously published data generated from a less sensitive Northern blot analysis where WAVE1 was expressed exclusively in the brain (18,60). Human WAVE2 expression was detected only in the placenta and lung. Similar to WAVE1, WAVE 2 is ubiquitously expressed in all human fetal tissues. WAVE3 is predominantly expressed in the brain, although a noticeable expression is also detected in other tissues such as lung, testis, kidney, and skeletal muscle.

6. Human WAVE protein sequence alignments

Human WAVE1 protein is 549 amino acids long, while the human WAVE2 and 3 are 488 and 501 amino acids, respectively. The overall similarity between all three proteins is >50%, but the similarity in the functional domains, i.e., the basic, the proline-rich, and the VCA domains, and the leucine zipper motif are >80%, which demonstrates a likely functional conservation of these proteins among mammals. Moreover, these functional domains were conserved in lower organisms such as *Drosophila*, *C. elegans*, and *Dictyostelium discoideum* apart from mice (61). The high degree of evolutionary conservation of these genes from yeast to mammals clearly indicates that their function is critical in the actin polymerisation and cytoskeleton organisation processes.

WAVE3 is currently the only gene associated with a human disorder. Sossey-Alaoui *et al* have previously shown that WAVE3 is inactivated in low-grade neuroblastoma tumours (62). This indicates that neuroblastoma might be a consequence of disruption of the actin polymerisation and cytoskeleton organisation machineries. This hypothesis is further supported by the findings that IGF-I, which is also involved in actin polymerisation and cytoskeleton organisation, promotes motility in neuroblastoma cells by increasing the amount of dynamic morphological changes, as measured by the formation of large lamellipodia (63).

7. Gene conservation among the WAVE family members

The human WAVE1, 2, and 3 genes map to chromosome regions 6q21, 1p36, and 13q12, respectively (18,62). While the WAVE1 gene maps distal to the human GPR6, 3 and 12, two other members of the extended GPR gene family, map distal to WAVE2 and proximal to WAVE3. HMG17 and 1, two members of the large, high-mobility gene family, are also found in the same order i.e., proximal to WAVE2 and distal to WAVE3 which suggests that WAVE genes may have a common origin and ancestor via duplication.

8. Clinical associations of WASPs with human cancer

Despite the pivotal role of the WASP family in cell and cell migration, its role in human and clinical cancer is only recently coming to light.

Expression of WAVEs and N-WASP in human breast cancer and association with clinical outcomes. Various reports showed associations between the expression of the WAVE family members with clinical human cancer. Sossey-Alaoui *et al* (64) recently reported that in tumour specimens from breast cancer patients of different stages and grades, immunohistochemistry with polyclonal antibodies to WAVE3 showed approximately 3-fold higher levels of WAVE3 were present in grade III tumours compared with grade I tumours. Little or no WAVE3 protein was detected in either normal breast or grade I tumours. Also, an inverse correlation was observed

between the oestrogen receptor/progesterone receptor status and WAVE3 levels (64). In a separate study using an orthotopic xenograft model (mammary fat pads of female SCID mice), they implanted MDA-MB-231 cells as well as cells with WAVE3 gene silencing. In the control group, palpable tumours were detected 15 days after inoculation. In the shWAVE3 groups, the tumours were first palpable much later (22 days and 40 days in 2 clones). Also, the tumour growth rate in the shWAVE3 cohorts was reduced by 40 and 80% for these clones. Finally, they also showed that spontaneous metastases to the lungs in WAVE3 knockdown clones were significantly reduced ($p<0.00001$). They concluded that suppression of WAVE3 attenuated the establishment of primary tumours of MDA-MB-231 cells in SCID mice (51).

A similar association was reported between WAVE members and breast cancer. In this analysis, the expression of WAVE molecules at the mRNA and protein levels in a cohort of 122 human breast cancer and 32 normal breast tissues, overexpression of WAVE2 was seen in node-positive cases as well as moderately and poorly differentiated tumours. A general trend of high expression of these proteins was observed in breast tumour tissues when compared with normal tissues. Finally, patients who died of breast cancer had high WAVE1, 2 and 3 levels and of these WAVE2 levels showed statistical significance ($p=0.02$) (41). The same group also reported that expression of N-WASP transcripts is reduced/lost in breast tumours, a change linked to a poor clinical outcome. In addition, the group demonstrated that forced expression of N-WASP in human breast cancer cells, MDA-MB-231, resulted in less invasive cells (62).

Expression of WAVEs in liver tumours. Expression of WAVE2 was investigated in clinical hepatocellular carcinoma (HCC) (65). Samples from 112 HCC patients were immunohistochemically stained for WAVE2. The findings correlated with clinical prognosis. Among these 112 cases, significant increases in WAVE2 expression levels were observed in 71 cases. More significantly, the increased WAVE2 expression correlated with the multinodularity, absence of capsular formation, higher Edmondson-Steiner grade, venous invasion and a shortened median survival time, all of which were statistically significant ($p<0.05$). By multivariable Cox regression analysis, high WAVE2 expression [risk ratio (RR), 1.68, $p=0.038$], high serum α-fetoprotein levels, multiple tumour nodules and vein invasion were independent prognostic factors for survival. This immunohistochemistry data were confirmed by results of reverse transcription-PCR and Western analysis of 31 HCC cases, in which the WAVE2 mRNA and protein in HCC tissues were significantly elevated when compared with paracarcinomatous liver tissue. The conclusion from this study was that WAVE2 could be a candidate prognostic marker for HCC.

Expression of WAVE2 and Arp2/3 in colorectal cancer. Another report studied a large cohort of colorectal cancer patients. This study used tissues collected over a 13-year period from 1987 (66). There were 29 cases for which tissue blocks of metastatic deposits in the liver as well as the primary tumour available. Seven of these were synchronous and the rest were metachronous. The immunohistochemical staining showed absence of distinct colocalisation of Arp2 and WAVE2 in normal epithelial cells, though the immunoreaction of either Arp2 or WAVE2 was seen focally. Because these cells are thought to function while migrating in tissues, cells that co-express Arp2 and WAVE2 may have greater ability to move freely than cells that do not. This could partially explain why cells which co-expressed both Arp2 and WAVE2 could migrate actively. This present study demonstrated that the colocalisation of Arp2 and WAVE2 is an independent risk factor for liver metastasis in colorectal cancer. This group obtained paired mirror sections of 154 cancer specimen matches for parameters such as the site of origin, the T stage, age and gender of patients. Expression of both Arp2 and WAVE2 was detected in the same cancer cells in nearly 36% of cases, but no co-expression was detected in normal colonic epithelial cells. Univariate analysis showed that the colocalisation was significantly predictive of liver metastasis (RR, 8.760). This risk ratio is higher than other currently known risk factors viz., histological grade (RR, 2.46), lymphatic invasion (RR, 9.95), and tumour budding (RR, 4.00). Among these, colocalisation and lymphatic invasion were independent risk factors by multivariate analysis. Further mRNA studies showed that high levels of Arp2, expressed by cancer cells, were significantly associated with liver metastasis. These results indicate that colocalisation of Arp2 and WAVE2 is an independent risk factor for liver metastasis of colorectal carcinoma. To further discuss the role of these proteins in metastasis, this significant study using a murine model is mentioned below.

WAVEs in melanoma. In their study on murine melanoma cells, Kurisu *et al* demonstrated that malignant B16F10 mouse melanoma cells expressed more WAVE1 and 2 proteins and showed higher Rac activity than B16 parental cells, which are neither invasive nor metastatic (67). Membrane ruffling, cell motility, invasion into the ECM, and pulmonary metastasis of B16F10 cells were suppressed by WAVE2 RNAi techniques. In B16 and B16F10 melanoma cells, WAVE1 was colocalised with F-actin in both peripheral and aberrant ruffles. WAVE2 was also concentrated in membrane ruffles but localised at the tips of both peripheral and aberrant ruffles. Cytosolic signals for WAVE2 were weaker than those of WAVE1, suggesting that WAVE2 functions at the very front of actin-rich protrusions and may have a distinct role in membrane extension. They further conducted *in vivo* studies to examine the effect of WAVE1 knockdown of metastatic deposits in the lungs of female C57BL/6 mice. When B16F10 cells were injected intravenously, they metastasised to the lungs and formed black spherical colonies. Two weeks post-injection, colonisation of the lung by B16F10 cells were observed in vector transfectants and in cells with WAVE1 RNAi. In contrast, WAVE2 RNAi suppressed metastasis of B16F10 cells to the lungs. This suggests that WAVE2 suppression may be an effective means to block invasion and metastasis. There are several events in which WAVE2 may be necessary. The colonisation cascade in lungs begins with attachment of tumour cells to endothelial cells of microvessels. Tumour cells then extend pseudopods into the subendothelial matrix and penetrate endothelial linings to adhere to the basement membrane. Finally, tumour cells extravasate with dissolution of the basement membrane (68).

in prostate cancer. We recently investigated the role of WAVEs and the pattern of expression in human prostate cancer. Immunohistochemical studies of prostate tissue specimens showed that prostate cancer tissue had a distinct staining when compared to normal prostate epithelial tissues. RT-PCR using the specific primer for WAVE1 showed good expression in both PC-3 and DU-145 cell lines. WAVE1 knockdown cell clones were generated from both cells, which showed a markedly reduced expression of WAVE-1 transcript, as confirmed by RT-PCR and Western blot. It was found that knockdown of WAVE1 was associated with significant reduction in invasion ($p<0.001$, in both cell lines) but not growth at 72 h ($p>0.05$, in both cell lines). Adhesion of cells to the Matrigel was not affected by the knockdown of WAVE1 ($p>0.05$), thus indicating its pivotal role in the control of cellular invasion (69). Our knockdown studies conducted on WAVE3 for these cell lines were associated with decreased invasiveness ($p<0.05$), but not cell growth (unpublished data).

9. Conclusions and perspectives

It is clear from the current evidence that the WASP family, particularly the WAVE proteins, are strongly linked to migration of a variety of cells including cancer cells. These proteins are also linked to the invasiveness and aggressiveness of cancer cells. However, it is acknowledged that not all pathways by which WAVEs accomplish actin nucleation are known. Evidence is emerging that the abnormalities of the WASP family proteins are linked to the clinical outcome of patients. Together, it is reasonable to conclude that the WASP family, and in particular the WAVEs, are key intrinsic regulators of cancer cells and bear important implications in the development of clinical cancers. Targeting these molecules is crucial to our efforts to achieve cancer control. Actin nucleation is a nodal point which is important for cell metastasis. Hence, methods to block this step could prevent metastasis. Further work needs to be carried out to elucidate if WAVEs have a similar role in different types of cancers.

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