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Review

Structure and Role of WASP and WAVE in Rho GTPase Signalling in Cancer

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Abstract. *A major factor controlling the metastatic nature of cancer cells is their motility. Alterations in the signalling pathways controlling its regulation can lead to tumor cell invasion and metastasis. Directional motility involves protrusion of the cell's leading edge, via formation of filopodia and lamellipodia, adhesion to the substrate followed by tail retraction and de-adhesion. Rho GTPase binding proteins function as activators of the actin cytoskeleton and are key players in the transendothelial migration of cancer cells. Activation of the specific GTPases Rho, Rac1 and Cdc42 results in formation of actin stress fibres, membrane ruffles, lamellipodia and filopodia respectively and in cortical actin assembly. Pathways through which Rho GTPases elicit these effects are through direct interaction with members of the Wiskott-Alrich Syndrome Protein (WASP) family which stimulates structures such as lamellipodia and filopodia. The present review explores the role and function of Rho GTPases, WASP and WAVE in cancer metastasis.*

Abbreviations: Arp, actin related protein; CRIB domain, Cdc42 and Rac interactive binding domain; FAK, focal adhesion kinase; GBD, GTPase binding domain; GTPase, Guanosine triphosphatase; N-WASP, neural-WASP; PIP2, phosphatidylinositol 4,5-bisphosphate; PSTPIP, proline-serine-threonine phosphatase interacting protein; ROCK, Rho associated serine threonine kinase; SCAR, suppressor of cAR; SH2, Src homology 2 domain; SH3, Src homology 3 domain; VASP, vasodilator-stimulated phosphoprotein; VCA domain, verprolin homology, cofilin homology acidic region domain; VPH domain, verprolin-homology domain; WAS, Wiskott-Aldrich syndrome; WASP, Wiskott-Aldrich Syndrome Protein; WAVE, WASP family verprolin homologous protein; WH1, WASP homology 1; WH2, WASP homology 2; WIP, WASP-interacting protein; WRC, WAVE regulatory complex.

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Rho Family of Proteins in Cancer

As members of the Rho family act as key regulators of cytoskeletal reorganisation, cell motility, cell-cell and cell-matrix (ECM) adhesion as well as of cell cycle progression, gene expression and apoptosis and, as each of these functions is of importance for the development and progression of cancer, it is not surprising that the Rho GTPases have been keenly studied for their role in cancer cell metastasis and invasion.

Rho and Cell Motility

It has been established that the acquisition of the migratory phenotype of invasive cancer cells is associated with an increased expression of several genes involved in cell motility (1). For migration to occur a cell must initiate polarisation, in response to a migration-promoting agent and extend protrusions in the direction of migration (2) with the resultant changes in cell shape involving a dynamic reorganisation of the cytoskeleton. The extension of the leading edge is instigated by protrusion of lamellipodia and/or filopodia, driven by actin polymerisation and filament elongation (3), and is often accompanied by membrane ruffling (4), which extends the cell body to create new, distal adhesion sites. This protrusion is followed by adhesion between the cell and substratum at the leading edge, which is accomplished mainly by integrin and non-integrin receptors binding to specific extracellular matrix protein domains (5,2). Actomyosin-mediated contraction of the cell drives the forward motion of the cell body with contractile forces being generated at or near the leading edge, associated with detachment of the trailing edge from the substratum, with the migrating cell secreting proteases to digest extracellular matrix proteins.

Studies on the involvement of Rho GTPases in these aspects of cell motility have firmly identified GTP-binding proteins as activators of the actin cytoskeleton (6), and to be key players in the transendothelial migration of cancer cells (7). It was originally demonstrated that activation of the specific GTPases Rho, Rac1 and Cdc42 resulted in the formation of actin stress fibres, membrane ruffles, lamellipodia and filopodia respectively in *in vitro* cell culture (8) and in cortical actin assembly (9). The activities of these three proteins were found to be linked to each other in a hierarchical manner. Thus, activation of Cdc42 was thought to lead to the formation of filopodia and to the activation of Rac, leading to the induction of lamellipodia. Rac subsequently activates Rho, leading to the formation of new sites of adhesion and to the assembly of stress fibres to enable cell contraction and retraction of the trailing edge (10).

Cdc42 and the WASP Proteins

Research has shown that the Rho protein Cdc42 has a major role as a regulator of cell polarity and its activity is found to be most prominent at the tip of the leading edge of the migrating cell (11). A group of molecules acting downstream of the Rho GTPases that are directly involved in actin reorganisation and form links between GTPases and the actin cytoskeleton are the Wiskott-Aldrich Syndrome protein (WASP) family. The first member of this family, WASP, was isolated in 1994 as a novel gene mutated in the X-linked recessive immunodeficiency disease Wiskott - Aldrich syndrome (WAS) (12). The human WASP gene is located at Xp11.22-p11.23 and is exclusively expressed in haematopoietic tissue. Following the discovery of the WASP gene, work from a number of research groups to unravel the disease mechanism of Wiskott-Aldrich syndrome identified the active, GTP-bound Rho GTPase Cdc42 as interacting with WASP (13-15). This link between WASP and Cdc42 was thought to be important as it had been shown that Cdc42 stimulated the formation and extension of finger-like protrusions, or filopodia, containing actin bundles (8, 16), and also to stimulate actin polymerisation *in vitro* (17, 18). However, further studies have demonstrated that neither N-WASP nor the Arp2/3 complex are necessary for filopodia formation (19-21) with knockdown of Cdc42 having no effect on filiopodia formation in fibroblastoid cells (22).

The WASP Family Proteins and the Arp2/3 Complex

In 1996, Miki *et al*., identified a 65kDa protein with 50% homology to WASP, which was termed neural-WASP (N-WASP) and is dominantly expressed in the brain. Similarly to WASP, N-WASP functions in regulating the cortical actin cytoskeleton (23).

During this time, five mammalian WASP family members were described; WASP, N-WASP and the WASP family verprolin homologous proteins WAVE-1/SCAR (24, 25) WAVE-2 and WAVE-3 (26).

At the same time in the mid-1990s, the actin-related proteins (Arps) were being studied in relation to the actin cytoskeleton (27, 28), with the Arp2/3 complex emerging as a key player in binding to actin *in vitro* and was found to co-localise with the actin cytoskeleton (28-30). Arp2 and Arp3 form a complex with five other proteins, the Arp complex (Arc) proteins, namely p41-Arc, p35-Arc, p19-Arc, p18-Arc and p14-Arc in *Acanthamoeba* (28, 29); p41-Arc, p34-Arc, p21-Arc, p20-Arc and p16-Arc in humans (and was found to be localised with actin-rich structures in *Acanthamoeba* (28, 31, 32) providing evidence for the association of the complex with actin polymerisation and thus in cytoskeletal organisation. All five WASP family proteins associate with the Arp2/3 complex, activating it by acting as nucleation promoting factors (NPFs), thus exhibiting an important role in actin polymerisation and cytoskeletal dynamics. More recently, three new NPFs have emerged; WASH (33), WHAMM (34) and JMY (35, 36) which contain an actin and Arp2/3 interacting WCA module. WASH (WASP and Scar homolog) has been linked with endosome trafficking (37, 38); WHAMM (WASP homolog associated with actin, membranes and microtubules), which appears to be important in maintaining Golgi structure and inducing actin assembly to promote tubule elongation (34); JMY (junction-mediating and regulatory protein) influences cellular motility and adhesion and drives actin nucleation by both Arp2/3 dependent and Arp2/3 independent pathways (35, 36, 39). The discovery of these novel NPFs has increased the complexity of Arp2/3 regulation and future work will undoubtedly aim to further clarify their functions and interactions within the cell.

Structure of WASP Family

The WASP and WAVE family proteins share common regions of homology (Figure 1): a proline-rich segment and a carboxy-terminal homologous sequence comprising three characteristic regions termed the VCA domain. The VCA region is comprised of the verprolin homology domain (also termed WASP homology 2 [WH2]) domain; the central homology domain and the acidic region. The V domain binds G-actin while the CA domain binds the Arp2/3 complex (40-42). Activation of the Arp2/3 complex is achieved by this binding, the resultant complex catalysing actin polymerisation. Importantly, N-WASP contains two WH2 domains and accordingly exhibits a much higher nucleation rate associated with the VCA region in contrast to the other members within the mammalian WASP protein family (43).

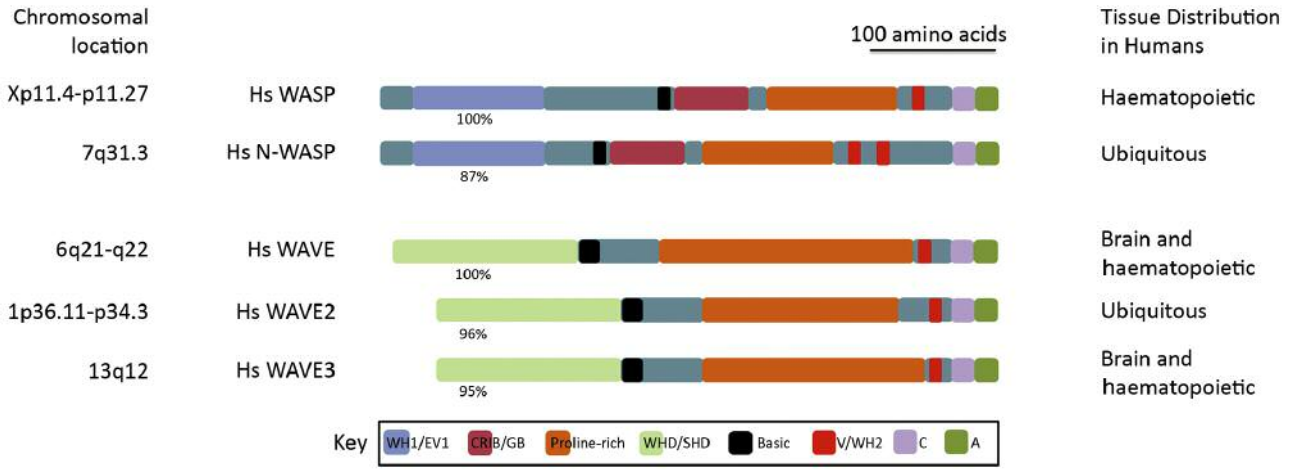

Figure 1. *Domain structure and tissue distribution of human WASP and WAVE family proteins. The percentage amino acid homology of the WH1/EV1 domain of WASP and N-WASP and of the WHD/SHD domain of the WAVE proteins is shown.*

The proline-rich region of these proteins allows binding of SH3-containing proteins such as Ash/Grb2 (23, 44), Nck (45) and proline-serine-threonine phosphatase-interacting protein (PSTPIP) (46). This stretch of residues separates the C-terminus from the amino (N-) terminus. At the N-terminus is the WH1 (WASP homology 1) domain which facilities association with WIP (WASP-interacting protein), which is implicated in protecting WASP from protease degradation (47, 48). Adjacent to this region is a basic stretch is involved with phospholipid PIP2 (phosphatidylinositol 4,5-bisphosphate) interaction and is postulated to co-ordinate with Cdc42 to drive actin polymerisation (49). Proximal to this basic stretch, and integral to the role played by WASP and N-WASP in actin polymerisation, is the presence of the CRIB (Cdc42 and Rac interactive binding) domain alternatively named GBD (GTPase binding domain) (Figures 1 and 2).

WASP Activation

Further studies have revealed that WASP and N-WASP exist in a closed conformation within the cell due to auto-inhibition of the VCA domain by the Cdc42/Rac interactive binding (CRIB) domain (50) (Figure 3A). This folded conformation masks the VCA region and therefore prevents the C and A domains from activating the Arp2/3 complex (51). Disrupting intramolecular interactions relieves the inhibited state of the protein and can be brought about *via* the competitive binding of various ligands such as the Rho GTPase, Cdc42 and phosphatidylinositol 4.5-bisphosphate (PIP2) which can associate with the GBD and basic-rich region of the protein, respectively and exposes the VCA region for subsequent Arp2/3 activation (49, 51, 52), inducing the assembly of a branching network of actin filaments that push the cell membrane forward (53).

Serine and tyrosine residues within WASP and N-WASP are subject to phosphorylation by numerous kinases that are able to influence their activity and localisation. For instance, it has been shown that releasing WASP and N-WASP allowed intramolecular interactions to occur following protein phosphorylation by the Src family of tyrosine kinases adjacent to the CRIB region (54). Additionally, focal adhesion kinase (FAK) phosphorylates tyrosine residue 256 of N-WASP, which affects its nuclear localisation and promotes cell migration (55). A potential explanation for this link between FAK and N-WASP is that activated FAK recruits Cdc42, which promotes N-WASP activation thus stimulating Arp2/3 and promoting actin polymerisation, a necessary step in cell motility (56). The equivalent conserved tyrosine residue described in WASP is at position 291 in N-WASP and is also subject to tyrosine kinase phosphorylation that leads to subsequent actin polymerisation (54). Furthermore, two serine residues found in the VCA domain of WASP are targeted by casein kinase 2. Phosphorylation of these serine residues dramatically increases VCA domain and Arp2/3 interaction, which significantly influences actin nucleation (57).

It would seem however, that these two modes of activation are not independent of each other as some interplay has been discovered. Coupling protein phosphorylation with Cdc42 intervention was found to have an enhanced effect on WASP activation (58); with Cdc42 shown to recruit WASP to the plasma membrane, where it was subjected to phosphorylation by Lyn and Btk (59).

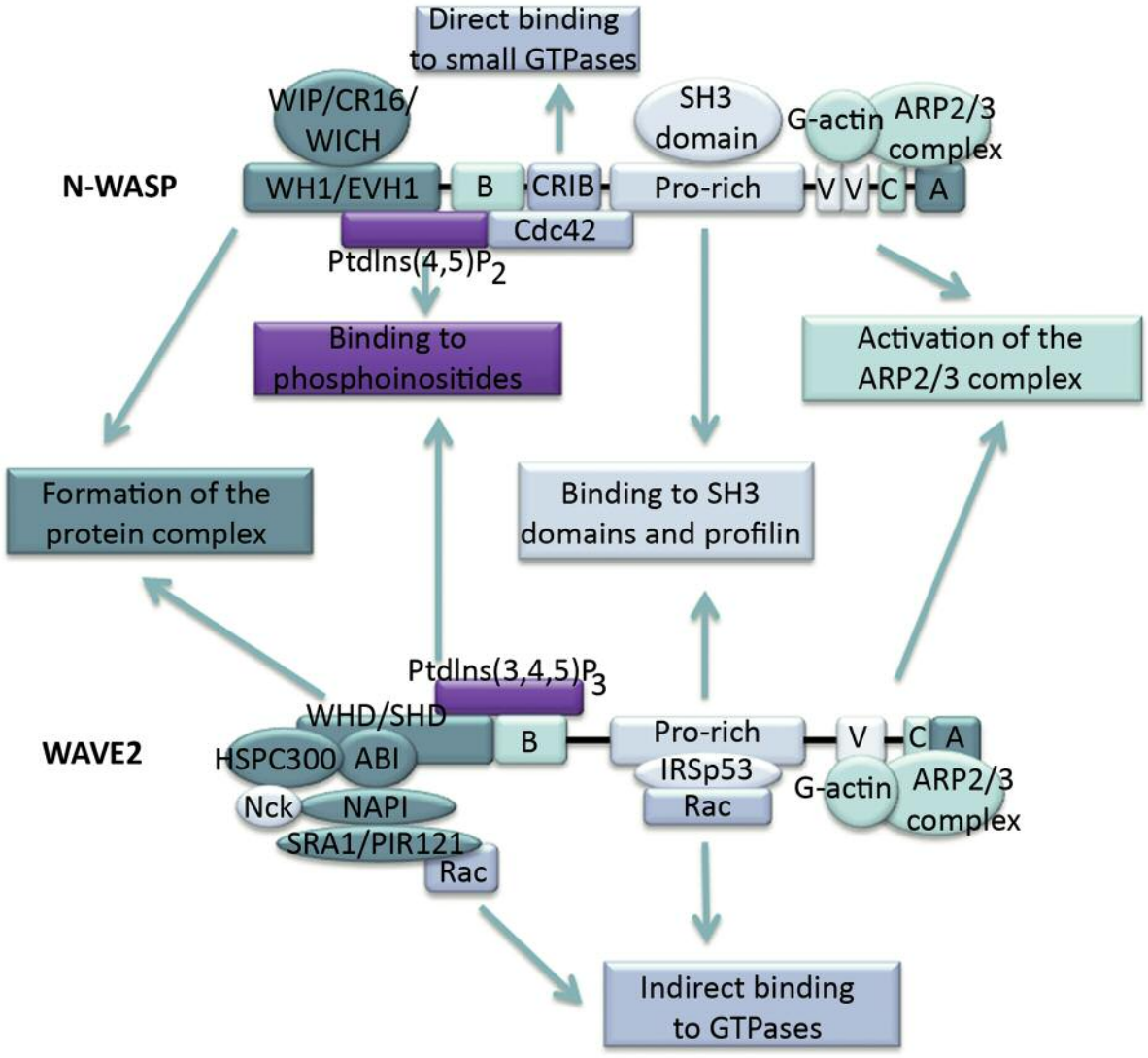

Figure 2. *Domain structure and binding partners of N-WASP and WAVE2. N-WASP contains an N terminal region (WH1/EVH1 domain), which binds WIP, CR16 and WICH. WAVE2 binds to Rac through SRA1/PIR121 in the WAVE2 complex and through IRSp53 binding to the proline-rich region of WAVE2.*

WASP Family Verprolin Homologous (WAVE) Protein Family

Following database searches using the verprolin-homology (VPH) domain sequence, a novel WASP-related WASP-family verprolin-homologous (WAVE) protein was identified due to its sequence conservation between WASP and N-WASP and its actin polymerising properties (24, 25).

Subsequent to this discovery, two additional WAVE proteins were identified, with the original protein re-named WAVE 1 (alternatively named suppressor of cAR; SCAR1) and the additional proteins named WAVE 2 and WAVE 3 (26). The *WAVE1* gene is located at chromosomal region 6q21 and encodes a gene product of 80,186 bp. The translated protein is 559 amino acids long and whilst there is evidence that it is widely expressed, it is expressed particularly in the brain. At chromosomal region 1p36.11 resides the WAVE2 gene which encodes a product of 85,940 bp. The corresponding protein is 498 amino acids long and is ubiquitously expressed but more so in peripheral blood leukocytes. The remaining protein of this sub-family is WAVE 3 whose gene, found at chromosomal region 13q12.13, encodes a product of 131,246 bp which when translated into protein, is found expressed mainly in the brain (60).

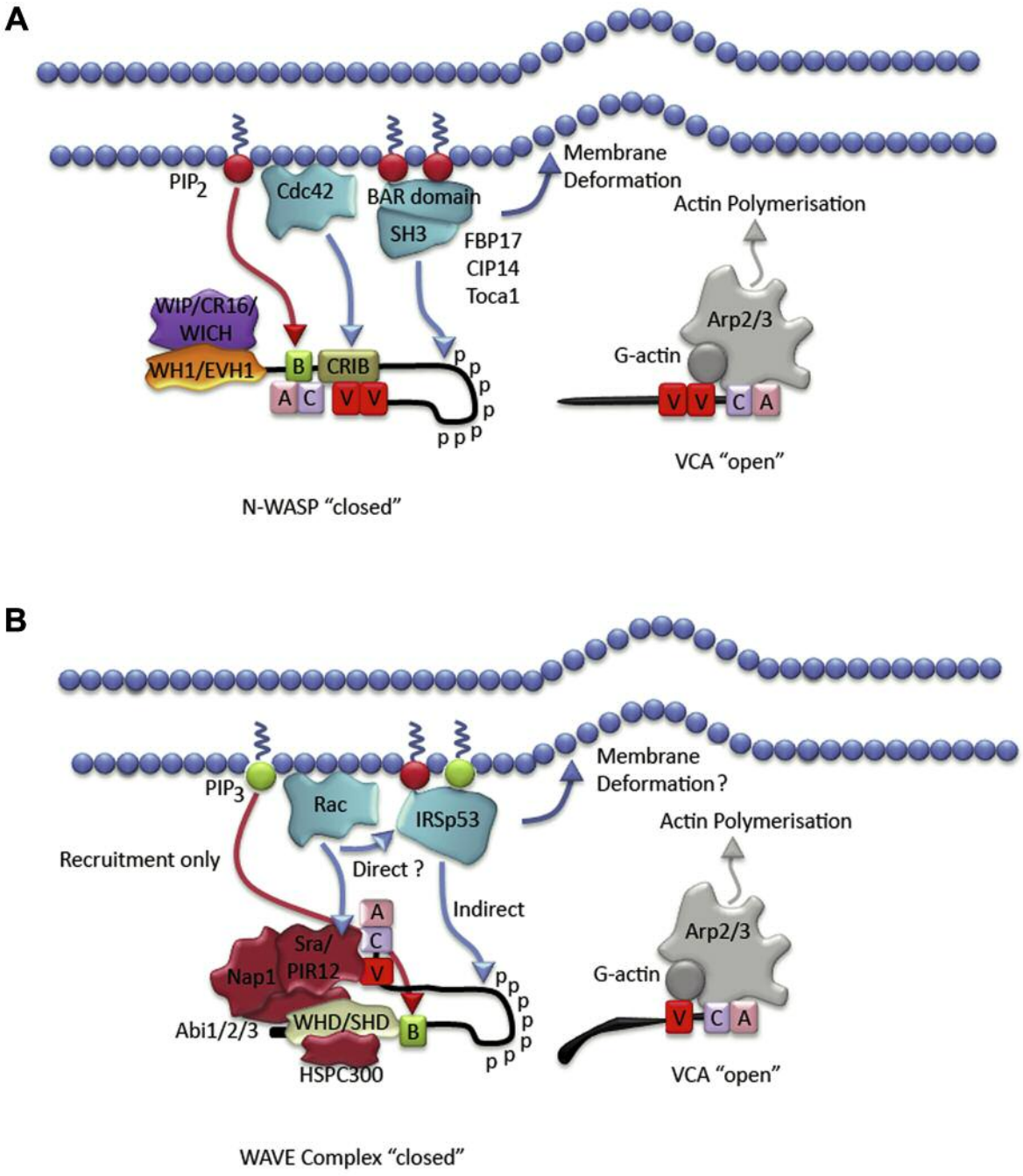

Figure 3. *Regulatory pathways for N-WASP and WAVE2 activation. A. Auto-inhibition of the VCA domain by the Cdc42/Rac interactive binding (CRIB) domain of N-WASP. SH3 domain binding to N-WASP can activate N-WASP. B. WAVE protein forms a complex within the cell with Nap, HSPC, PIR and Abi2 which keeps WAVE inactive. Locally-activated Rac binds to the Nap-PIR-Abi2 subcomplex releasing the HSPC-WAVE subcomplex, which binds to the Arp2/3 complex to stimulate actin polymerisation.*

All WAVE proteins share a common carboxyl- (C-) terminus comprised of the verprolin homology domain (V) also known as WASP homology 2 (WH2) domain, central homology sequence (C) and an acidic region (A), which together cumulate as the VCA region that is homologous and serves the same purpose as the WASP and N-WASP C-terminal in actin monomer and Arp2/3 complex interaction. Moreover, the similarity seen in protein domains

of all five WASP and WAVE members extends to the presence of a highly basic region and a long proline region between the amino- (N-) terminus and C-terminus of these proteins. The distinguishing factor between the WASP and WAVE proteins is the WH1 and GBD/CRIB domains characteristically seen at the N-terminus of WASP proteins that are absent in the WAVE proteins. In contrast, the N-terminus of WAVE proteins possesses the WAVE homology domain/SCAR homology domain (WHD/SHD). (Figure 1 shows the domain structure of the WAVE proteins).

WAVE activation

Unlike the WASP proteins that exist independently in cells, each WAVE protein is found to be associated with four additional proteins *via* its WHD to form the WAVE regulatory complex (WRC). The components of this 400kDa pentameric heterocomplex were described by Eden *et al*., in 2002 as comprising Abi (Abelson-interacting protein), Nap1/Hem-2, Sra1/Cyfip1 and HSPC300/Brick1 (61). These authors proposed that this complex keeps WAVE inactive within the cell with the addition of locally activated Rac relieving the inhibition by binding to the Nap-PIR-Abi2 subcomplex releasing the HSPC-WAVE subcomplex, which also binds to the Arp2/3 complex to stimulate actin polymerisation (61) (Figure 3B). This trans-inhibitory model controlling WAVE activation may explain how actin nucleation in the cytoplasm can be prevented but does not explain how the activity of the complex in actin nucleation is site-directed in regions of membrane protrusions. Characterisation of the various partners of WAVE within the WRC has identified Abi1 as an essential component of the complex (62), and increases WAVE2 actin polymerisation activity by directly binding with the WHD domain of WAVE2. Furthermore, it has been shown that the Abi1-Nap1-PIR21complex with WAVE1 and WAVE2 were not disrupted following the addition of activated Rac (62,63). This is in contrast to the findings of Eden *et al*., and further results have given added support to the belief that, in Drosophila and mammalian cells, WAVE proteins form stable complexes that are not disrupted following activation by Rac. Individual deletion of the expression of WAVE, Nap1, PIR121/Sra1 or Abi1 by RNAi methods led to the failure of Rac-induced actin remodelling and lamellipodia formation (62-64). This seems to indicate that PIR121/Sra-1, Nap1 and Abi have positive, rather than inhibitory, roles to play in WAVE regulation. Other studies have shown the WAVE2 complex, isolated from the membrane fraction of cells, to be fully active without dissociation of the complex, however, the WAVE 2 complex isolated from the cytosol was inactive (65). In order to clarify the intrinsic activity of the WAVE complex, Derivery *et al*., (2009) (66) employed an approach whereby the human WAVE complex was purified using a stable cell line expressing a tagged subunit. The endogenous complex formed around this subunit was then purified by affinity chromatography. The activity of this WAVE complex was then assessed using pyrene-actin assay in the presence of purified Arp2/3 complex. These assays unequivocally demonstrated that the WAVE complex in its native conformation is intrinsically inactive. These authors concluded that the discrepancies in the state of activity of the WAVE complex may be a result of differences in purification procedures and risks of denaturation. Similarly, Ismail *et al*. (67) showed that WAVE activity is inhibited within the WRC complex. Utilising actin assembly assays it was shown that WAVE1 VCA concentrations of 100-500 nM resulted in substantial activation of the Arp2/3 complex whereas the same concentrations of WRC components produced practically no activation (67). Work by other authors has identified an interaction between the transmembrane cell adhesion molecules protocadherin 10 and 19 (PCDH10 and PCDH19) and the WAVE regulatory complex *via* Nap-1 (68, 69). Recently, the biochemical interactions between these molecules and the WRC have been investigated and a conserved peptide motif termed the WRC interacting receptor sequence (WIRS) has been identified (70). A further 120 membrane-associated proteins, have been identified including protocadherins, which contain the WIRS motif. This sequence directly binds a conserved surface on the WRC formed by the Sra and Abi subunits and provides an important link between diverse membrane proteins, the WRC and the actin cytoskeleton (70). The importance of the WIRS can also be seen where synaptic adhesion molecule SYG-1 interacts with the WRC through the WIRS in its cytoplasmic tail leading to local F-actin assembly. This may provide a means of limiting the activity of the WRC to specific cell regions (71).

Integration of the WAVE protein with other subunits to create multi-protein complexes would also appear to act as an intra-complex mechanism to inhibit WAVE. Within the WRC, the V and C regions of WAVE1 are sequestered by Sra1 as a means of blocking WAVE activation. Actin-binding residues of the V region are concealed by Sra1 binding making it impossible for monomeric actin to associate (Figure 3B and Figure 4). Coupled with a combination of inter-protein contacts within the WAVE1 structure, the V region is rendered inactive and the WRC is induced into a configuration which is incompatible with actin association and therefore suppresses actin polymersiation. Studies have shown that mutations of certain Sra1 residues, important for actin V region binding, enables WRC association with the Arp2/3 complex and consequently stimulates actin filament branching. Moreover, the effects of mutations of particular residues of the C region have been found to reduce WRC activity towards the Arp2/3 complex (72).

The WRC is constitutively inactive without intervention by Rac GTPases, phosphatidylinositols and/or kinases. Recruitment of the WAVE proteins to the plasma membrane

is facilitated by Rac GTPases. However, WAVE proteins are unable to interact directly with Rac in the same way WASP and N-WASP do with Cdc42. Instead, WAVE relies on components of the WRC to elicit these effects. RNA interference studies have shown how the removal of either Sra-1 or Nap1 prevented the ability of cells to produce Rac-dependent lamellipodia (63). Whether Rho GTPase-dependent activation of WASP or WAVE is direct or not, regulating their activity is controlled *via* the competitive binding of GTPases.

The competitive binding of Cdc42 which disrupts intramolecular interactions between the CRIB domain and VCA region, induces WASP out of its intrinsically inactive state. In a similar way, deletion of the VCA region of WAVE was found to increase the affinity between WRC and Rac1 as VCA deletion released Sra1 for Rac1 binding (72). This could explain previous findings that certain Sra1 mutations impeded Arp2/3 complex activation by WRC; this is likely to be due to reduced affinity of Rac1 for WRC.

Although the relationship between Rac and WAVE in cell motility has been long established, it would seem that Rac is not the sole GTPase activator of the WRC. *In vitro* approaches have demonstrated the affinity of Rac1 for WRC interaction was relatively low as was the case for WRC activation by Arf1 GTPase alone. However, upon the coordinated efforts of Rac1 and Arf1 together, WRC recruitment and activity at the plasma membrane were greatly enhanced (73).

Additionally, proteins comprising the WRC have shown the ability to become phosphorylated at various residues with some modifications showing enhanced signalling activity of the complex (74-76). Phosphorylation of residues within regions of WAVE has been shown to be an influential factor in WRC activity with the potential to facilitate actin polymerisation (76). It has been proposed that specific phosphorylation modifications could affect the stability of helix structures of the VCA motif and thus Sra1 interaction. WAVE1 phosphorylation of serine residues by cyclin-dependent kinase 5 (Cdk5) suppresses its ability to activate actin polymerisation through the Arp2/3 complex (77). However, phosphorylation of WAVE1 at tyrosine residue 125 by the non-receptor tyrosine kinase Src was shown to enhance both Arp2/3 complex association and activity *in vitro* and *in vivo* (74). Likewise, phosphorylation of the tyrosine residue 150 in WAVE2 by Ableson (Abl) non-receptor tyrosine kinase was found to be essential in actin polymerisation and cytoskeletal remodelling as Y150 mutations hindered these effects (75). Furthermore, Abl-mediated WAVE3 phosphorylation was shown to phosphorylate four tyrosine residues in WAVE3 (Y151, Y248, Y337 and Y486) and promoted lamellipodia formation and cell motility (76) (Figure 2 shows WAVE2 binding partners).

WAVE and Cancer

Work analysing WAVE and its association with breast cancer, carried out in our laboratories, demonstrated an overall trend of elevated expression in all three isoforms in breast tumor tissues relative to normal breast tissue. This pattern of expression was also evident for patients who died from breast cancer with WAVE2 levels reaching statistical significance. Furthermore, node-positive specimens and moderately and poorly differentiated tumors exhibited significant WAVE2 overexpression (78).

The clinical significance of WAVE in cancer was further implicated by Fernando *et al*. who demonstrated higher expression levels of WAVE1 and WAVE3 in the metastatic prostate cancer cell lines, PC-3 and DU-145 in comparison with epithelial prostate cancer cells. Accordingly, immunohistochemistry techniques revealed stronger staining for WAVE1 and WAVE3 in prostate tumor specimens compared to normal prostate specimens. WAVE1 knockdown in PC-3 and DU-145 cells revealed a significant reduction in growth rate and invasive capacities of the cells whilst the same approaches were utilised to knockdown WAVE3 expression which showed a significant decrease in cell invasion (79, 80). An independent research group also demonstrated suppression of *in vitro* cell invasion following WAVE3/WASF3 gene inactivation in metastatic prostate cancer cells, PC-3 and DU-145. Furthermore, they were also able to show a reduction in cell motility as well as decreased proliferative abilities which contrast with findings published by Fernando *et al.,* which showed no significant change in cell growth. The same group also evaluated the *in vivo* effects by injecting WAVE3 knockdown prostate cancer cells into the flanks of mice. Tumor growth rate was significantly reduced as well, as there was no evidence of metastatic spread to the lungs in mice injected with WAVE3 knockdown cells compared to the control group (81). These findings mirror those on *in vivo* breast cancer and the effects of WAVE3 knockdown (76).

However, the role of WAVE proteins in cancer is not clear and may be different in different types of cancer. Spence *et al*. (82) show that WAVE3 knockdown does not affect invasion of cancer cells *in vitro*. Tang and colleagues show that NWASP and WAVE2 have different roles in migration and invasion of cancer cells; the WRC actually inhibits invasive migration of epithelial cells in 2D, which triggers an N-Wasp-dependent invasion program involving FAK (83).

Looking more closely at the members of the WAVE regulatory complex, Silva *et al*., (2009) (84) have shown that Cyfip1 is deleted in human epithelial cancers and has been identified as a potential tumor suppressor. Loss of Cyfip1 was shown to lead to changes in WAVE-regulated actin dynamics and changes in cell-cell adhesion and cell-ECM contact. These authors have shown a clinical association with

poor prognosis in colon and breast cancer cohorts, with low expression of Cyfip1 significantly associated with higher tumor stage and with lymph node metastasis in IDC and with vascular invasion and higher stage in colon cancer patients (84). Interestingly, members of the WRC have been shown to have a variety of expression levels in different breast cancer cell lines, with higher expression levels of Abi1 present in more invasive breast cancer cell lines compared with less invasive breast cancer cell types (85). Furthermore, down-regulation of Abi-1 expression in MDA-MB-231 cells lead to a decrease in adhesion, proliferation, migration and invasion.

WASP and Human Disease

Discovery of the WASP gene stemmed from the identification of mutations in the gene in patients affected by Wiskott-Alrich syndrome (WAS). Affected individuals present a broad spectrum of symptoms and severity, with some patients exhibiting the full triad of clinical manifestations with poor survival rates compared to those with a milder phenotype who survive to adulthood. The genetics underlying WAS have been linked to several hundred mutations in the WASP gene with some evidence of a genotype to phenotype relationship (86). For example, missense mutations within the first three exons of the WASP gene are associated with individuals displaying mild symptoms whilst those with nonsense, frameshift, splice site, insertion or deletion mutations in the WASP gene are linked with symptoms of a more aggressive nature (87). On a molecular level, such mutations within the WASP gene would result in a defective protein product and could cause a decline in WASP activity. Alternatively, mutations within important domains of WASP could disrupt its specified function. For instance, amongst the missense mutations identified in the WASP gene, the vast majority of these are found within the regions that encode the WH1 domain. Aberrations within this protein domain could potentially interfere with WIP interaction.

In addition to platelet abnormalities, immunological defects and eczema being commonly observed in affected individuals, many WAS patients are at an increased risk of developing malignancies, especially those presenting with autoimmune disorders (88). Accordingly, the majority of these malignancies are lymphoreticular in origin and such malignant tumors can establish at a young age, although the frequency at which they occur is higher in adolescents through to adulthood. Statistics from a North American group of WAS patients found malignancies were present in 13% of the cohort with a mean age of onset of 9.5 years. The most common malignancy reported is B-cell lymphoma testing positive for Epstein-Barr virus (88).

Beyond the human malignancies associated with the WAS clinical phenotypes, the WASP family of proteins have also been linked to other cancers. Using immunohistochemical approaches, N-WASP expression was demonstrated to be lower in breast tumor tissue compared to normal mammary epithelial cells. The same study also revealed a link between tumors from patients with a poor prognosis and significantly lower N-WASP levels compared to those with a good prognosis. Forced expression of N-WASP was induced in the breast cancer cell line MDA-MB-231 that displayed significantly reduced invasive and motility abilities (89). The outcome for patients with breast cancer has been shown to be related to the levels of N-WASP within the tumor tissues, with a stepwise decrease of the levels of N-WASP seen from good prognosis through to poor prognosis (89). Another study using the metastatic breast cancer cell line, MTLn3 revealed the use of either dominant negative N-WASP cells or treating cells with shRNA targeting N-WASP considerably decreased the ability of invadopodia formation, fundamental cell protrusions for cell invasion (90). Similarly, N-WASP expression was found to be higher in invasive breast cancer samples than in normal tissue and this study outlined a role for N-WASP in invasion by controlling the spatial arrangement of MT1-MMP in relation to actin-based pseudopodia. Despite the contradicting conclusions drawn from these studies, it would be logical to associate WASP abnormalities with human cancer considering their roles in actin polymerisation, a driver of cell motility, a contributory trait to cancer progression.

WAVEs and WASP as Therapeutic Targets

More and more, data is emerging confirming that abnormalities of the WASP and WAVE proteins are linked to clinical outcome in cancer. Increased WAVE1 is associated with invasiveness and growth of prostate cancer cells (79). Similarly, enhanced cell motility stimulated by abnormal upregulation of WAVE2 has been linked to cancer invasiveness and metastasis (92), with Arp2/WAVE2 colocalisation providing a risk factor for liver metastasis in colorectal cancer (93). Targeting these molecules has been highlighted as a fundamental step in the journey towards cancer control. Further research to elucidate the role played by WAVE in cancer pathology could lead to novel cancer therapeutics.

Summary

The present review highlighted the role of the small GTPases of the Rho family and their activation of WASP family proteins in relation to cell motility. It is clear from the evidence presented that the WASP family, especially WAVE proteins, are strongly associated with migration of a range of tumor cells, and are implicated in tumor cell invasion and metastasis. They are shown to be linked with the

aggressiveness and invasiveness of cancer cells, in relation to their function as nucleators of actin *via* association with the Arp2/3 complex, driving lamellipodia formation and cell motility. As more evidence emerges to link the WASP family proteins as regulators of cancer cell motility, targeting these molecules may be an important step in preventing cancer cell metastasis.

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