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NME genes in epithelial morphogenesis

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Abstract

The *NME* family of genes encodes highly conserved multifunctional proteins that have been shown to participate in nucleic acid metabolism, energy homeostasis, cell signaling, and cancer progression. Some family members, particularly isoforms 1 and 2, have attracted extensive interests because of their potential anti-metastasis activity. Unfortunately, there have been few consensus mechanistic explanations for this critical function because of the numerous molecular functions ascribed to these proteins, including nucleoside diphosphate kinase, protein kinase, nuclease, transcription factor, growth factor, among others. In addition, different studies showed contradictory prognostic correlations between *NME* expression levels and tumor progression in clinical samples. Thus, analyses using pliable *in vivo* systems have become critical for unraveling at least some aspects of the complex functions of this family of genes. Recent works using the *Drosophila* genetic system have suggested a role for *NME* in regulating epithelial cell motility and morphogenesis, which has also been demonstrated in mammalian epithelial cell culture. This function is mediated by promoting internalization of growth factor receptors in motile epithelial cells, and the adherens junction components such as E-cadherin and β -catenin in epithelia that form the tissue linings. Interestingly, *NME* genes in epithelial cells appear to function in a defined range of expression levels. Either down-regulation or over-expression can perturb epithelial integrity, resulting in different aspects of epithelial abnormality. Such biphasic functions provide a plausible explanation for the documented anti-metastatic activity and the suspected oncogenic function. This review summarizes these recent findings and discusses their implications.

Keywords

NME genes; Awd; *Drosophila*; Epithelial morphogenesis; Follicle cell; Trachea

Introduction

The *Nm23* family of genes (or using the unified name for the genomic locus, *NME*) encodes nucleoside diphosphate kinases (NDPKs), which generate nucleoside triphosphates from cognate nucleoside diphosphates using ATP as the phosphate source. This enzymatic activity was originally recognized as a house-keeping function involved in nucleic acid synthesis and energy metabolism, described by two Nobel laureates in their respective fields, Paul Berg and Hans Krebs (Berg and Joklik 1953; Krebs and Hems 1953). From a completely different line of study, the first mutant allele of *NME* was discovered by another scientific giant Alfred Sturtevant, who in 1956 described a curious *Drosophila* mutant that conferred lethality to the otherwise viable eye color mutant *prune* (Sturtevant 1956). This mutant, termed *Prune-killer* (subsequently renamed *Killer-of-prune*), later turned out to be

an allele of the *Drosophila* homolog of *NME* gene, *abnormal wing discs (awd)*. Since *Drosophila* eye color is determined by the level of drosoterpine, the synthesis of which requires hydrolysis of GTP by the enzyme GTP cyclohydrolase (Fan et al. 1976), the genetic interaction between *NME/awd* and the eye color mutant seemed to also implicate the NDPK function. The exact nature of this early example of “synthetic lethality” is still unknown, however. Indeed, the functional complexity of this small (17–19 kD) protein has been the hallmark of this field of research from the very beginning. In this review, I will attempt to summarize only one aspect of this fascinating family of genes: the function related to its role in regulating epithelial cancer formation.

***NME* as a metastasis suppressor**

In the late 1980s, mouse *NME1* (*Nm23M1*) was identified as a potential metastasis suppressor gene when it was isolated as a cDNA clone down-regulated in metastatic derivatives of a murine melanoma cell line K-1735 (Steeg et al. 1988). This clone was named *Nm23* for genes expressed in non-metastatic cells. At about the same time, a *Drosophila* gene involved in imaginal disc development was cloned (Dearolf et al. 1988a, b). The *Drosophila* gene, *awd*, is highly conserved (~78% amino acid identity) compared with mammalian *NME1* and *NME2* (Biggs et al. 1990; Rosengard et al. 1989) and is the gene mutated in *Killer-of-prune* (Biggs et al. 1988). It was soon discovered that this “new” family of genes in fact encodes the old nucleoside diphosphate kinases (Biggs et al. 1990; Wallet et al. 1990). In human, the gene family consists of ten related members (Desvignes et al. 2009), but the *NME1* (*Nm23-H1*) and *NME2* (*Nm23-H2*) isoforms are the most closely related and are the ones most implicated in tumor progression. They are also evolutionarily highly conserved. Indeed, the *awd* lethal phenotype can be rescued by exogenously expressed human *NME2* (Xu et al. 1996).

In xenograft tumor models, *NME* has been shown to inhibit metastasis but not primary tumor growth of cell lines derived from human breast cancer, murine melanoma, rat colon cancer, and human oral squamous cancer (Ouatat et al. 2003). However, in clinical cancer samples, the situation is not as clear-cut. While in earlier breast cancer studies, there were statistically significant correlations between reduced *NME* expression levels and metastasis [reviewed in (Heimann and Hellman 2000)], in later studies, no clear correlation comparing benign and metastatic breast cancers could be discerned [e.g., (Sirotkovic-Skerlev et al. 2005)]. There are many other such discrepancies [for a few examples, see (An et al. 2006; Anwar et al. 2004; Galani et al. 2002; Ouellet et al. 2006)]. Furthermore, in other cancer cohorts, particularly those of ovarian cancers, up-regulated *NME* levels have been correlated with poor prognosis (Harlozinska et al. 1996; Mandai et al. 1994), suggesting an oncogenic function for *NME*. While many of the discrepancies can be attributed to the method used (Northern vs. IHC, for example) or quality of the reagents (antibody specificity, for example), these observations also suggest that the physiological and developmental functions of *NME* family of genes operate in a defined range of expression levels. Thus, either over-expression or loss of expression of *NME* may result in abnormal cellular phenotypes.

Multiple functions of *NME*

Further complicating the issue of *NME*'s role in tumor progression is the multiple enzymatic functions assigned to this gene: (a) nucleoside diphosphate kinase (NDPK) that transfers the terminal phosphate group from ATP to a nucleoside diphosphate (such as GDP), through the formation of an intermediate histidine-phosphate linkage (Ouatat et al. 2003); (b) DNA binding and DNA nuclease that are involved in transcriptional regulation (Ma et al. 2002; Postel et al. 2000); (c) DNase activity that is activated by granzyme A in caspase-

independent apoptosis (Fan et al. 2003); (d) 3' exonuclease activity that may confer an anti-mutating function (Zhang et al. 2011); (e) secreted growth factor that binds to the oncogenic form of MUC1 surface protein (Hikita et al. 2008; Okabe-Kado et al. 2009); (f) histidine-dependent protein kinase (Engel et al. 1995; Inoue et al. 1996; Wagner et al. 1997). The protein kinase enzymatic activity is similar to that of NDPK but toward protein substrates (Besant et al. 2003). Some tantalizing kinase targets have been identified such as aldolase C, kinase suppressor of Ras (Ksr) (Steeg et al. 2003), the G β subunit of the heterotrimeric G protein (Cuello et al. 2003; Hippe et al. 2003; Hippe et al. 2009), and the Ca²⁺-activated K⁺ channel KCa3.1 required for Ca²⁺ influx and activation of B and T cells (Srivastava et al. 2006). Importantly, some evidence suggest that it is the protein kinase activity, not NDPK, that is important for cell motility control (Freije et al. 1997; MacDonald et al. 1996). It is important to note that NDPK and protein kinase activities are not necessarily mutually exclusive. Both nucleoside triphosphate production and protein modulation may be important in different cellular contexts.

It is at present difficult to reconcile such diverse and seemingly unrelated molecular functions. Besides NDPK and histidine-dependent protein kinase, which can be demonstrated at the molecular level, others cannot be explained enzymatically. NME proteins primarily function as homo- or heterohexamers. One plausible model is that the NME complex may work as a scaffold that facilitates the activities of many diverse enzymes, secreted protein action notwithstanding.

Early genetic studies in *Drosophila*

To date, the most physiologically relevant function of *NME* was revealed by genetic studies in *Drosophila* [reviewed in (Nallamotheu et al. 2009)]. Initially, *awd* mutations were shown to cause imaginal disc defects, hence its name *abnormal wing discs* (Dearolf et al. 1988a, b). Subsequent studies showed that *awd* transgene carrying the NDPK-dead mutation in the active site histidine residue (residue 119 in the *Drosophila* protein) failed to rescue the *awd* lethal phenotypes (Xu et al. 1996). It was also shown that primordial ovaries (containing both germline and somatic cells) from *awd* mutants failed to develop when transplanted into wild-type larvae, although primordial germ cells alone from mutant early embryos were capable of forming functional ovaries in wild-type surrogates (Dearolf et al. 1988a). Since in the latter case, the wild-type surrogate embryos provided the normal somatic cells to encase the mutant germ cells and reconstitute functional ovaries, the observation suggested that *awd* was not involved in germ line development but might play a specific role in the development of somatic follicle cells in the egg chamber. This predicted somatic function of *awd* later turned out to be the one that regulates epithelial morphogenesis (see below).

In an unrelated genetic screen for second-site mutations that exacerbate the neurological phenotype of a temperature-sensitive *dynammin* mutant (paralysis at 29°C due to defects in endocytosis-mediated neurotransmitter uptake), Krishnan et al. (2001) set out to isolate mutations that made *dynammin* mutant pass out at a lower temperature (25°C). Three lines of such *dynammin* phenotypic “enhancers” were isolated. Incredibly, all three are alleles of *awd*. This suggests that the functional relationship between *awd* and endocytosis is exceedingly specific and almost exclusive.

In the late 1990s, our laboratory demonstrated the first developmental function of the von Hippel-Lindau (*VHL*) tumor suppressor gene in *Drosophila* trachea formation (Adryan et al. 2000). Mutations in human *VHL* gene are the major genetic defect underlying renal cell carcinoma of the clear cell type (Kaelin 2002). The function of *Drosophila VHL* in tracheal morphogenesis was highly notable because of the tubular origin of the renal cancer. The *Drosophila* trachea is an epithelial tubule system for disseminating oxygen. Formation of

primary and secondary tracheal branches follows a stereotypical morphogenic program. The major chemotactic system in tracheal branching morphogenesis is mediated by fibroblast growth factor receptor (FGFR; encoded by the *Drosophila* *breathless* gene *btl*) (Affolter et al. 2003; Ghabrial et al. 2003; Uv et al. 2003). The guidance cue is provided by the FGF homolog Branchless (Bnl) expressed in target tissues distal to the migrating tips (Ribeiro et al. 2002; Sutherland et al. 1996) and is received by the receptor Btl expressed in the tracheal cells (Glazer and Shilo 1991; Klambt et al. 1992; Lee et al. 1996; Ohshiro and Saigo 1997). In an attempt to further characterize the function of *VHL* in *Drosophila*, a yeast two-hybrid screen was conducted to isolate VHL-interacting proteins, and Awd was isolated as such (Hsu, personal communication). It was subsequently shown that the NME protein is stabilized by VHL in *Drosophila* and in human kidney and renal carcinoma cells (Hsouna et al. 2010; Hsu et al. 2006; Nallamotheu et al. 2009; Urakami et al. 1997). Whether *NME* is involved in renal carcinoma progression requires further investigation, although a few clinical studies have suggested a link between loss of *NME* and renal cell carcinoma (Ayhan et al. 1998; Hiasa et al. 1996; Nakagawa et al. 1998). Meanwhile, the interaction of VHL and Awd prompted us to examine the role of *awd* in tracheal development.

Awd function in motile epithelial tissues

As expected, *awd* mutants exhibited the same ectopic tracheal tubule branching phenotype as observed in the *VHL* mutant (Dammai et al. 2003). Taking the cues from the *awd*-*dynammin* genetic interaction observed in the neurons, as described above, it was speculated that a defective endocytic process in *awd* mutant could result in over-accumulation of the chemotactic receptor Btl, elevated signaling, and unrestrained tubule cell migration. This proved to be true (Dammai et al. 2003). In *awd* mutants and in cultured *Drosophila* S2 cells, *awd* loss-of-function caused over-accumulation of Btl on all tracheal cell surface, leading to increased ERK activity, mesenchymal cell morphology, and ectopic cell migration (for an example, see Fig. 1). Importantly, the ligand Bnl expression in the stromal cells was not affected, neither was the proliferation and differentiation of the tracheal cells. Genetic analysis indicated that *awd* functionally interacted with *dynammin* as predicted. That is, the severity of the *awd* phenotype is exacerbated by the *dynammin* mutant. This study was the first in vivo demonstration of the potential anti-metastatic function of *NME*, as it negatively modulates the motility of tubule epithelia, but not the proliferation and fate determination of the cells. In fact, the disrupted tubule epithelium in *awd* mutants resembles the process of epithelial-to-mesenchymal transition (Fig. 1).

The finding of *awd* function in tracheal cells led to examination of similar roles in another motile epithelial cell system: the migration of border cells during *Drosophila* oogenesis, which has been considered an in vivo model for clustered epithelial cell invasion and epithelial-to-mesenchymal transition (Montell 2003; Rørth 2002). Each *Drosophila* egg chamber contains a germ cell complex—one oocyte and 15 nurse cells—that originates from one of two germline stem cells (Dobens and Raftery 2000). The germ cell complex is enveloped by a single layer of somatic epithelial follicle cells (Fig. 2a). The follicular epithelium mechanically supports the integrity of the egg chamber and communicates with the germ cells to provide positional cues for the developing oocyte. One key morphogenic event involving the follicular epithelium is the migration of border cells (Montell 2003; Rørth 2002). At the beginning of stage 9 (about 42 h after the egg chamber is formed and about 24 h before egg laying), two anterior polar cells recruit 4–8 neighboring cells to form a border cell cluster. The border cell cluster delaminates from the epithelium and invades through the nurse cell complex (Fig. 2a, b) until it reaches the anterior of the oocyte about 6 h later at stage 10, traversing a linear distance of ~100–150 μm . In its final location, the border cell cluster is critical for forming the micropyle (an eggshell structure for sperm entry) and providing anterior spatial cues for the future embryo. The precise movement of

the border cells is guided by the *Drosophila* PDGF/VEGF signaling pathway (ligand Pvf emanating from the oocyte and received by receptor Pvr on the border cells) (Fig. 2b) (Duchek et al. 2001; McDonald et al. 2003).

Interestingly, Awd is expressed in all somatic follicle cells but conspicuously lost in the migrating border cells immediately after they delaminate from the epithelium (Fig. 2a) (Nallamotheu et al. 2008). This observation led to the speculation that Awd may, as in trachea, regulate the internalization and signaling of the chemotactic receptor Pvr. Reduced Awd in migrating cells allows for surface localization of Pvr to receive the chemoattractant Pvf. Forced expression of Awd in the border cells using specific promoter predictably reduced border cell movement (Fig. 2c) (Nallamotheu et al. 2008). This was accompanied by a reduction of the Pvr level and decreased Pvr signaling as measured by the level of phospho-tyrosine. Interestingly, over-expression of constitutively active Pvr or a dominant-negative mutant of dynamin resulted in over-active signaling and “round-about” movement of the border cells (Bianco et al. 2007; Duchek et al. 2001; Nallamotheu et al. 2008); that is, the border cells cannot migrate toward the oocyte but instead spin around or move sideways. Forced expression of *awd* in these conditions reduced the levels of Pvr signaling and rescued the ectopic migration phenotype (Nallamotheu et al. 2008).

Thus, in intrinsically motile epithelial cells such as the tracheal cells and the border cells, *awd* regulates the surface levels of growth factor receptors, which in turn modulates cell motility.

***awd* function in organ-lining epithelium**

In the course of studying the function of Awd during oogenesis, we also noted prominent epithelial defects in *awd* mutant follicle cells, indicating a role in epithelial morphogenesis (Woolworth et al. 2009). The *Drosophila* follicle cells in fact have been a recognized and important model for studying epithelial establishment and maintenance.

The key characteristic of epithelial cells is asymmetrical specification of membrane domains, marked by domain-specific proteins (Fig. 3) (Knust and Bossinger 2002; Nelson 2003; Tepass 2002). The epithelial morphogenic mechanism, although with minor variations in different epithelial tissues, is highly conserved from worm to mammal. The critical initial step in establishing epithelial polarity is the specification of the apical domain, which is defined by the function of a complex containing atypical PKC, Bazooka (Baz; mammalian and worm PAR-3) and PAR-6 [reviewed in (Suzuki and Ohno 2006)]. The PAR complex is required for the localization of another apical complex containing Crumbs (Crb), Stardust (Std; mammalian Pals1), and Discs lost (Dlt; mammalian PATJ) (Tepass 2002). The PAR- and Crb-containing complexes occupy the apical-most region of the lateral membrane, just apical to the adherens junction (AJ).

The apical complex in turn restricts the localization of a third complex containing Scribble (Scrib; mammalian Scribble/Vartul), Discs large (Dlg) and Lethal(2) giant larvae (Lgl) (Betschinger et al. 2005; Bilder et al. 2003) to the basolateral domain, while Lgl also antagonizes the apical components and prevents their spreading to the basolateral side (Humbert et al. 2006; Yamanaka et al. 2003). The antagonistic actions of apical and basolateral complexes help define the apical-lateral locus eventually occupied by AJ (Humbert et al. 2006). However, it is not entirely clear how membrane distribution of AJ components is refined and precisely restricted to the apicolateral domain. Also curiously, the Par and Scribble/Dlg/Lgl complexes become agonists in facilitating cell migration at the periphery of motile cells (Humbert et al. 2006).

In *awd* mutant follicle cells, AJ components β -catenin (encoded by the *armadillo* gene, *arm*), E-cadherin (DE-cadherin) and α -spectrin are mislocalized (Woolworth et al. 2009). They spread to the apical membrane domain and the overall lateral membrane level also increases (for an example, see Fig. 2d). By contrast, apical complex components such as Dlg and basal-lateral component Lgl are largely unaffected. Concomitant with AJ component spreading, *awd* mutant follicle cells exhibit cell shape change and piling-up phenotype typical of adenoma (Woolworth et al. 2009). The mechanistic correlation between AJ spreading and epithelial cell piling-up is not entirely clear. However, it is known that maintenance of epithelial monolayer requires cell division along the plain of the epithelium. This is accomplished by anchoring mitotic spindles at the AJs (Harris and Peifer 2007) so that the cytokinetic furrow constricts perpendicularly to the epithelial planar axis. Spreading of the AJ components can thus randomize the specification of the plain of cell division, resulting in piling-up.

Opposite to *awd* loss-of-function, over-expression of Awd can promote turnover of the engaged AJ components. This is marked by loss of surface levels of AJ components, which lead to disruption of the epithelium and change of follicle cell morphology toward more spindle shape (Woolworth et al. 2009) (for an example, see Fig. 2e). The over-expression induced phenotype can therefore be considered pro-tumorigenic. In other words, over-expression of Awd renders an oncogenic function to this anti-metastasis suppressor. Thus, both loss-of-function and over-expression of Awd can cause disruption of epithelial characteristics, albeit in opposite manner leading to different abnormalities. Such biphasic functions may provide an explanation for the conflicting results in the correlation between *NME* expression levels and cancer progression.

The function of Awd in epithelial cells is particularly interesting when considered in the context of its dynamic expression pattern (Woolworth et al. 2009). In early egg chambers (pre-stage 5), Awd is expressed throughout the follicle cell body. After stage 5 (~27 h after egg chamber is formed and ~43 h before egg laying), Awd gradually accumulates to the basal side of the cell. Basal localization becomes prominent at stage 6 (~27–30 h after egg chamber is formed) and is firmly established after stage 8 (at ~36 h after egg chamber formation) (illustrated in Fig. 2f). Stage 6 is a critical time in epithelial morphogenesis as these follicle cells cease to proliferate and become fully differentiated. A dramatic morphogenic event then occurs at stage 9 (42 h after egg chamber formation), in which ~95% of the 1,000 or so follicle cells gradually adopt a columnar cell shape and move posteriorly as a sheet until they precisely envelop the oocyte. The remaining ~5% of the anterior follicle cells become stretched and squamous. The repositioned columnar follicle cells mechanically support the integrity of the oocyte and provide positional cues for the developing oocyte (Dobens and Raftery 2000). We propose that the role of Awd in the follicle cells is to remove mislocalized E-cadherin- β -catenin complex from the membrane before the epithelial polarity is established before stage 6 and during columnarization and the sheet movement at stage 9. The latter activity is relevant to the finding that internalization and recycling of E-cadherin is an important mechanism allowing for disassembly and reassembly of AJ during epithelial cell movement or cell shape change (Kamei et al. 1999; Le et al. 1999). In early egg chambers before the epithelial polarity is completely established, Awd is needed in the apical and lateral domain to remove the unengaged E-cadherin and Arm. After stage 6 when membrane domains are established and apical membrane is sealed from the lateral domain by the Crb-containing apical complex, Awd relocates to basolateral region and continues to retrieve laterally localized, uncomplexed adherens junction components during epithelial sheet movement.

Awd regulates Rab5

The relationship between expression levels of Awd and the surface levels of β -catenin and E-cadherin resembles that of Awd and surface receptor levels described previously—*awd* loss-of-function results in membrane accumulation, and over-expression of *awd* results in reduction of membrane localization. It was therefore suspected that the endocytic function of Awd is also at play in AJ modulation. As such, Awd may be involved in retrieving and internalizing β -catenin and E-cadherin that are not complexed in the AJ. This was confirmed as RNA duplex-induced knockdown of *awd* could rescue the phenotype induced by over-expressing a constitutively active *Rab5* mutant. Rab5 protein is a Ras-like small GTPase that mediates the fusion of endocytic vesicles into early endosomes (Stenmark 2009). Conversely, *awd* knockdown exacerbated the phenotype induced by over-expressing a dominant-negative mutant of *Rab5* (Woolworth et al. 2009). Importantly, in *awd* mutant follicle cells, the endogenous Rab5 protein level is reduced. Therefore, Awd is involved in stabilizing and enhancing the activity of Rab5. Such a function raised the possibility that Awd may directly modify Rab5 via its protein kinase activity, as opposed to the conventional view that NME proteins can promote GTPase functions by locally supplying GTP. Future studies are needed to address this possibility.

Epithelial function of *NME* in the mammalian systems

In an early study, human *NME1* was shown to reconstitute the epithelial characteristics of metastatic breast cancer cell MDA-MB-435 in a three-dimensional matrix, including formation of organized acinus-like epithelial organoids, deposition of the basement membrane components type IV collagen and laminin, and growth arrest (Howlett et al. 1994). Thus, *NME/awd* appears to be important for maintaining the epithelial characteristics. Loss of this function can lead to transition of the epithelial cell to a more mesenchymal state.

The study in follicle cells also recalls an earlier study demonstrating the *NME1* function in internalization of E-cadherin in the basolateral domain of Madin–Darby Canine Kidney (MDCK) cells (Palacios et al. 2002). In the MDCK model, NME1 protein is recruited to the cell periphery by activated Arf6. Arf6 belongs to the ADP-ribosylation factor (ARF) family of GTPases, which plays a critical role in the regulation of membrane traffic and the organization of the cytoskeleton (Hiroi 2009). Arf6 in particular is known to induce E-cadherin endocytosis (Palacios et al. 2001) and promotes dynamin-dependent internalization of E-cadherin. Importantly, it was shown that Arf6-NME1 could promote both clathrin- and caveolin-dependent endocytosis, although E-cadherin is internalized by the clathrin-based vesicles only (Palacios et al. 2002). It is also important to note that manipulation of Arf6 and NME activities in confluent monolayer MDCK cells did not result in disruption of the epithelial structure. This indicates that in the context of MDCK cells, NME1 has little effect on the existing AJ in a stable epithelium. Its main function, as observed in *Drosophila* follicle cells, is required for the formation of AJ, by retrieving un-incorporated AJ components, in morphogenic processes involving epithelial sheet movement and dynamic disassembly–reassembly of AJs.

More recently, using human non-invasive hepatoma (HepG2) and colon cancer (HCT8/S11) cell lines, which normally express high levels of NME1 and NME2, it was also demonstrated that reduced NME1 but not NME2 could lead to dispersion of E-cadherin and β -catenin (Boissan et al. 2010). The reduction of AJ in cell–cell contacts is correlated with reduced cell aggregation and increased cell motility. Unlike in *Drosophila* follicle cells, however, reduced NME1 in the human tumor cells resulted in cytoplasmic relocation, not apical and lateral membrane accumulation of the AJ components. This appears to be a

phenotype similar to over-expression of *awd* in *Drosophila* follicle cells. It is unclear why the two systems exhibit such discrepancy. One likely explanation is that in these human tumor cells, NME1 does not regulate AJ localization via endocytosis. Instead, NME1 may be required for translocalization or recycling of cytoplasmic E-cadherin to the AJ. Nonetheless, this study demonstrated that in human hepatocyte carcinoma and colon cancer patient samples, NME1 expression is low in normal tissues but up-regulated in tumor cells, while the expression is reduced at the invasion front of the carcinoma, and the expression levels of NME1 in different regions of the tumor mass are correlated with the surface levels of E-cadherin. As such, NME1 indeed may possess both oncogenic and anti-metastasis activities.

Perspectives

Genetic studies using *Drosophila* as an in vivo model has revealed a role of *NME* in epithelial morphogenesis. This function is also confirmed in cultured mammalian epithelial cells. It is interesting to note that in *Drosophila* follicular epithelium, loss of *awd* or over-expression of *awd* can induce different aspects of epithelial defects. However, neither of the two conditions alone generated metastatic tumors. It is likely that additional genetic changes are needed. This multi-component metastasis progression model is not unique. For example, over-expression of constitutively active Ras (Ras^{V12}) can induce proliferative tumors in *Drosophila* (Pagliarini and Xu 2003), while mutations in epithelial adhesion gene *scribble* can result in adenoma-like epithelial piling-up (Bilder et al. 2000), much like the *awd* mutant phenotype. However, only when the two genetic defects were combined did severe metastatic cancer occur (Wu et al. 2010). It will be therefore interesting to examine the outcome of *awd* loss-of-function in the oncogenic background (such as over-expression of Ras^{V12}), which would provide a clear demonstration of the anti-metastasis function of *NME*. Analogous phenomenon has been demonstrated in the mouse model (Boissan et al. 2005), in which induced hepatocarcinoma developed metastasis to lung in *Nme1* knockout mice but not in wild-type counterparts.

It is not yet clear what molecular activity of NME is involved in epithelial morphogenesis. *Drosophila* studies indicate that *awd* genetically interacts with *dynammin* but regulates the protein level of Rab5. There are at least two ways Awd can regulate Rab5 function: either as a GTP supplier or as a protein modifier. We do not favor the GTP supplier role in this case since Rab5 protein level is dramatically reduced in *awd* mutant cells and it is not known that the availability of GTP can influence the level of Ras-like proteins. On the other hand, it would be highly interesting if Awd stabilizes Rab5 via phosphorylation. Although phosphorylation of Rab5 in cells has not been documented, phosphorylation of other Rabs has been shown to regulate their activities and subcellular distributions (Bailly et al. 1991; Cormont et al. 1994; Karniguian et al. 1993). Demonstrating Rab5 regulation by NME/Awd via phosphorylation will be exceedingly novel. One difficulty in separating the GTP supplier and the protein kinase functions in vivo is that the two activities utilize the same histidine-dependent phosphate transfer active site, and presently, there are no point mutations that can distinctly separate the two activities. It is therefore critical that comprehensive structural analyses of this small but enigmatic protein be conducted so that key amino acid residues specific for each activity can be predicted and tested. Such information indeed will be of ultimate importance for the NME studies in general.

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Fig. 1.

Tracheal phenotypes in *awd* mutant. Wild-type and *awd*^{*2a4*} alleles, either heterozygous or homozygous as indicated, were combined with β -gal reporter gene specifically expressed in the trachea. Embryos were stained with anti- β -Gal antibody. *awd* mutation results in mild abnormal branch migration (in heterozygote) with ectopic sprouting of cellular processes (*arrows*) or complete disruption of the trachea tubules with dispersed, rounded tracheal cells (in homozygote)

Fig. 2.

awd function in the follicular epithelium. All egg chambers were stained for β -catenin (red), Awd (green), and DNA (blue). **a** Wild-type stage 8–9 egg chambers. Relevant cell types are indicated. At stage 8 (left panel), border cells remain in the anterior tip (i.e., the opposite end relative to the oocyte) of the epithelium and express Awd. At the transition to stage 9 (middle panel), the border cell complex delaminates from the epithelium and loses Awd expression. At mid-stage 9 (right panel), the Awd-negative border cell complex moves toward the oocyte. The progress of border cell complex is in step with the follicular epithelial sheet movement toward the posterior (dashed line). **b** A diagram illustrating the border cell migration. Red dots depict the diffusion of Pvf from the oocyte, and the blue line depicts the receptor Pvr expressed on the border cell surface. The chemotactic signaling drives the directional migration of border cells. **c** A stage 9 egg chamber in which *awd* is ectopically expressed from the border cell-specific promoter *slbo*. The border cells delaminate but remain at the anterior end (arrow), instead of migrating along with the follicle cell sheet (dashed line). **d** Homozygous *awd* mutant follicle cells were generated by mitotic recombination induced in the heterozygous egg chambers and are identified by a lack of Awd expression. The *awd* mutant clone exhibits significantly increased β -catenin expression on the cell surface and irregular cell size and shape, instead of the regular hexagonal normal cells. **e** An egg chamber over-expressing *awd* from the *hsp70* promoter. Follicle cells with increased levels of Awd (arrows) show rounded and spindle cell shapes. **f** A diagram depicting the dynamic expression pattern of Awd (shown in green) in the follicle cells during oogenesis. Between stage 5 and stage 9, Awd gradually relocates to the basal domain

Fig. 3. Illustration of mature *Drosophila* follicle cells, depicting domain-specific membrane proteins. Adherens junction is shown with attached actin bundles. See text for details