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review article

A young researcher's guide to NME/Nm23/NDP Kinase

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Abbreviations:
NDPK – nucleoside diphosphate kinase
MIF – macrophage migration inhibitory factor
MDM2 – mouse double minute 2 homolog
EBNA 1 – 3C – Epstein-Barr nuclear antigen 1–3C
TGF-β – transforming growth factor β
LPA1 – lysophosphatidic acid receptor 1
MPA – medroxyprogesterone acetate
STRAP – Serine/Threonine Kinase Receptor Associated Protein
MLS – mitochondrial localization signal domain
TRF1 – Telomeric Repeat Binding Factor 1
ICAP1α – integrin cytoplasmic domain-associated protein 1alpha

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Abstract

Nucleoside diphosphate kinases (NDPKs) catalyze the exchange of the terminal phosphate from trinucleotides to dinucleotides through a high-energy phosphohistidine intermedier. They are encoded by NME genes and have been found, with a few exceptions, in all living beings. Besides their well-known function as key regulators of the cellular nucleotide homeostasis, they have been appointed numerous additional biochemical and biological functions. The discovery of NME1/NDPK A as the first metastasis suppressor opened new avenues in cancer research. Although the precise role of NME genes/proteins in cancer dissemination is not yet revealed, it seems that further intensive research in this field may lead to new advances in cancer diagnosis and prognosis, as well as encourage new therapeutic strategies.

INTRODUCTION

Nucleoside-diphosphate kinases (NME/Nm23/NDPK) constitute a family of evolutionary conserved enzymes present in all three domains of life *(1)*. The NDPK was discovered in the mid-20th century by its biochemical activity consisting of the removal of the terminal phosphate from a nucleoside triphosphate (mainly ATP) and its addition to all other (d)NDPs. It is generally considered to be a housekeeping enzyme since it is in charge of the maintenance of the cellular NTP pool *(2)*. A huge interest for this enzyme started in early nineties when it was discovered that a gene named *nm23* (non-metastatic clone 23, also known as *NME*), responsible for metastasis suppression in the murine melanoma model system *(3)* is actually encoding one of the subunits of NDPK, NDPK A. The NME/Nm23/NDPK family members have been assigned a vast range of biological functions spanning from metastasis suppression, proliferation and development to ciliary functions and vesicular transport *(4)*. Despite comprehensive studies done so far in this field, there are still essential questions that have not been answered. The exact mechanism by which the members of the NME/Nm23/NDPK family execute their biological functions still remains to be unraveled.

Structure, function and intracellular localization of NME/NDPK members

Nucleoside diphosphate kinases (NDPKs) catalyze the exchange of γ-phosphate between triphosphates and diphosphates through a high-energy phosphohistidine intermedier *(5)*. They are encoded by the *NME* genes. Ten such genes have been found in humans *(4)*. On the basis of phylogenetic studies, the exon-intron structure and the presence/absence of specific protein domains, the vertebrate NME proteins have been separated into two groups (Figure 1) *(6)*.

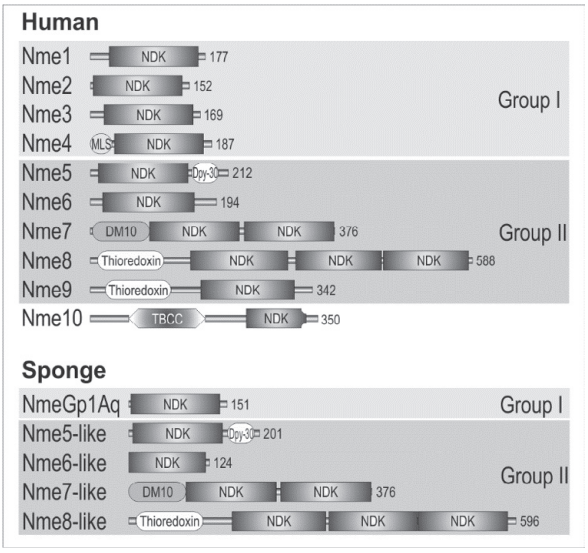

Figure 1. *Schematic representation of NME proteins domains in human and sponge. Proteins are represented on a scale 1:10 (1mm = 10 amino acids). The number of amino acids is displayed on the right side of the schematic representation of the gene. Protein domains have been indicated with boxes, and each protein has been searched against SMART/Pfam databases. Abbreviations of domain names are retrieved from SMART/Pfam databases and indicated in the figure. Shortened name MLS stands for mitochondrial localization signal domain.*

Group I protein members (*NME1-4*) are generally highly homologous among themselves and compared to their orthologs in different species (58-88% amino acid homology). Their corresponding genes have a similar exon-intron structure, as well *(7)*. The NME1/NDPK A and NME2/NDPK B subunits form the main cellular nucleoside diphosphate kinase which executes at least 80% of the cellular NDPK function. The identically folded subunits can assemble into enzymatically active hexamers in all possible combinations (A6, A5B1,....B6) *(2)*. The 17 kDa subunits A and B are 88% identical in their amino acid sequence. Both proteins are ubiquitously expressed and display mainly cytoplasmic localization but can also be found elsewhere in the cell (Figure 2) *(8)*. A transcript encoding a longer form of NDPK A *(9)*, as well as a readthrough transcript encoding a part of the NDPK A and the complete NDPK B have been reported *(10)*. The third gene/protein in the Group I named DR-Nm23 (NME3 or NDPK C) has also been reported and seems to be at least partially localized in the mitochondria. NME3 possesses a 17 amino acid N-terminal hydrophobic sequence which could anchor the protein in the membrane but it is not considered to be a regular mitochondrial signaling sequence *(11)*. The NDPK D (NME4/Nm23-H4) is, however, a true mitochondrial NDPK with a canonical sequence which targets the protein to mitochondria *(12)*. All Group I members exhibit the NDPK activity in their hexameric form with similar kinetic parameters. They possess nine specific residues that have been essential for the stability of the NDP kinase and its activity *(13)*. Besides this housekeeping role in the synthesis of nucleoside triphosphates, the NME/NDPK proteins have been assigned several additional biochemical functions. One of them is the His-protein kinase which includes the transfer of a high-energy phosphate from the phosphorylated intermediate to another protein *(14, 15)*. There has also been evidence that the NME proteins may function as transcriptional modulators *(16)*, exonucleases *(17)* and have a scaffold function *(18)*.

NME Group II encompasses NME5-9 and, in contrast to the Group I members, they are more divergent among themselves (28-45% amino acid identity) and dissimilar to Group I proteins (25-34% amino acid identity). They possess either a single or several NDK domains. Their tentative multimeric forms have not yet been elucidated. So far, the enzymatic activity was reported only for the NME6 *(19)*, although the evidence for its NDPK activity is questionable. Compared to the human NME1 and NME2, the NME6 protein has seven additional

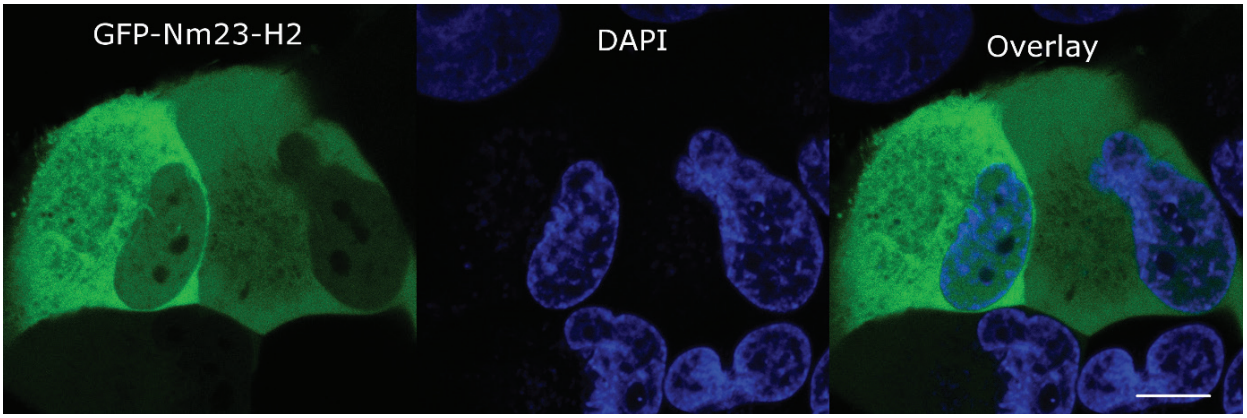

Figure 2. *Subcellular localization of GFP-Nm23-H2. Human melanoma cell line WM793B transfected with pEGFPC1-nm23-H2. Fluorescent staining is visible in the cytosol and the nuclei with greater incidence in the cytoplasmic region. Scale bar represents 10 μm.*

residues at the N-terminus and 23 additional residues at the C-terminus. It has one extra amino acid at the position Leu-30 and three additional residues in the Kpn-loop which lies in the catalytic site and is essential for the maintenance of the hexameric structure, a prerequisite for the NDPK activity. It is also possible that the elongated C-terminus in NME6 (and NME5 as well) disrupts the hexameric form *(20)*. Human NME6 does not possess the canonical mitochondrial signaling sequence but potential mitochondrial targeting sequences have been observed in homologs of NME6 in non-bilaterian metazoans *(21)*. The Group II members usually do not possess all nine residues considered to be crucial for the NDPK activity. They differ considerably from the Group I by having either one or several NDK domains (NME7 and NME8 have two and three NDK domains, respectively), while some of the NME representatives have additional domains. Almost all metazoan NME5 proteins possess a C-terminal DPY-30 domain, NME7 has a DM10 domain while NME8 and NME9 have thioredoxin domains *(7)*. The Group II members differ from the Group I members especially in the length of their N and C termini (Figure 1). As mentioned earlier, the NME6 was reported to be located in the mitochondria. Interestingly, all members of the Group II, except NME6, encode proteins mainly located in cilia or flagella of spermatids or spermatozoa *(22)*. Their role in these cellular structures remains to be elucidated.

Evolutionary history of NME genes/proteins

It has been suggested that the Group I *NME1/2* and *NME3/4* genes arose from a single ancestor gene common to all chordates by the first round of whole genome duplication (1R) which occurred early in the vertebrate lineage *(23)*. *NME3* and *NME4* arose from a cis-duplication of *NME*3/4 gene that occurred probably before or around teleost radiation, while *NME1* and *NME2* separated through cis-duplication after the emergence of amphibians. Our work *(24)* suggests that the sponge Group I NME gene/protein of the last common ancestor of all Metazoans was structurally and functionally similar to the multifunctional enzyme it is today. The analysis of the sponge *NMEGpISd* (*NME* homolog from the marine sponge *Suberites domuncula*) promoter revealed that some of the motifs crucial for human promoter activity are also present in sponges. Further, the sponge protein has a hexameric structure and a NDPK activity similar to the human homolog NME1. NMEGpISd shows DNA binding properties of NME1 and/or NME2 and interacts with the human NME1 ortholog/homolog in human cultured cells. Moreover, it shows the same subcellular localization as the human NME1 and significantly diminishes migration potential of human cells, which leads to the conclusion that the sponge NME protein can replace the human partner in, at least, some biological functions which are usually associated with "higher" metazoans. The possible function of the NMEGpI (ancestral type protein, the precursor of Group I proteins) is not yet established in simple metazoans but it is possible that it participates in ancient precursor processes, which can be viewed as a foundation for the complex signaling networks in higher animals. Our recent work on the NME homolog from filasterean *Capsospora owcarzaky*, a close unicellular relative of animals *(25)* reveals similar features which indicate that the NME protein did not change significantly in the transition to multicellularity.

It has been shown that *NME5-8* have already been present in the common ancestor of choanoflagellates and metazoans and emerged at the time of eukaryote radiation *(23)*. Most Group II members are present in early-branching eukaryote lineages. Two exceptions are *NME*8, which is probably a choanoflagellate/metazoan innovation, and *NME*9, which originated from an incompletely translocated duplication of *NME*8 after separation of eutherians and metatherians. The *NME*10 displays a separate evolutionary history since it seems that its NDK domain was inserted relatively recently *(7)*. The evolutionary studies of the Group II genes/proteins underscore their necessity in the physiology of every living cell although there has been no systematic research to reveal their function.

NME1 – a pioneer in metastasis suppression

Since its discovery as the first metastasis suppressor gene *(3)*, *NME*1 has been in focus of scientific research *(26)*. In order to inhabit new locations, metastatic cells must detach from the original tissue, break through the basal lamina and invade the surrounding tissue, enter the nearby blood or lymphatic vessels, survive the transit through the lymphatic or blood system, extravasate from blood/lymphatic vessels into distant tissue and form a colony of cells (micrometastasis). Metastasis suppressors are specifically involved in regulation of one or several steps of this metastatic cascade. The key feature of a metastasis suppressor gene is that its expression inhibits metastasis but it does not influence primary tumor growth. Upon restoration of its function, the cell is no longer metastatic although it remains tumorigenic *(27)*. In the recent years, numerous studies have shown that down-regulation or even loss of expression of *NME1* correlates with metastasis formation and poor clinical outcome in many tumor types such as breast cancer, melanoma, ovarian carcinoma, hepatocellular and laryngeal carcinoma as well as several others *(28, 29)*. The exception to the rule is childhood neuroblastoma where overexpression and mutation of *NME1* (and *NME2*) were detected and linked to poor patient prognosis and unfavorable clinical outcome *(30, 31)*. Additionally, hematological malignancies also exhibit similar features: overexpression of *NME1* correlates with disease progression and unfavorable prognosis *(32)*. From these data it is obvious that *NME1* plays

a tissue-specific role, and, therefore, different regulatory mechanisms act in different tumors. It has been reported that *NME1* is implicated in invasion and colonization *(33)*.

Although *NME2* was not studied so much in this context, it does exhibit metastasis suppressor activity. It has been reported that NME2 has been involved in several human cancers including hepatocarcinoma, endometrial and cervical carcinoma, leukemia, breast and gastric carcinoma, pleural mesothelioma, colorectal, lung, sarcoma, and giant cell tumors *(34)*. Similar to NME1, NME2 is not only a metastasis suppressor but also may be capable of promoting and modulating tumorigenesis in different cellular environments. The mechanisms by which either of these proteins execute their functions in tumorigenesis and metastasis development remain to be elucidated by future studies.

NME protein partners

Although being proposed a plethora of biochemical functions, the NDPK activity is the only biochemical function of the NME proteins that has been thoroughly investigated *(2)*. These findings are critically evaluated by a part of the scientific community which considers the evidence, concerning the relatively vast variety of biochemical functions, to be partial and potentially inadequate *(35)*. It is, however, obvious that the vast variety of cellular functions in which NME proteins are involved, can hardly be explained solely by its enzymatic activity. It is more likely that they are a consequence of protein-protein interactions, or a combination of the two which could support the hypothesis that NME proteins may suppress tumor metastasis by binding and inactivating signaling pathways promoting aggressiveness *(35)*.The interacting partners of NME family members include a number of oncogenic, viral and cytoskeletal proteins which are, considering the diverse NME subcellular localization, spatially defined *(36)*. In the recent years, the most thoroughly investigated members of the NME family, NME1 and NME2 have both been reported to interact with dynamin *(37)*. Additionally, NME1 seems to interact with prune *(38)*, MIF *(39)*, p53/STRAP *(40)*, WHL *(41, 42)* and a number of small GTPase interacting proteins *(43)*. NME2 has been reported to interact with TRF1 *(44)*, MDM2 *(45)*, ICAP1α *(46)* and several others. An excellent review on potential NME partners and possible confusion in the interpretation of their interactions has been published by Vlatković et al *(36)*. Although having 88% amino acid homology and identical 3D structures the two proteins seem to be quite selective in choosing their interacting partners which clearly confirms that, although forming a functional NDP kinase together, each of them participate in distinct signaling pathways and execute additional, dissimilar functions in the cell. The number of interactions involving NME proteins in processes contributing to carcinogenesis is considerable and represents a potential pool of biomarkers or therapeutic targets for disease monitoring and treatment.

Potential translational approaches

Treatment of metastatic cancers is one of the major challenges in therapy of malignant diseases. Metastases are often inoperable so the choice of treatment relies on chemotherapy or radiotherapy with usually very modest results and fatal outcome. Therefore, the development of new therapeutic strategies for the metastatic disease is of utmost importance.

One of the crucial elements of metastasis formation is the depletion of metastasis suppression, therefore, it is crucial to re-express the missing gene/protein in order to inhibit metastasis. Several drugs have been reported to elevate *NME1* expression, such as, for example, *all-trans* retinoic acid in hepatocellular carcinoma cell line *(47)*. The injection of *thujone*, a natural product, into nude mice bearing B16F-10 melanoma cells, upregulated *NME1* and increased overall survival *(48)*. Several other compounds have been reported to have a similar effect on *NME1 in vitro*: anti-inflammatory compounds such as indomethacin and acetylsalicylic acid, antioxidant L-carnosine, gamma linoleic acid and several others *(49)*. The treatment with medroxyprogesterone acetate (MPA) induced re-expression of *NME1* in triple negative breast cancer cells *(50)* and soft agar colonization was reduced by 50% *in vitro*. Xenograft experiments introducing MDA-MB231 cells *in vivo* revealed 43% less pulmonary metastases in MPA treated, versus untreated mice *(51)*. Based on these data, a phase II clinical trial with MPA treatment was conducted *(29)*, however, unfortunately, the patients failed to achieve the necessary plasma levels of MPA *(52)*. Another approach that included a cell permeable *NME1* construct, suppressed pulmonary metastases development in mice while increasing overall survival *(53)*.

A number of other approaches have been introduced over the years of investigation. It has been suggested that the NME1/h-Prune protein complex regulates cancer progression and that its specific impairment could be a new therapeutic strategy. A peptide that disrupts the interaction of NME1 and h-Prune was identified. Preclinical studies on breast and prostate cancer and neuroblastoma have proven effectiveness and safety of this procedure in a mouse prostate cancer model system *(54, 55)*. Another approach is based on the identification of different gene targets whose expression is inversely correlated to *NME1*. Lysophosphatidic acid receptor 1 (LPA1) was identified as such a target in multiple cancer cell lines and breast carcinoma. The use of LPA1 antagonists was verified in preclinical studies *(56)*.

Although it has been proven that *NME1* is downregulated in many aggressive tumor types, until this date it hasn't been established as an independent diagnostic or

prognostic marker. Due to its interaction with a number of binding proteins, NME1, although expressed, might be unavailable, which provides an explanation of why *NME1* expression is not of independent prognostic importance and, therefore, its expression level, at least for now, does not have any clinical value *(57)*. However, since the number of interactions involving NME proteins in processes contributing to carcinogenesis is considerable it is possible that interactions themselves represent a potential pool of biomarkers or therapeutic targets for disease monitoring and treatment.

Concluding remarks

In the last decades, extensive research has been done in order to elucidate the structure, biochemical and biological functions, evolution and translational research of NME/NDPK. Although the knowledge about this gene/protein family is extensive we are still facing many questions to be answered in order to fully understand its multifunctional nature. Besides NME1, NME2 and NME4, the knowledge about other family members, including the Group I protein NME3, is rather limited. Therefore, to get insights on the physiology of others, especially the Group II family members, more basic research should be supported.

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