J Bioenerg Biomembr (2006) 38:163–167
DOI 10.1007/s10863-006-9040-3

MINIREVIEW

Potential roles of 3′-5′ exonuclease activity of NM23-H1 in DNA repair and malignant progression

David M. Kaetzel · Qingbei Zhang · Mengmeng Yang · Joseph R. McCorkle · Deqin Ma · Rolf J. Craven

Published online: 13 October 2006

Abstract NM23-H1 is a metastasis suppressor protein that exhibits 3′-5′ exonuclease activity *in vitro*. As 3′-5′ exonucleases are generally required for maintenance of genome integrity, this activity represents a plausible candidate mediator of the metastasis suppressor properties of the NM23-H1 molecule. Consistent with an antimutator function, ablation of the yeast NM23 homolog, *YNK1*, results in increased mutation rates following exposure to UV irradiation and exposure to the DNA damaging agents etoposide, cisplatin, and MMS. In human cells, a DNA repair function is further suggested by increased NM23-H1 expression and nuclear translocation following DNA damage. Also, forced expression of NM23-H1 in NM23-deficient and metastatic cell lines results in coordinate downregulation of multiple DNA repair genes, possibly reflecting genomic instability associated with the NM23-deficient state. To assess the relevance of the 3′-5′ exonuclease activity of NM23-H1 to its antimutator and metastasis suppressor functions, a panel of mutants harboring defects in the 3′-5′ exonuclease and other enzymatic activities of the molecule (NDPK, histidine kinase) have been expressed by stable transfection in the melanoma cell line, 1205Lu. Pilot *in vivo* metastasis assays indicate 1205Lu cells are highly responsive to the metastasis suppressor effects of NM23-H1, thus providing a valuable model for measuring the extent to which the nuclease function opposes metastasis and metastatic progression.

Keywords NM23 · Exonuclease · DNA repair · Metastasis · Metastasis suppressor

D. M. Kaetzel (✉) · Q. Zhang · M. Yang · J. R. McCorkle · D. Ma · R. J. Craven
Department of Molecular and Biomedical Pharmacology, College of Medicine, University of Kentucky, Lexington, Kentucky 40536-0298, USA
e-mail: dmkaetz@uky.edu

Introduction

NM23-H1 was the first metastasis suppressor gene to be identified, exhibiting an ability to suppress metastatic growth without significant impact on the transformed phenotype (Steeg et al., 1988). Over 18 years of investigation have uncovered multiple enzymatic activities for the NM23-H1 molecule, but the mechanism(s) underlying its ability to suppress metastasis remain under active investigation. Fig. 1 portrays the primary biochemical activities of NM23-H1 as understood at present, and highlights potential antimetastatic functions for each. This diversity of enzymatic activities is in accord with the notion that prototypical metastasis suppressor genes will impact upon many aspects of cell signaling and function (Shevde and Welch, 2003). Of these activities, the strongest case for antimetastatic activity to date has been made for the histidine kinase activity (hisK) of NM23-H1. Mutations in NM23-H1 which ablate the hisK, such as proline-96 to ser ($P_{96}S$) and $S_{120}G$, lack the motility-suppressing activity of the wild-type protein when overexpressed in MDA-MB-435 breast carcinoma cells (MacDonald et al., 1993; Wagner et al., 1997). Moreover, the kinase suppressor of Ras (KSR) is a substrate for the hisK of NM23-H1, suggesting a potential mechanism underlying motility-suppression (Hartsough et al., 2002). However, definitive validation of the role played by the hisK awaits detailed characterization of relevant downstream hisK substrates and their relationships to metastasis suppression. The nucleoside diphosphate kinase (NDPK) activity of NM23-H1 has been indicated to be not essential for metastasis suppression (MacDonald et al., 1993), although this has yet to be demonstrated using the forced expression model used above, due to lethality associated with overexpression of NDPK mutants.

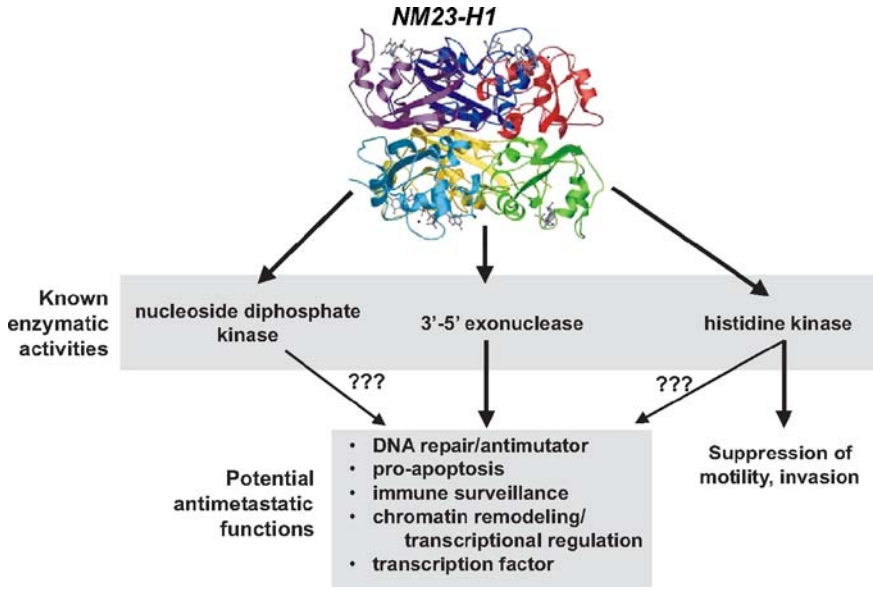

Fig. 1 Potential antimetastatic functions of the primary enzymatic activities of NM23-H1

We reported recently that preparations of recombinant NM23-H1 possess significant 3′-5′ exonuclease (3′-5′ *EXO*) activity (Ma et al., 2002; Ma et al., 2004), analogous to that demonstrated with the NM23-H2 molecule (Postel et al., 2001; Postel, 1999). The 3′-5′ *EXO* activity of NM23-H1 has been validated rigorously, by virtue of its precise coelution with NM23-H1 protein during column chromatography (including size exclusion). In addition, site-specific mutation of a residue shown previously to inactivate the NM23-H2 isoform ($K_{12}Q$) results in a reproducible loss of 3′-5′ *EXO* activity. Also compelling was the original observation of highly selective binding of a C-rich, single-stranded oligodeoxynucleotide to NM23-H1-expressing clones in a HeLa cDNA expression library (Ma et al., 2002). The 3′-5′ *EXO* activity is very intriguing in light of the association of these enzymes with DNA repair processes, and the mutator phenotypes which often arise as a consequence of their deficiencies (Shevelev and Hübscher, 2002). Progression to the metastatic phenotype is well-recognized to require the accumulation of mutations that permit tumor cells to overcome numerous barriers to metastatic growth. In addition to antimutator activity, the 3′-5′ *EXO* could be involved in immune elimination of tumor cells, as suggested by a report that NM23-H1 provides a nuclease ("nicking") activity required for induction of cytotoxic T cell-induced apoptosis (Fan et al., 2003). Also noteworthy is the ability of NM23 expression to regulate transcription via interactions with nuclease-hypersensitive elements in such growth-relevant genes as c-*myc* (Berberich and Postel, 1995) and platelet-derived growth factor A-chain (Ma et al., 2002). In this minireview, we will summarize our progress toward understanding the physiological role of the 3′-5′ *EXO* activity of NM23-H1, as well as its potential relationship to metastasis suppressor activity.

Potential role of the 3′-5′ exonuclease activity of NM23-H1 in DNA repair

NM23 deficiency results in mutator phenotypes in bacteria and yeast

DNA repair and the maintenance of genomic integrity represent logical cellular functions for the nuclease activities of NM23-H1 and NM23-H2. In this regard, a previous study in *Escherichia coli* provided an early indication that the NM23 homologue, *ndk*, is required for maintaining genomic integrity (Lu et al., 1995). When the *ndk* gene was disrupted, frequencies of spontaneous mutations conferring resistance to the antibiotics rifampicin and nalidixic acid were significantly increased (20- to 50-fold). Disruption of *ndk* alone, or in combination with other genes required for NTP synthesis (e.g. pyruvate kinase, succinyl CoA synthetase), had no effect on cell growth or morphology. The mutator phenotype observed in the *ndk*-disrupted strain was atttributed to an imbalance in nucleotide pool sizes as a consequence of NDPK deficit, most notably a 20-fold elevation in intracellular concentrations of dCTP. However, the exact enzymatic activity(s) of ndk required for antimutator activity was not identified directly in this study. The strong conservation of amino acid residues in ndk implicated previously in the NDPK, hisK and 3′-5′ *EXO* activities of NM23-H1 strongly suggests similar functions for the *E. coli* enzyme and, thus, the possibility that any one or more of these activities may be relevant to DNA repair.

In an effort to quantify the contribution of NM23-H1 to DNA repair, we have initiated experiments in the yeast *Saccharomyces cerevisiae*. Yeast provide excellent eukaryotic

models for DNA repair research, with considerable progress made in defining their DNA repair pathways and mechanisms. Moreover, knockout strains are now available for essentially all open reading frames in the *S. cerevisiae* genome, including the NM23 homologue *YNK1*. These characteristics of yeast provide a wealth of options for genetic approaches to determining the role of NM23 proteins in DNA repair. Prior to the initiation of our studies, a number of relevant observations had been obtained about potential DNA repair functions of the *YNK1* gene in yeast. For example, treatment with the DNA methylating agent, methyl methanesulfonate (MMS) elicits a marked increase in *YNK1* expression, consistent with a role in the DNA repair response (Gasch et al., 2001). Interestingly, the MMS-induced increase is dependent on expression of *MEC1*, the yeast homologue of the human ATR kinase which plays a central role in transduction of DNA damage-induced signaling. Also potentially significant is the detection of physical interactions between *Ynk1p* and two proteins implicated in DNA repair (Gavin et al., 2002), a histone H4 protein (*HHF2*) and RNA polymerase I subunit A135 (*RPA135*). HHF2 is one of two nearly identical H4 proteins that are required for chromatin assembly and telomeric silencing, and is of particular interest because of its well-recognized role in maintenance of genomic integrity (Grunstein, 1990).

We are currently assessing the role of *YNK1* in DNA repair and maintenance of genomic integrity by measuring the impact of ablation of the *YNK1* gene on mutation rates in *S. cerevisiae*. *YNK1*-null (*YNK1*Δ) mutants have been shown previously to exhibit no defects in growth rate, spore formation, mating ability or morphology (Fukuchi et al., 1993), analogous to the lack of viability phenotype in *E. coli* (Fukuchi et al., 1993). We have observed that the *YNK1*Δ strain exhibits no increase in spontaneous mutation rates, as determined using the conventional *CAN1* forward mutation assay. However, significantly higher mutation rates were observed for the *YNK1*Δ strain in response to treatment with MMS (3-fold) and UV (7-fold) as compared to the wild-type counterpart. Current efforts are being devoted to analyzing DNA repair activity in *YNK1*Δ strains that have been engineered with second knockouts in a variety of known DNA repair genes (e.g. *DDC1*, *RAD17*, *MEC3*, *MSH2* and *RAD27*), in an effort to magnify the *YNK1*-dependent mutator phenotype. Such evidence of functional cooperativity has proven useful in establishing relevance of numerous candidate proteins to the DNA damage response (Craven et al., 2002; Morrison et al., 2004). These double-knockout strains should also provide models for complementation with wild-type and enzymatically-defective mutant forms of NM23-H1 in efforts to identify repair-relevant biochemical activity(s) of the molecule.

Cell culture models for assessing DNA repair activity of NM23 proteins in mammals

While yeast provide clear advantages for analysis of DNA repair activity, observations obtained in this model will require validation in cells derived from higher vertebrates, preferably in human lines. Our laboratory has devoted considerable efforts to identifying human cell lines devoid of NM23 expression in which DNA repair activity can be assessed, and complementation experiments conducted with a minimum of background NM23 expression. We have identified three human metastatic cell lines, WRO82 (thyroid carcinoma), WM1158 and 1205Lu (melanoma), that are deficient in expression of both NM23-H1 and NM23-H2. Interestingly, another cell line (WM793) which represents the advanced vertical growth phase (VGP) of melanoma, but is non-metastatic in experimental rodent models, has also been observed to be devoid of detectable expression of either NM23-H1 or NM23-H2. Also made available to this project are mouse embryo fibroblasts derived from mice harboring a lesion of the NM23-M1 gene, the murine homologue of NM23-H1 (Arnaud-Dabernat et al., 2003). We have recently engineered stably transfected lines of 1205Lu and WM793 that express a range of NM23-H1 variants, including wild-type and the mutants $H_{118}F$ ($NDPK^-$, $hisK^-$, EXO^+), $P_{96}S$ ($NDPK^+$, $hisK^-$, EXO^+) and $K_{12}Q$ ($NDPK^-$, $hisK^-$, EXO^-). The $K_{12}Q$ mutant is the only variant deficient in 3′-5′ *EXO* activity and, thus, has the potential to provide insights into the role of this enzyme in DNA repair. Genomic stability is being assessed in these cell lines by conventional measurements of spontaneous and DNA damage-induced mutation rates at the hypoxanthine phosphoribosyl transferase (hprt) and ouabain-resistance (Oua^R) loci (Glaab and Tindall, 1997; Mankovitz et al., 1974; Rossman et al., 1995).

DNA damage induces nuclear translocation of NM23 proteins and colocalization with mediators of DNA repair

If NM23 proteins play an important and direct role in DNA repair, they must be present in the nucleus. Moreover, expression and nuclear localization of NM23 proteins would be expected to be induced during periods of genomic stress, such as following exposure to DNA damaging agents. Indeed, nuclear localization of NM23-H1 has already been described in cells induced to undergo granzyme A-mediated apoptosis following cytotoxic T cell attack (Fan et al., 2003). In our laboratory, immunoblot analyses have revealed that NM23-H1 expression is induced dramatically in two tumor cell lines (HepG2, HeLa) by the DNA damaging agents etoposide and cisplatin. Both elicit peak inductions within 2 h of treatment, with expression returning to near baseline levels by 24 h.

The induction is accompanied by similar kinetics of nuclear accumulation of NM23-H1. Immunocytochemical studies in HeLa cells demonstrate that these agents promote localization of NM23 proteins to both perinuclear regions and distinct nuclear foci. More recently, we have observed that UV irradiation appears to induce NM23-H1 expression and localization in nuclear foci to an even greater extent than etoposide and cisplatin. Moreover, NM23-H1-containing foci appear to colocalize with Rad 1, a component of the 9-1-1 complex implicated DNA damage induced by UV and other agents. Taken together, these results strongly suggest a role for NM23 proteins in the DNA damage response, particularly in UV damage repair. Studies are actively underway to demonstrate physical interactions between NM23-H1 and proteins implicated in the DNA damage response using standard "pull-down" approaches. Novel interactions are also being probed by proteomic analysis of nuclear factors that are associated with NM23-H1 following genomic insult.

Also underway is an investigation of the nature of NM23-H1 action upon translocation to the nucleus after genomic insult. While DNA repair is an obvious potential function of the 3′-5′ *EXO* activity, an alternative or complementary role in promoting apoptosis must also be considered, especially in light of its proapoptotic function in cells targeted by cytotoxic T cells (Fan et al., 2003). In this regard, the melanoma cell line panels described above are being employed to address this question. A prediction is that forced expression of NM23-H1 will impart increased sensitivity to DNA damage via apoptotic mechanisms, and that the 3′-5′ *EXO*-deficient mutant $K_{12}Q$ will not complement the NM23-H1-deficient condition. Indeed, increased cisplatin sensitivity has been described previously in response to forced expression of NM23-H1 in a melanoma cell line (Ferguson et al., 1996). In addition, the nature of NM23-H1-dependent modifications of DNA will be assessed. Precedent with the cytotoxic T cell mechanism suggests that DNA nicking similar to that seen in the granzyme A-mediated apoptotic pathway will be evident in melanoma cells that overexpress wild-type or other mutant forms of NM23-H1 in which the 3′-5′ *EXO* is intact.

Forced expression of NM23-H1 in NM23-deficient, metastatic cell lines results in downregulation of genes associated with DNA repair and replication

To address the potential physiological relevance of the 3′-5′ *EXO* activity of NM23-H1, we considered whether modulation of NM23-H1 expression affects expression of other genes involved in DNA repair, replication and gene transcription. Using adenoviral vectors engineered to provide robust expression of wild-type NM23-H1, we have recently determined by microarray analysis that NM23-H1 elicits profound effects on the transcriptome in NM23-deficient, metastatic cell lines. While the effects of NM23-H1 re-expression were exerted across all major functional classes of genes, of particular relevance was the demonstration of coordinate downregulation of numerous genes implicated in DNA repair. These include such well-known participants in DNA repair as Rad51 (dsDNA break repair), HUS1 (9-1-1 complex), MLH1 (mismatch repair), BRCA1 (MRE11 complex), PCNA, flap endonuclease 1 (FEN1), DNA polymerase θ, and methyl-CpG binding protein. In light of the apparent association of NM23-H1 with Rad1 (see above), the regulation of another 9-1-1 complex member, HUS1, is particularly noteworthy.

Although further study is necessary, it is tempting to speculate that downregulation of these DNA repair genes following reexpression of NM23-H1 may reflect genomic instability associated with the NM23-deficient state. It will be of prime interest to better understand the mechanism underlying the relationship between NM23-H1 and DNA repair genes, which could be mediated by direct repression of the promoters and transcription rates for these genes, in keeping with the proposed transcription factor properties of NM23-H1. Alternatively, the relationship may be less direct, with genomic instability resulting from the NM23-H1-deficient state leading to activation of more generalized regulatory mechanisms for transcription of DNA repair genes (El Deiry, 2002; Ford, 2005; Rosen et al., 2003). In either case, a transcriptional mechanism for regulation of these DNA repair genes would be novel, as many appear to be regulated by post-translational modifications of prexisting protein pools and by their translocation to sites of DNA repair.

Conclusions

Evidence is accumulating across a wide range of prokaryotic and eukaryotic organisms in support of a role for NM23-H1 in maintaining genomic integrity, although the mechanisms underlying such a function remain to be elucidated. The NM23-H1 molecule is exceedingly versatile, possessing at least three distinct enzymatic activities that could participate in DNA repair and other aspects of protecting the genome. The *in vitro* 3′-5′ *EXO* activity we have characterized represents a very plausible candidate activity, as most other examples of these enzymes are indeed implicated in mechanisms of DNA repair. However, the NDPK activity must also be considered, in light of its potential role in maintaining balance in nucleotide pool sizes. Moreover, the hisK activity may also play an important role in direct and/or indirect signaling to repair pathway components, although evidence for such possibilities is currently lacking. A panel of NM23-H1 mutants have been constructed, characterized and expressed in yeast and mammalian cell lines to address these important issues, and will hopefully contribute to a fuller understanding of the antimetastatic functions of NM23-H1.

Acknowledgements The work described here was supported by grants from the National Cancer Institute (CA83237) and the Kentucky Lung Cancer Research Fund.

References

Arnaud-Dabernat S, Bourbon PM, Dierich A, Le Meur M, Daniel J-Y (2003) J Bioenerg Biomembr 35:19–30
Berberich SJ, Postel EH (1995) Oncogene 10:2343–2347
Craven RJ, Greenwell PW, Dominska M, Petes TD (2002) Genetics 161:493–507
El Deiry WS (2002) Cancer Biol Ther 1:490–491
Fan Z, Beresford PJ, Oh DY, Zhang D, Lieberman J (2003) Cell 112:659–672
Ferguson AW, Flatow U, MacDonald NJ, Larminat F, Bohr VA, Steeg PS (1996) Cancer Res 56:2931–2935
Ford JM (2005) MutatRes 577:195–202
Fukuchi T, Nikawa J, Kimura N, and Watanabe K (1993) Gene 129:141–146
Gasch AP, Huang M, Metzner S, Botstein D, Elledge SJ, Brown PO (2001) MolBiolCell 12:2987–3003
Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G (2002) Nature 415:141–147
Glaab WE, Tindall KR (1997) Carcinogenesis 18:1–8
Grunstein M (1990) Annu Rev Cell Biol 6:643–678
Hartsough MT, Morrison DK, Salerno M, Palmieri D, Ouatas T, Mair M, Patrick J, Steeg PS (2002) J Biol Chem 277:32389–32399
Lu Q, Zhang X, Almaula N, Mathews CK, Inouye M (1995) J Mol Biol 254:337–341
Ma D, McCorkle JR, and Kaetzel DM (2004) J Biol Chem 279:18073–18084
Ma D, Xing Z, Liu B, Pedigo N, Zimmer S, Bai Z, Postel E, Kaetzel DM (2002) J Biol Chem 277:1560–1567
MacDonald NJ, De La Rosa A, Benedict MA, Freije JM, Krutsch H, Steeg PS (1993) J Biol Chem 268:25780–25789
Mankovitz R, Buchwald M, Baker RM (1974) Cell 3:221–226
Morrison AJ, Highland J, Krogan NJ, Arbel-Eden A, Greenblatt JF, Haber JE, Shen X (2004) Cell 119:767–775
Postel E, Abramczyk BM, Levit MN, Kyin S (2001) Proc Natl Acad Sci USA 97:14194–14199
Postel EH (1999) J Biol Chem 274:22821–22829
Rosen EM, Fan S, Pestell RG, Goldberg ID (2003) J Cell Physiol 196:19–41
Rossman TG, Goncharova EI, Nadas A (1995) Mutat Res 328:21–30
Shevde LA, Welch DR (2003) Cancer Lett 198:1–20
Shevelev IV, Hübscher U (2002) Nat Rev Mol Cell Biol 3:1–12
Steeg PS, Bevilacqua G, Kopper L, Thorgeirisson UP, Talmadge JE, Liotta LA, Sobel ME (1988) J Natl Cancer Inst 80:200–204
Wagner PD, Steeg PS, Vu ND (1997) Proc Natl Acad SciUSA 94:9000–9005