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EDITORIAL

The NDPK/NME superfamily: state of the art

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Nucleoside diphosphate kinases (NDPK) are nucleotide metabolism enzymes encoded by *NME* genes (also called *NM23*). Given the fact that not all *NME*-encoded proteins are catalytically active NDPKs and that NM23 generally refers to clinical studies on metastasis, we use here NME/NDPK to denote the proteins. Since their discovery in the 1950's, NMEs/NDPKs have been shown to be involved in multiple physiological and pathological cellular processes, but the molecular mechanisms have not been fully determined. Recent progress in elucidating these underlying mechanisms has been presented by experts in the field at the 10th International Congress on the NDPK/NME/AWD protein family in October 2016 in Dubrovnik, Croatia, and is summarized in review articles or original research in this and an upcoming issue of *Laboratory Investigation*. Within this editorial, we discuss three major cellular processes that involve members of the multi-functional NME/NDPK family: (i) cancer and metastasis dissemination, (ii) membrane remodeling and nucleotide channeling, and iii) protein histidine phosphorylation.

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Nucleoside diphosphate kinases (NDPK) are nucleotide metabolism enzymes encoded by *NME* (also named *NM23*) genes. NDPKs are ubiquitous enzymes catalyzing the transfer of a phosphate from nucleoside triphosphates (NTPs) to nucleoside diphosphates (NDPs), in particular GDP, by a ping-pong mechanism involving the formation of a phospho-histidine intermediate.[1–3] In this reaction, the main donor of phosphate is ATP, mainly provided by the mitochondrial oxidative phosphorylation, since its intracellular concentration is much higher than that of any other nucleoside triphosphate.[4] In mammals, 10 genes have been identified to be a part of the NME/NDPK family.[5,6] They encode proteins with one or two NDPK domains or a truncated NDPK domain, eventually associated with other domains whose functions remain largely unknown. Phylogenetic analysis shows that the *NME* genes are divided into two different groups.[5,7] The group I genes encode proteins (NME1 to NME4) sharing 58 to 88% identity with each other. The four proteins NME1, NME2, NME3, and NME4 are ubiquitous and possess NDPK catalytic activity. They are mainly localized in the cytosol and at the plasma membrane except the NME4 isoform, which possesses a specific mitochondrial targeting sequence directing it to mitochondria (Lacombe *et al*, upcoming issue[8]). NME1, NME2, NME3, and NME4 form hexamers as the catalytically active form (Ćetković *et al*, next issue[9]). Group II is comprised of more divergent proteins sharing only 22 to 44% identity with group I enzymes and between each other. These proteins are found in ciliated structures except for NME6, which is ubiquitous. NME1 and NME2 are the most abundant by far, and also the most studied, particularly NME1.

Constitutive knockout (KO) mice have been generated and reported for *NME1*, *NME2*, *NME1 +NME2*, *NME5*, and *NME7* genes.[10] *NME1* KO mice exhibit a normal overall phenotype, with only a mild global hypotrophy and impaired mammary gland development.[11,12] *NME2* KO mice are phenotypically normal, except a defect in K^+ channel Kca3.1 activation and cytokine production in T cells.[13] In striking contrast, the double KO mice for both *NME1* and *NME2* genes die at birth, probably due to major defects in erythroid cell maturation.[14,15] *NME5* and *NME7* knockout mice harbor a phenotype suggestive of primary ciliary dyskinesia, which is consistent with expression of NME5 and NME7 in ciliated structures.[16] Finally, knockdown of *NME2* in zebrafish induces severe cardiac dysfunction.[17–19] Given the existence of multiple *NME* genes encoding various NME/NDPK proteins with possibly redundant functions, future

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generation of conditional knock-out and knock-in models should help us to understand their specific functions in a particular tissue or developmental stage, or for a given pathological process.

Besides their known function in the control of intracellular nucleotide homeostasis, which was discovered in the 1950's, NDPK proteins have been shown to be involved in multiple physiological and pathological cellular processes, mostly without a clear knowledge of the underlying molecular mechanisms. Based on the reports made at the 10th International Congress on the NME/NDPK/Awd proteins in October 2016 in Dubrovnik, Croatia, experts in the field have summarized their ideas and views in review articles or contributed novel original research to two special issues of *Laboratory Investigation*. Within this editorial, we will focus on three major topics that emphasize recent progress in the field: (i) cancer and metastasis dissemination, (ii) membrane remodeling and nucleotide channeling, and (iii) protein histidine phosphorylation.

NME IN METASTASIS

Metastasis is the main cause of death in cancer patients. The first step in metastasis is the conversion from *in situ* carcinoma into invasive cancer, as the basement membrane is breached and invasive migration of tumor cells occurs through stromal fibrillar type I collagen. The tumor cells then enter the circulation and exit to colonize a secondary organ and form a metastasis.[20–23] The mechamisms of the switch from *in situ* to invasive carcinoma remain largely unknown, although membrane type 1-matrix metalloproteinase (MT1-MMP) is involved in this process.[24,25] The identification of *NME1* as the first metastasis suppressor gene[26] has opened the field of this new class of metastasis dissemination regulators ([27–30]; Ćetković *et al*, upcoming issue;[31] Khan and Steeg,[32] this issue). Unlike tumor suppressor genes, metastasis suppressors inhibit *in vivo* metastasis without globally inhibiting primary tumor growth. In several solid tumor types, an inverse association between NME1 expression and metastatic spread has been observed. Specifically, three major findings have verified the anti-metastatic activity of *NME1*. First, the overexpression of *NME1* in several metastatic cell lines including melanoma, breast, colon, lung, liver, ovary, prostate, and oral carcinoma cell lines, reduced their metastatic potential in xenograft models.[33–39] Second, the incidence of lung metastases is significantly increased in *NME1* knockout mice prone to develop hepatocellular carcinoma.[40] Third, the silencing of *NME1*, but not of the highly homolog *NME2*, by RNA interference confers a metastatic phenotype to several non-invasive human epithelial tumoral cell lines by altering cell–cell adhesion, migration, invasion, and signaling linked to cancer progression.[41] Invalidation of *NME1* induced the destabilization of E-cadherin-mediated adherens junctions, and the nuclear translocation of β-catenin associated with the transactivation of the transcription factor TCF/LEF-1, all these events are hallmarks of epithelial–mesenchymal transition 'EMT'. *NME1* silenced hepatic and colon tumor cells acquired the ability to invade Matrigel, which mimics the basement membrane, and native type I collagen, the major component of the stroma. Similar increased invasiveness in Matrigel due to *NME1* silencing has also been reported for uveal melanoma, lung, and breast cancer cells.[41–44] *NME1* silencing induced the formation of invadopodia, which are specialized plasma membrane protrusions endowed with MT1-MMP-dependent matrix degrading activity.[41] Thus, several mechanisms converge to promote matrix proteolysis upon *NME1* silencing. Pro-invasive signaling pathways that are activated upon *NME1* silencing included Rac1, MAPK/SAPK, Akt, and TCF/LEF-1-mediated transcription.[41] *NME1* silencing promotes upregulation of several MMPs including MT1-MMP. Accordingly, pharmacological inhibitors targeting Rho-GTPases, Src, PI3K/Akt, MAPK/SAPK, and MT1-MMP abrogated the pro-invasive signaling induced by *NME1* deficiency. Thus, it appears that multiple mechanisms induce a strong invasive and motile phenotype in response to *NME1* silencing, in agreement with the multifunctional properties of this metastasis suppressor protein. In conclusion, NME1 emerges as a major upstream regulator of the metastatic signaling cascade.

The molecular mechanisms involved in the control of metastatic potential by NME/NDPK proteins are largely unknown (Farkas *et al*,[45] this issue). They could involve several known NME1 enzymatic activities (NDPK, histidine kinase, and 3′–5′ exonuclease), protein-protein interactions, and/or downstream gene regulation properties. A granzyme A-activated DNAse (GAAD) activity involved in caspase-independent apoptosis was also reported for NME1.[46,47] Accumulating evidence suggests that cytosolic NME/NDPKs NME1 but also often NME2, interact with and affect different components and regulators of the cytoskeleton, including actin-binding proteins, intermediate filaments, and cytoskeleton

attachment structures (adherens junctions, desmosomes, and focal adhesions) in cells from a variety of organisms and tissues, and in the course of development, suggesting that this association is evolutionarily conserved and may serve an essential function.[48–55] The interactions of NDPK with components of the cytoskeletal machinery are highly relevant, given the well-established role of the cytoskeleton in cell motility, a critical determinant of the metastasis process. NME1 has also been reported to bind proteins belonging to small and heterotrimeric G-proteins ([56–61]; and Filic[62] upcoming issue; Abu-Taha *et al*,[63] this issue), transcriptional complexes ([64–69]; Sharma *et al*,[70] Pandey and Robertson,[71] and Puts *et al*,[72] all in this issue), and components of signaling pathways of MAPK,[73–75] TGF-β[76–78] and Notch,[79] as well as other factors promoting invasion and metastasis ([80,81]; Ferrucci *et al*[82] upcoming issue). Accordingly, the list of protein/protein interactions involving NME1 is huge and could yield new information about the anti-metastatic activity of NME1, and more generally about the metastatic process. The role of the highly homologous isoform NME2 in metastasis dissemination remains much less documented and controversial.[83–87] Our most recent data demonstrate a major role of NME1, not of NME2, during the transition of breast carcinoma from *in situ* to invasive (Mathieu Boissan, unpublished data).

Numerous clinical studies reported a crucial role of NME1 in cancer metastasis; indeed, an inverse association between NME1 expression and the metastatic potential for human solid tumors of epithelial origin such as breast, liver, colorectal, ovarian, and lung carcinoma and for melanoma has been reported.[88] However, the link between NME, cancer and metastasis is more complicated, because of the biphasic expression of NME in cancer and metastasis: (i) an overexpression of both NME1 and NME2 isoforms in most human solid tumors at early stages of tumoral development[41,89] as well as murine tumors,[40] and (ii) a specifically decreased expression of NME1 in the primary tumor correlated with metastatic spread.[89,90] Interestingly, a loss of NME1 was observed at the invasive front of hepatic and colorectal tumors.[41] If for liver, breast, colon and lung carcinoma as well as for melanoma, the vast majority of studies reported an inverse correlation with metastasis and/or poor overall survival (Leonard *et al*,[91] next issue), this is less marked in gastric and ovarian carcinoma, for which disparate results were found.[88,92] In other types of cancers such as neuroblastoma, hematopoietic malignancies, and osteosarcoma, high tumoral expression was noted, which was most often correlated with poor outcome (Tan and Chang,[93] this issue). The conflicting data described in the literature, might be due, at least partially, to the presence of the two closely related isoforms NME1 and NME2, which are most often not discriminated by antibodies and probes; heterogeneous expression in the primary tumors; and/or the criteria used to evaluate and grade NME1 expression in human clinical samples.

An important question little documented in the literature pertains to how NME1 is lost during invasive stages. Three mechanisms have been proposed: First, downregulation of *NME1* expression may be the result of increased methylation of CpG islands mostly found around the transcription start site of the *NME1* promoter as DNA methylation inhibitors can increase NME1 expression in tumor cells.[94] Second, SWI/SNF chromatin remodelers-associated PRMT5 (protein arginine methyltransferase 5) is directly involved in transcriptional repression of *NME1*.[95] Third, NME1 could be regulated at the protein level by (i) lysosomal cysteine cathepsins, proteases with known roles in invasion and metastasis, which directly cleave and degrade NME1,[96] and (ii) the E3 ubiquitin ligase SCF-FBXO4, which interacts with NME1 to mediate its polyubiquitination and subsequent proteosomal degradation.[97] Finally, a field worth investigation in regard to NME1 tumoral expression is the potential role of the tumoral stroma, in particular, cancer-associated fibroblasts (CAF), which have been shown to promote cancer invasion and migration, and metastasis in many different models.[98] Taken together, these data strongly suggest that re-expression of NME1 in invasive cancer cells could constitute a promising strategy for anti-metastatic therapy.[99,100]

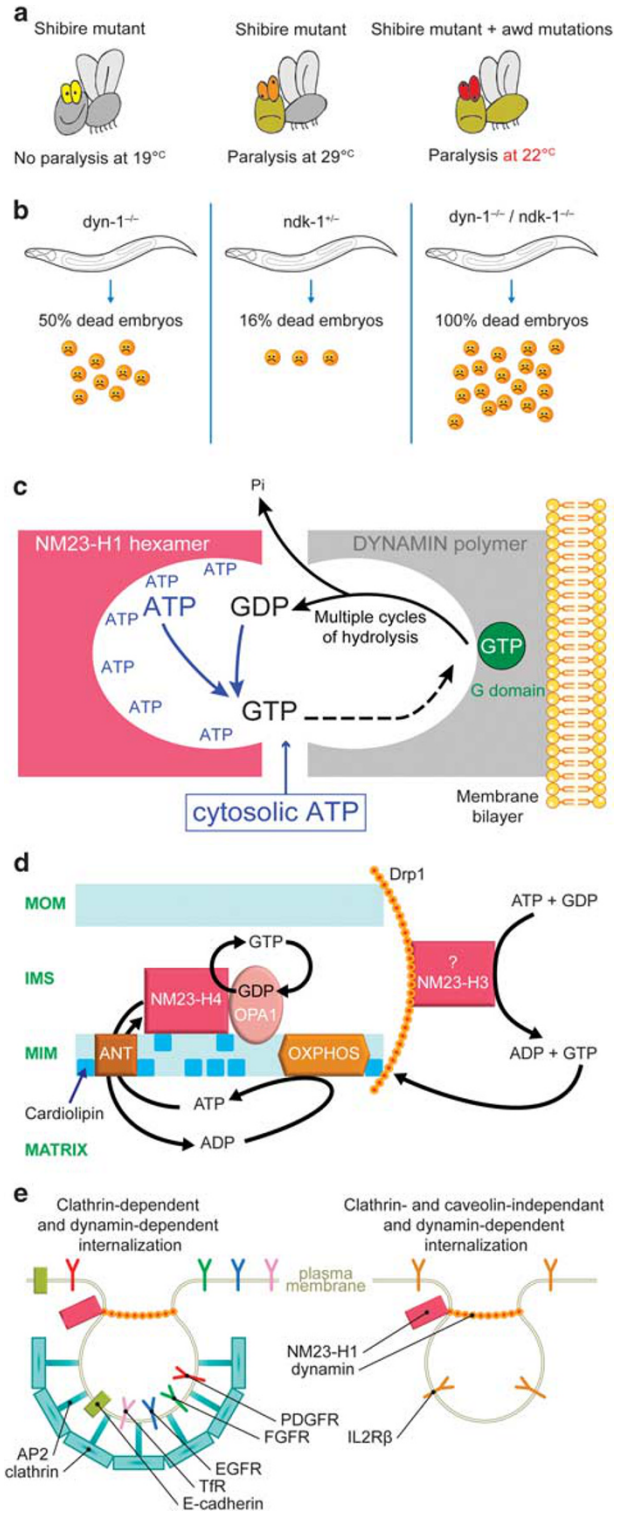
NME IN MEMBRANE REMODELING AND NUCLEOTIDE CHANNELING

Studies using several model systems, including *Drosophila melanogaster*, *Caenorhabditis elegans*, and mouse, as well as human, have demonstrated a fundamental role of the NME/NDPK family in endocytosis, intracellular trafficking, and nucleotide channeling (Figure 1). The starting point was a report in 2001 by neurobiologists, demonstrating a genetic and functional interaction between *Awd* and *Shibire*, the NME/NDPK and dynamin homologs, respectively, in *D. melanogaster*.[101] Mutations in *Awd* (the corresponding protein Awd sharing 78% amino-acid identity with the human NME1 and NME2

isoforms) enhance paralysis of the *Shibire* mutant, a temperature-sensitive mutant of dynamin that blocks its function, resulting from defects in endocytosis-mediated neurotransmitter uptake at synaptic junctions.[101] Remarkably, in a genetic screen to identify mutations that modify this phenotype, only *Awd* mutations were found, indicating that the functional relationship between *Shibire* and *Awd* is highly specific. More recent work in *Drosophila* epithelial cells, such as tracheal cells and border cells, confirmed the link between *Shibire* and *Awd* for internalization of growth factor receptor homologs for FGF and PDGF/VEGF.[102,103] Awd-dependent endocytosis also contributes to ensure epithelial integrity of follicular cells in the egg chamber by modulating the levels of adherens junction components.[104] A number of further studies suggest links between NME and dynamin. The endocytic function of VHL (Von Hippel-Lindau) protein, a tumor suppressor that serves as a negative regulator of hypoxia-inducible factor-alpha subunits, regulates surface localization of FGF (fibroblast growth factor) receptor 1 via NME1,[105] and ARF6-GTP, the active form of ARF6, interacts with and recruits NME1 to facilitate dynamin-mediated endocytosis.[58] A novel genetic interaction was also found between *Dyn-1* and *NDK-1,* the homologs of dynamin and NME/NDPK, respectively, in the nematode *C. elegans* during engulfment of apoptotic corpses.[106] This process requires reorganization of cytoskeleton and membrane remodeling to extend the cell surface of the engulfing cell. Indeed, Dyn-1 and NDK-1 loss-of-function mutant embryos show phenotypic similarities, ie an accumulation of apoptotic cell corpses, and *Dyn-1:NDK-1* double mutants are lethal. Moreover, in a genome-wide RNAi screen for genes involved in membrane trafficking, knockdown of *NDK-1* caused failure in receptor-mediated endocytosis.[107] In mammals, knockdown of *NME1/NME2* impairs dynamin-mediated endocytosis of surface receptors such as the transferrin and EGF receptors, as well as of IL-2 receptor β subunit in human and monkey cells;[108] however, knockdown appears not to affect recycling of receptors since recycling of the transferrin receptor from endosomes to the plasma membrane is not altered. Taken together, these data clearly indicate that dynamin and NME/NDPKs are close functional partners involved in membrane remodeling and trafficking across different species.[109]

Dynamin superfamily proteins are unique molecular motors that use GTP instead of ATP. They have a very low affinity for GTP and a high basal rate of GTP hydrolysis. Polymerization into helical structures around the membrane neck of clathrin-coated pits (CCP) *in vivo* stimulates their GTPase activity. Accordingly, dynamin activity should be very sensitive to variations in the GTP level.[108,110,111] This raises the hypothesis that NDPK activity of NME/NDPK proteins could directly affect the function of dynamin superfamily proteins through spatiotemporally controlled GTP production and availability, to meet elevated GTP turnover rates of dynamins. In support, loss of *NME1/NME2* inhibits dynamin-mediated endocytosis and phenocopies the dynamin-null phenotype[108,112] ie, the augmentation of the density of CCPs and the tubular elongation of CCPs connected to the plasma membrane in comparison to controls. Thus, in the absence of NME1 and NME2 proteins, CCPs form properly but fail to detach from the plasma membrane, indicating a role for these NDPKs in dynamin-mediated membrane fission at the CCPs. Local delivery of NME/NDPK-generated GTP to dynamins is further supported by the following findings:[108,113] (i) NME1/NME2 colocalize at CCPs with the AP-2 complex, a major component involved in clathrin-mediated endocytosis, and dynamin, and they interact physically with dynamin, (ii) NME1/NME2 are recruited to dynamin-coated tubules and stimulate GTP-loading on dynamin, and (iii) NME1/NME2 trigger dynamin-mediated membrane fission in the presence of ATP and GDP. Thus, cytosolic NME1/NME2 channel GTP to classical cytosolic dynamins at plasma membrane CCPs to power their GTPase activity during endocytosis.

Besides the functional link between the two cytosolic NDPKs (NME1 and NME2) and classical endocytic dynamins (dynamin-1, expressed at high levels specifically in neuronal tissues and involved in synaptic vesicle endocytosis and dynamin-2, ubiquitously expressed and involved in clathrin-mediated endocytosis (CME) as well as in some clathrin-independent endocytic pathways), the GTP channeling mechanism from NME/NDPK toward dynamins has been also shown in another subcellular compartment, the mitochondria. Here NME4 and the dynamin-related GTPase OPA1 are co-localized in the intermembrane space, bound to the inner mitochondrial membrane. Knockdown of *NME4*, but not of *NME1/NME2*, induces alterations of the mitochondrial morphology including fragmented and swollen-blebby mitochondria, which is reminiscent of a defect of mitochondria fusion.[108,114] Furthermore, NME4



NDPK co-localizes and interacts with OPA1 at the inner membrane,[115] and increases GTP-loading on OPA1, indicating a scheme in which NME4 provides a local GTP supply to OPA1 at the inner mitochondrial membrane.[108] Taken together, the evidence reported above supports a model in which NMEs/NDPKs physically interact with dynamin superfamily proteins in the same subcellular compartment to maintain a high local concentration of GTP for dynamin's function in

Figure 1 (**a**) Genetic interaction between NM23 and dynamin homologs in *Drosophila*. Left: a mutant of the dynamin homolog in *Drosophila* (shibire), which is temperature sensitive, present no paralysis at a permissive temperature, 19 °C. Middle: the shibire mutant present paralysis of the flies due to defects in endocytosis-mediated neurotransmitter uptake at a non-permissive temperature, 29 °C. Right: combination of mutations of awd, the homolog of NM23-H1/-H2 in *Drosophila*, and mutations of shibire induces paralysis at a lower temperature 22 °C, indicating that mutations of NM23 make synapses more sensitive to dynamin mutations. (**b**) Genetic interaction between NM23 and dynamin homologs in *C. elegans*. In order to test whether NDPK/NDK-1 and DYN-1/Dynamin also interact in the worm genetically, ndk-1 −/−;dyn-1 −/− double mutants were generated. The viable thermosensitive ky51 allele of dyn-1 (dyn-1 −/−) results in 50% embryonic lethality at the restrictive temperature (25 °C). These embryos arrest development at a late embryonic stage due to endocytosis defects and display an accumulation of cell corpses. In the progeny of ndk-1+/− heterozygotes 16% of ndk-1 −/−;dyn-1 −/− homozygotes die as embryos showing the Dyn-1-like late embryonic lethality phenotype with persistent cell corpses. Double mutants at the restrictive temperature resulted in no viable progeny (100% embryonic lethality). (**c**) NM23-H1 a GTP dealer for dynamin at clathrin-coated pits during endocytosis. Dynamin is recruited at the plasma membrane clathrin-coated pits, polymerizes into helix which strongly stimulates its GTPase activity inducing GTP hydrolysis; multiple cycles of GTP loading and hydrolysis are necessary for constriction and fission. To load very efficiently and locally the GTP on dynamin, NM23-H1 physically interacts with dynamin at clathrin-coated pits and uses the GDP coming from the GTP hydrolysis and the cytosolic ATP pool to produce GTP in a closed circuit. Finally, to increase the efficiency of the reaction, a hexamer of NM23-H1 interacts with dynamin, this hexamer containing six active sites that can function in concert, ensuring in a same time six catalytic reactions. (**d**) NM23-H4 controls mitochondrial fusion through a functional coupling with the dynamin-related GTPase OPA1. A hexamer of NM23-H4 binding to the mitochondrial inner membrane harbors NDPK activity for regeneration of NTP, mainly GTP, in the mitochondrial intermembrane space, and for direct and local GTP fueling (channeling-tunneling) of the dynamin-related protein OPA1. NM23-H4 also channels ADP via adenylate translocase (ANT) into the matrix space for stimulation of respiration and ATP regeneration through oxidative phosphorylation (OXPHOS). The localization of NM23-H3 at the mitochondrial surface where the dynamin-related pro-fission protein Drp1 acts could suggest that the NDPK NM23-H3 could assist Drp1 by GTP fueling during mitochondrial fission. MOM, mitochondrial outer membrane; IMS, intermembrane space; MIM, mitochondrial inner membrane; ANT, adenylate translocase; OXPHOS, oxidative phosphorylation. (**e**) NM23-H1 controls dynamin-mediated endocytosis. Left: NM23-H1 facilitates clathrin-dependent and dynamin-dependent internalization of E-cadherin, transferrin receptor (TfR), epidermal growth factor receptor (EGFR), fibroblast growth factor (FGFR), and platelet-derived growth factor receptor (PDGFR). Right: NM23-H1 facilitates clathrin- and caveolin-independent and dynamin-dependent internalization of the β subunit of the interleukin 2 receptor (IL2Rβ).

membrane remodeling. Interestingly, another mode of action of NME4 is also related to its capacity to bind membranes. The NME4 hexamer enables intermembrane transfer of the mitochondrial phospholipid cardiolipin between inner and outer mitochondrial membrane, which can serve as a signal for mitophagy or apoptosis, depending of the cardiolipin oxidation state. The bioenergetics and lipid signaling functions seem to be mutually exclusive, making this protein a sort of bifunctional nanoswitch ([115,116]; Schlattner *et al*,[117] this issue).

NMEs AS PROTEIN HISTIDINE KINASES

Besides their well-established role as NDP kinases, there is emerging evidence that NME/NDPK proteins act as protein histidine kinases in mammals (Attwood and Muimo[118] and Muimo *et al*,[119] upcoming issue). For NME2/NDPK-B, at least two substrates were identified and studied in more detail: the β subunit of heterotrimeric G protein[120–122] and the potassium channel KCa3.1.[123–125] In the first case, NME2 forms a complex with β/γ dimers of heterotrimeric G proteins, in which a phospho-relay occurs from His118 of NME2 to His266 of the Gβ subunit. This ultimately leads to the formation of GTP from GDP and thus receptor-independent activation of the G protein. The second well-documented example is the NME2-mediated activation of the K^+ channel KCa3.1, inducing cytokine production in $CD4^+$ cells. After stimulation of the T cell receptor TCR, the PI3 kinase-C2β subunit synthesizes phosphoinositol from which the phosphate is transferred on His118 of NME2.[125] The kinase directly binds and activates KCa3.1 by transferring the phosphate from His118 to His358 of the channel. It has also been shown that the mammalian histidine phosphatase PGAM5 dephosphorylates NME2 on His118, thereby inhibiting phosphorylation and activation of KCa3.1.[125] These findings reveal a novel regulatory mechanism for a mammalian potassium channel and T-cell activation, and highlight a unique feature of histidine *versus* serine/threonine and tyrosine as a regulatory phosphorylation site.[126] However, exactly how bulky protein substrates can gain access to the active site of NME/NDPK proteins with the essential His118 situated in a cleft remains to be studied and explained by biochemical and crystallographic studies. More recently, anti-phosphohistidine antibodies have been obtained that will provide unique tools to

scrutinize the role of histidine phosphorylation by NME/NDPK and dephosphorylation by phosphatases in various cellular processes ([126,127]; Makwana *et al*,[128] next issue; Adam and Hunter,[129] this issue).

CONCLUDING REMARKS AND PERSPECTIVES

Over the last three decades, extensive analyses of the NME/NDPK family started to reveal its multifaceted roles in cellular pathophysiology and the underlying molecular mechanisms. Beginning from a simple kinase activity, the field faces today the complexity of 10 different isoforms, all having specific features and often alternative, additional activities. The roles of these various isoforms undoubtedly require specific targeting and location within the cell. Most studies addressed the specific roles of NME1 and NME2, the two most abundantly expressed isoforms, with particular emphasis on the anti-metastasis function of NME1. Since Steeg's pioneering work on the anti-metastasis potential of NME1, most studies confirmed its invasive and metastasis suppressor function in many different cellular and murine models using transfection experiments and knockout mouse strategies. However, translation of these findings to the clinics, eg, by modulating NME1 expression levels, is still lagging behind. Indeed, medroxyprogesterone acetate (MPA) has been previously identified as a potential candidate compound for clinical setting as NME1 expression increased *in vivo* in mice treated by MPA and contributed to the diminution of metastasis, but unfortunately it showed no therapeutic benefit in women with metastatic breast cancer.[130,131] Thus, to date NME1 is not 'drug-able'. If the anti-metastasis role of *NME1* has been clearly reported in a large variety of tumor types, we do not know how its expression is regulated and in particular downregulated in the invasive component. Thus, it is of utmost importance to discover how it is modulated at the transcriptional, translational, and posttranslational level. Also, it would be of great interest to determine the role of the stromal microenvironment mainly composed of type I fibrillary collagen and cancer-associated fibroblasts (CAF), and its stiffness in the regulation of *NME1* expression. Moreover, selective silencing of *NME1* or *NME2* does not alter the protein level of the other isoform, indicating that either protein does not interfere with the expression or stability of the other isoform.[108] It is of great importance to remind that these isoforms show 88% identity at the protein level; sixteen of the eighteen different amino acids between NME1 and NME2 are located at the lateral surface of the hexamer, which could give rise to different interactions with other proteins and hence different cellular functions. NME1 and NME2 recombinant proteins form heterohexamers (Ioan Lascu, Marie-Lise Lacombe, unpublished data). If this is also the case *in vivo*, formation of such heterohexamers could introduce a supplementary level of cellular regulation. Indeed, NME1 can be co-immunoprecipitated with NME2 and vice versa *in vivo* (Mathieu Boissan, unpublished data), and thus the NME1/NME2 ratio could be crucial for cellular functions of NME/NDPK.

The role of the NME/NDPK family in membrane remodeling and nucleotide channeling has become widely recognized as another feature of several family members, including at least NME1, NME2, NME4, and potentially NME3.[108] Here the classical NDPK activity as the main source of GTP is extended to a direct GTP fueling of GTP-dependent members of the dynamin superfamily through direct protein/protein interaction. This was shown in different species and model systems, including the fruit fly *D. melanogaster,* the nematode *C. elegans* and human, indicating an evolutionary conserved mechanism of membrane remodeling controlled by NME/NDPK proteins. GTP fueling to cytosolic dynamins (through cytosolic NME1 and NME2) affects endocytosis of cell surface receptors, while GTP fueling to mitochondrial dynamin-related OPA1 (through mitochondrial NME4 isoform) affects mitochondrial inner membrane fusion and cristae remodeling. The localization of NME3 at the outer mitochondrial membrane, where the dynamin-like protein DRP1 is recruited to mediate mitochondrial fission, suggests that NME3 might, likewise, assist DRP1 in this process. These observations provide a biochemical and thermodynamic explanation of why dynamin superfamily members are dependent on NDPKs. However, to fully validate this model, more comprehensive structural analyses of the interactions between NDPKs in their hexameric state and dynamin superfamily proteins in their polymeric state are needed. In addition, NME4 has an alternative signaling function. By binding to and transferring cardiolipin between inner and outer mitochondrial membrane in damaged mitochondria, it can trigger mitophagy or apoptosis. Recruitment of NME proteins to dynamin superfamily proteins as well as lipid transfer induced by the NME4 protein could suggest a general role of NME proteins in providing an interaction scaffold as well as nucleoside triphosphate channeling.[113] It remains

to be shown how these molecular mechanisms contribute to pathological states at the level of organisms, since the novel mitochondrial functions have potential for the development of interventions in various human pathologies.

Further, during the last decade, it became evident that certain NME/NDPK family members could act as a protein histidine kinase. In particular, NME2 has at least two defined substrates, the β subunit of heterotrimeric G proteins and the intermediate conductance potassium channel KCa3.1. In all these proteins, the phosphorylation of a specific histidine residue is of regulatory importance for protein function, and NME2 seems to be the only known kinase able to catalyze this phosphorylation. There is apparently a direct transfer of the phosphoryl group between the phosphohistidine in the active site of the autophosphorylated NME2 and the histidine in the substrate protein. As mentioned above, the primary function of NME/NDPK is to catalyze phosphoryl transfer between NTPs and NDPs. Hence, how can this function be reconciled with phosphoryl transfer from NTPs to histidine on substrate proteins through NDPK activity is an important so far unresolved question. Specific anti-phosphohistidine antibodies will help to decipher the physiological relevance of histidine phosphorylation and more accurately the precise role of NME proteins in this process.

Finally, since more than one decade, it has been shown that some tumor cell lines overexpressing NME1 secrete this protein into the extracellular environment ([132]; Romani *et al*,[133] this issue; Khanim and Bunce,[134] upcoming issue). Indeed, high concentrations of NME1 were found in the sera of patients with tumors overexpressing NME1. Tumor cells may secrete NME1 through unknown mechanisms, since there is no signal peptide sequence for secretion in the NME1 protein. In particular, the serum level of NME1 has been shown to be an important prognosis factor in acute myeloid leukemia. Secreted NME1 by tumor cells may affect tumor growth as an autocrine growth factor. For now, we need to understand how NME1 is secreted and how secreted NME1 affects tumor progression.

In conclusion, it is fascinating to observe how NME/NDPK proteins regulate complex cellular events from development, nucleotide channeling, and histidine phosphorylation to metastasis dissemination, indicating that these proteins are definitely not just 'housekeeping enzymes'. The mechanisms underlying these biological functions are now emerging from a body of working models, slowly but steadily validated by experimental studies, including several high-profile publications. Further biochemical, developmental, and molecular biology studies, targeting in particular the specific subcellular compartmentalization of NME/NDPK proteins, mutational assays, and finally animal models will undoubtedly further disentangle and validate NME/NDPK functions in human health and disease.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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