MINI-REVIEW

WAVEs: A Novel and Promising Weapon in the Cancer Therapy Tool Box

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Abstract

The Wiskott-Aldrich Syndrome Protein family Verprolin - homologous proteins (WAVEs), encoded by a metastasis promoter gene, play considerable roles in adhesion of immune cells, cell proliferation, migration and destruction of foreign agents by reactive oxygen species. These diverse functions have lead to the hypothesis that WAVE proteins have multi-functional roles in regulating cancer invasiveness, metastasis, development of tumor vasculature and angiogenesis. Differentials in expression of WAVE proteins are associated with a number of neoplasms include colorectal cancer, hepatocellular cancer, lung squamous cell carcinoma, human breast adenocarcinoma and prostate cancer. In this review we attempt to unify our knowledge regarding WAVE proteins, focusing on their potentials as diagnostic markers and molecular targets for cancer therapy.

Keywords: Wiskott-aldrich family verprolin- homologous protein - WAVE - actin-cytoskeleton - metastasis

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Introduction

Cancer is one of the most dreadful diseases of the 20th century and the second leading cause of mortality worldwide. World Health Organization (WHO) reports that death from cancer accounting for 7.6 million deaths in 2008 and it could increase with 12 million deaths by the year 2030. Cancer arises through multistep processes in which tumor invasion and metastasis become an imperative phase. Tumor metastasis is accomplished through a series of processes where tumor cells leave a primary tumor to colonize other sites of the body, which is a major cause of death for cancer patients and seems to be a major obstacle for successful cancer therapy. Tumor invasion and metastasis, the most essential biological characteristics, are related directly to poor prognosis and mortality of patients (Peng et al., 2011; Zhang et al., 2011). Metastasizing cells must first disseminate from the primary tumor, invade the surrounding tissue, intravasate and extravasate the circulatory system, initiate angiogenesis and colonize distant sites while evading the immune system. Each steps must successfully completed to give rise to a metastatic tumor. The WAVE protein (Wiskott-Aldrich family Verprolin-homologous protein) an actin-cytoskeleton and remodelling protein identified in humans in the year 1994. Therefore, in this review we discuss the roles of WAVE proteins during tumor progression and as a potential molecular target for cancer therapy (Derry et al., 1994; Miki et al., 1996; Miki et al., 1998; Kurisu et al., 2009).

WASP and WAVE: Two Molecular Adaptor Complexes

In mammals, the Wiskott-Aldrich Syndrome Protein family consists of two subfamilies, WASP and WAVE. WASP subfamilies possess WASP and Neural-WASP (N-WASP). WAVE subfamily possesses $WAVE_1$/ $SCAR_1$, $WAVE_2$, and $WAVE_3$. Human $WAVE_1$ and $WAVE_3$ gene are located in the brain and moderately present in hematopoietic lineages and $WAVE_2$ gene is expressed ubiquitously in mammals. WAVE protein consists of 498 and 559 amino acids and are encoded by 9 to 12 exons and the length of $WAVE_3$ gene are 131.2 Kb, where as N-WASP and WASP are around 67.1 Kb and 7.6 Kb respectively (Millard et al., 2004; Vartiainen et al., 2004; Deeks & Hussey, 2005; Stradal & Scita, 2006; Takenawa & Suetsugu, 2007). WAVE proteins are also known as suppressor of cAMP receptor (SCAR) proteins. In humans the multifunctional WAVE complex protein act as key factor for reorganization of the actin cytoskeleton which are essential for broad spectrum of cellular functions includes immune response for the formation of cell proliferation, adhesion of immune cells, migration, formation of phagosomes and phagocytosis of foreign agents and destruction of foreign agents by reactive oxygen species (ROS). In addition WAVE has been identified as a key mediator in the development of cancer by regulating cancer invasiveness, metastasis, tumor vasculature and angiogenesis (Bokoch, 2005; Burkhardt et al., 2008; Fooksman et al., 2010; Harwood &

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Batista, 2010; Hidalgo & Frenette, 2007). In order to find more effective target for anticancer drugs, it is essential to discuss WASPs and WAVE proteins in greater detail and its relevance with cancer invasion (Kurisu and Takenawa, 2010).

WAVE in Actin Dynamics

Actin is the most abundant protein in the eukaryotic cells and it acts as dominant structural components of lamellipodia. The actin polymerization and reorganization have an important role in the regulation of many cellular events (Connor et al., 2008). WASPs and WAVEs protein involves in initiation of actin polymerization process. The association of WASP family scaffolding proteins with actin cytoskeleton occurs through two conserved binding motifs such as verprolin homology (VPH) domain and C-terminal acidic domain that binds to Arp2/3 complex which is a major actin nucleator in cells. The Arp2/3 complex consists of two actin like proteins Arp2 and Arp3, which serves as an actin pseudodimer (Machesky et al., 1998). WAVEs are thought to act downstream of the Rac GTPase (Rho-family small GTPase), connecting Rac activation to the induction of Arp2/3-mediated actin polymerization (Suetsugu et al., 2003). Regulation of dynamic filamentous actin remodelling by $WAVE_1$ and $WAVE_2$ is necessary for the formation of mature immunological synapse (a stable contact between two T-cells). Thus, if the WAVE proteins are defective and failure of actin polymerization results in loss of function of T-cells lead to immunodeficiency. In eukaryotic cells, the cell motility and invasion require highly coordinated regulation of actin dynamics process. The deregulations of WAVE proteins lead to aberrant enhancement of cell motility phenotypes which are correlated with cancer invasiveness and metastasis. Cancer cells extend cell protrusions which are driven by actin polymerization and thus, co-ordinated inactivation of cell-cell adhesion and actin polymerization is required for active invasion and metastasis. The functions of Arp2/3 complex are enhanced during cancer conditions and the co-localization of Arp2 and $WAVE_2$ are associated with tumor budding and irregular invading growth (INF) for higher metastatic potential during tumor progression (Keiichi et al., 2007).

WAVE in Tumor Progression

WAVEs, the fascinating proteins play an important role in cell motility, migration, invasion, tumor progression and metastasis. Yet the exact mechanism behind the role of WAVEs in promoting tumor progression has not been defined. Deregulation of WAVE proteins has been implicated in a number of cancers. $WAVE_1$ involves in the pathogenesis of childhood acute lymphoblastic leukaemia and with acute myeloblastic leukaemia (Sossey et al., 2007; Wang et al., 2008; Yang et al., 2010) and $WAVE_2$ expression are higher during colorectal cancer and in hepatocellular carcinoma tissues (Yang et al., 2006). The $WAVE_2$-Arp2/3 and Rac-$WAVE_2$ signalling enhanced in facilitating invasion and metastasis during breast cancer and in murine melanoma cells respectively in experimental animals (Kurisu et al., 2005; Yokotsuka et al., 2011). The interaction of $WAVE_2$ with HSPC300 (Haematopoietic stem/progenitor cell protein 300) increases metastatic potential of lung squamous cell carcinoma (Cai et al., 2009). Therefore these evidences provide a better understanding towards the human $WAVE_1$ and $WAVE_2$ protein that have a greater potential role during tumour progression and metastasis. $WAVE_3$ is highly expressed in advanced stages of breast cancer (adenocarcinoma MDA-MB-231 cells) and also involve in regulating tumor volume, metastasis, vascular endothelial growth factor (VEGF) and angiogenesis. The expression level of $WAVE_3$ is higher in grade III tumors whereas no $WAVE_3$ are detected in normal breast and grade I tumors. Together, these findings reveal that the expression level of $WAVE_3$ is correlated with breast cancer progression (Sossey et al., 2007). Cell migration plays an important role in embryogenesis, angiogenesis, wound repair and during cancer metastasis (Lauffenburger & Horwitz 1996; Christopher & Guan 2000). Focal adhesion kinase (FAK) a cytoplasmic tyrosine kinase involves in regulating the cell migration (Parsons et al., 2000; Hauck et al., 2002) but FAK signalling pathway is poorly understood. The N-WASP which is also essential for regulating the actin cytoskeleton remodelling involves in cell migration and during cancer progression (Takenawa & Miki 2001; Ren et al., 1999). The over expression of N-WASP decreases the growth, adhesion and invasiveness of cancer cells thereby knockdown of N-WASP may increases in the growth, adhesion and invasiveness of the cancer cells. Blocking of FAK by using the FAK inhibitor increases the invasion due to the knockdown of N-WASP (Lauffenburger & Horwitz 1996). FAK plays a major role in cancer progression through the activation of N-WASP by activating FAK-N-WASP-ARP2/3 complex which regulates cell migration (Sanchez et al., 2010). This provide an ideal mechanism by which FAK act as substrate for N-WASP protein interacts to regulate cancer progression which enables the cancer cells to behave more aggressive (Martin, 2011). The interaction between N-WASP and FAK regulated in phosphorylation of Tyr^{256} in a FAK dependent manner. The N-WASP is regulated through nuclear translocation and tyrosine phosphorylation (Wu, 2004). Tyr^{256} in WASP which is corresponds to Tyr^{256} of N-WASP is phosphorylated by Fyn, Btk, Lyn and Hyc. Thus the regulation of tyrosine phosphorylation is conserved with other member of WASP family regulating actin cytoskeleton in differential cell (Wu, 2004). The knockdown of N-WASP disrupts the formation of podosomes or invadopodia by which cancer cells facilitate elongated motility (Kurisu & Takenawa, 2010).

WAVE: Target for Cancer Therapy

Genetic studies in animal model and in cancer cell lines reveals that WAVE have direct role over tumor progression and metastasis. Yet, the precise molecular mechanisms behind the role of WAVE in promoting tumor progression are still being elucidated. Studies investigated the effects of silencing these WAVE family proteins in cell lines reported that knockdown of $WAVE_3$ expression in

human breast adenocarcinoma MDA-MB-231 cells using small interfering RNA (siRNA) resulted in a significant reduction of cell motility, migration and invasion which are correlated with the reduction in the levels of active p38 mitogen-activated protein kinase. Research on breast cancer MDA-MB-231 cells expressing short hairpin RNA to $WAVE_3$ ($shWAVE_3$) shows that significant reduction in matrigel invasion and lung colony formation in mice. In the orthotropic model there was a greater reduction in growth rate of the primary tumor, as well as in the metastasis to the lungs. These genetic data collectively provide clear insight in the $WAVE_3$-p38MAPK pathway that have direct role in breast cancer progression and metastasis. Down regulation of $WAVE_3$ inhibits lung metastasis of MDA-MB-231 cells in experimental animal models (Sossey et al., 2007).

Interestingly, the effect of $WAVE_3$ knockdown on the metastatic potential of MDA-MB-231 cells reveals that there is a reduction in tumor colonies on the lung surface indicating reduced *in vivo* metastasis of the experimental animals. A close correlation has been proved between VEGF-C/VEGFR-3 expression and lymph node metastasis in primary laryngeal carcinoma (Wang et al., 2012). The knockdown of $WAVE_3$ decreases the levels of VEGF and angiogenesis which are critical for the establishment and tumor progression. This provides convincing evidence that $WAVE_3$ could play a vital role in the acquisition of the metastasis tumor cells. Furthermore, $WAVE_3$ have direct effect on motility and invasion of breast cancer cells. The proteins of this family regulate actin polymerization through the recruitment of the Arp2/3 protein complex via a verprolin-cofilin-acidic domain at the C terminus. It is thought that the formation of the multimeric complex comprising the verprolin-cofilin-acidic domain, the actin monomer, and activation of Arp2/3 complex is a critical step in actin polymerization (Pollard & Borisy, 2003; Takenawa & Miki, 2001).

The suppression of $WAVE_3$ by siRNA in breast cancer MDA-MB-231 cells dramatically reduces lamellipodia (membrane rufflings) formation at the migrating edge while increasing actin stress fibers linked to focal adhesion, which is generally associated with static cells. This in vitro phenotype was accompanied by a significant decrease of cell migration and invasion, suggesting that $WAVE_3$ is a critical factor that regulates the motility and adhesiveness of cells. These findings further support, $WAVE_3$ could also play a vital role in the acquisition of the metastatic potential of tumor cells and this provide an direct evidence for the functional importance of $WAVE_3$ in tumor cell invasion and metastasis (Sossey et al., 2005). As recently pointed out by Fernando et al., the expression of $WAVE_1$ and $WAVE_3$ in prostate cancer cell lines and in prostate tissues respectively, reveals the importance of $WAVE_1$ and $WAVE_3$ in controlling the invasiveness of prostate cancer cells (Fernando et al., 2008 and 2010).

The presence of Arp2/3 complex and $WAVE_2$ in colorectal cancer cells disclose that the co-localization of Arp2 and $WAVE_2$ appears as an independent risk factor for liver metastasis. The interaction of Arp2 and $WAVE_2$ are highly associated with tumor budding which leads to loss of cell-cell adhesion. Further by blocking the interaction of this complex in cancer conditions may prevent invasion and metastasis (Keiichi et al., 2007). The ectopic expression of RacCA (GTP-binding protein Rac) and $WAVE_2$ in malignant B16F10 mouse melanoma cells increased the invasiveness and silencing the $WAVE_2$ through RNAi on the B16F10 cells resulted in suppression of membrane ruffling, cell motility and pulmonary metastasis suggest that suppression of $WAVE_2$ expression could prevent the cancer invasion and metastasis (Kurisu et al., 2005).

Conclusions

Despite the detailed knowledge about molecular and cellular mechanism of WAVE and WASP super family proteins in tumor growth; invasion and metastasis context is still quite limited. This review highlights that the WAVE proteins are expressed in higher levels in experimental cancer models and thereby suggests that WAVE proteins can be used as a better diagnostic marker and could be a potential molecular target for cancer therapy. Further investigations into cellular mechanisms of WAVE proteins and how critical signaling pathways interact will explore a new therapeutic approach in diverse range of tumors.

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