Quantitative Analysis of the Expression of Human N-myristoyltransferase 1 (hNMT-1) in Cancers

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Abstract: Human N-myristoyltransferase 1 (hNMT-1) catalyzes the covalent attachment of myristic acid to N-terminal glycine residues (myristoylation) of numerous protein substrates. Overexpression of hNMT-1 in colorectal and gallbladder cancers makes it a potential biomarker and drug design target for such cancers. In this study, we investigated hNMT-1 expression during the progression of eight different human cancers using quantitative RT–PCR. The study results showed that hNMT-1 was up-regulated in breast, colon, lung and ovarian cancers but not kidney, liver, prostate and thyroid cancers. This suggests a role for hNMT-1 as a biomarker for detection of breast, colon, lung and ovarian cancers. This study also suggests the available hNMT-1 inhibitors may be potential therapeutic agents against breast and lung cancers through all disease stages, although their use would likely be limited to early stage colon and ovarian cancers.

INTRODUCTION

Protein modification is an important component of genetic regulation. The structural alterations resulting from protein modification may regulate the biological activity, sub-cellular localization, stability, and turnover of proteins [1-5]. Unlike the common modifications (phosphorylation, nucleotidylation and glycosylation), myristoylation is a relatively rare type of acylation. Myristoylation involves the covalent attachment of myristic acid (C14:0) to the N-terminal glycine residue of protein substrates *via* an amidic linkage [6-8]. Co- or post-translational myristoylation increases protein lipophilicity and facilitates the protein's association with cellular membranes and/or other hydrophobic protein domains [9, 10].

Protein myristoylation is catalyzed by a family of highly conserved enzymes, N-myristoyltransferases (E.C. 2.1.3.93). These enzymes are ubiquitously distributed in eukaryotic cells [6-10]. Previous biochemical and structural studies revealed that N-myristoyltransferases belong to the GCN5-related N-acetyltransferase superfamily and possess a pseudo 2-fold symmetry in their catalytic domains [11-13]. The N-terminal half is more conserved in amino acid residues and is responsible for the binding of myristoyl-coenzyme A (myristoyl-CoA). The C-terminal half has fewer conserved amino acid residues and is responsible for protein substrate recognition. N-myristoyltransferases follow an ordered bi-bi sequential catalytic mechanism (Fig. **1**). Myristoyl-CoA binds to the enzymes first, followed by binding of the protein substrate to form a ternary complex. Upon completion of the reaction, coenzyme A (Co-A) is released prior to release of the myristoylated protein substrate. No covalent bonds are formed between myristoyl-CoA and the N-myristoyltransferases during the reaction [14].

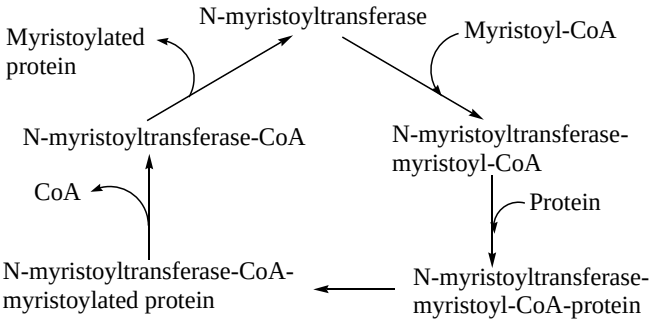

Fig. (1). Ordered bi-bi sequential catalytic mechanism for N-myristoyltransferases. Myristoyl-CoA binds prior to the protein substrate and the release of CoA occurs before the release of the myristoylated protein substrate.

Human N-myristoyltransferase 1 (hNMT-1) is encoded by a single gene copy located on chromosome 17 (17q21.31) [15, 16]. hNMT-1 protein substrate specificity can be broadly divided into two groups: signal transduction proteins such as the protein tyrosine kinases $pp60^{c\text{-}src}$, $pp60^{yes}$, $pp56^{lck}$, $pp59^{fyn/syn}$ and c-Abl, and viral proteins such as the HIV-1 and SIV Nef proteins and the HIV $Pr53^{gag}$ precursor [17-28]. hNMT-1's involvement in tumorgenesis was first reported with colon cancer where colorectal cancer cells demonstrated enhanced hNMT-1 expression and activity, as well as increased activity of its substrate $pp60^{c\text{-}src}$ [29, 30]. Furthermore, inhibition of $pp60^{c\text{-}src}$ myristoylation using N-myristoyltransferase inhibitors depressed the colony formation of colonic cancer cell lines [29]. Subsequent reports indicated hNMT-1 upregulation in gallbladder and brain cancers [31, 32]. Such studies identified hNMT-1 as a possible anti-cancer drug design target, which has promoted the development of several families of inhibitors [33-36]. Since substrates of hNMT-1 participate in different signal transduction pathways, abnormal expression and activity of hNMT-1 might be involved in the pathogenesis of other cancer types. In addition, no previous studies have addressed the

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Table 1. Patient Demographic Characteristics and Cancer Stage for the Origene TissueScan Oncology qPCR Cancer Survey Panel 96

Patient Number	Tissue	Age	Gender	Stage	Patient Number	Tissue	Age	Gender	Stage
A01	Breast	44	F	0	E01	Lung	49	F	0
A02	Breast	45	F	0	E02	Lung	79	M	0
A03	Breast	32	F	0	E03	Lung	62	F	0
A04	Breast	47	F	I	E04	Lung	71	M	Ia
A05	Breast	52	F	I	E05	Lung	64	M	Ib
A06	Breast	57	F	IIa	E06	Lung	85	F	Ib
A07	Breast	42	F	IIa	E07	Lung	72	M	Ib
A08	Breast	63	F	IIIa	E08	Lung	63	M	IIb
A09	Breast	61	F	IIIa	E09	Lung	71	M	IIb
A10	Breast	55	F	IIIc	E10	Lung	58	M	IIIa
A11	Breast	45	F	IV	E11	Lung	80	M	IIIb
A12	Breast	42	F	IV	E12	Lung	51	M	IV
B01	Colon	61	M	0	F01	Ovary	70	F	0
B02	Colon	91	M	0	F02	Ovary	31	F	0
B03	Colon	37	F	0	F03	Ovary	42	F	0
B04	Colon	93	F	I	F04	Ovary	29	F	Ia
B05	Colon	65	M	IIa	F05	Ovary	43	F	Ib
B06	Colon	78	M	IIa	F06	Ovary	51	F	Ic
B07	Colon	66	M	IIa	F07	Ovary	80	F	IIb
B08	Colon	86	M	III	F08	Ovary	46	F	IIIa
B09	Colon	42	F	IIIb	F09	Ovary	52	F	IIIb
B10	Colon	61	M	IIIc	F10	Ovary	74	F	IIIc
B11	Colon	63	M	IIIc	F11	Ovary	77	F	IIIc
B12	Colon	51	F	IV	F12	Ovary	79	F	IV
C01	Kidney	71	M	0	G01	Prostate	68	M	0
C02	Kidney	66	M	0	G02	Prostate	65	M	0
C03	Kidney	54	F	0	G03	Prostate	76	M	0
C04	Kidney	52	M	I	G04	Prostate	70	M	I
C05	Kidney	55	F	I	G05	Prostate	63	M	II
C06	Kidney	52	M	I	G06	Prostate	70	M	II
C07	Kidney	57	F	II	G07	Prostate	71	M	II
C08	Kidney	59	M	III	G08	Prostate	56	M	II
C09	Kidney	37	M	III	G09	Prostate	63	M	II
C10	Kidney	64	M	III	G10	Prostate	53	M	III
C11	Kidney	70	M	IV	G11	Prostate	65	M	III
C12	Kidney	51	M	IV	G12	Prostate	61	M	III
D01	Liver	81	M	0	H01	Thyroid	30	F	0
D02	Liver	86	M	0	H02	Thyroid	68	F	0
D03	Liver	33	F	0	H03	Thyroid	46	F	0
D04	Liver	79	M	I	H04	Thyroid	15	F	I
D05	Liver	58	F	I	H05	Thyroid	28	F	I
D06	Liver	66	M	I	H06	Thyroid	39	F	I
D07	Liver	63	F	II	H07	Thyroid	57	M	II
D08	Liver	68	M	II	H08	Thyroid	74	M	II
D09	Liver	62	F	II	H09	Thyroid	76	F	III
D10	Liver	71	M	IIIa	H10	Thyroid	52	F	III
D11	Liver	21	M	IV	H11	Thyroid	52	M	IVa
D12	Bile duct	66	M	IV	H12	Thyroid	45	F	IVa

variations in hNMT-1 expression levels over the progression of human cancers. In order to investigate the role hNMT-1 plays in the progression of different cancer types, hNMT-1 mRNA expression levels were evaluated in a 96-patient-sample cancer survey panel by quantitative RT-PCR in the current study.

MATERIALS AND METHODOLOGY

hNMT-1 mRNA expression levels were evaluated in an Origene 96-sample TissueScan Oncology qPCR Cancer Survey Panel (Table **1**) by quantitative RT-PCR using an Applied Biosystems 7300 Real-Time PCR System (Foster City, California, USA). hNMT-1 expression was normalized to the internal control, β-actin in the different patients. Primer sequences (forward: GATGATGACAACATGTTCCGATTTGA-TT; reverse: GCCGGAGAGCCCACAAA) and the TaqMan probe (FAM-ATTCCCCGGAGTTTCT) for gene *NMT1* (GenBank accession number: AF043324) encoding hNMT-1 were designed and synthesized by Applied Biosystems based on the ITS region. The TaqMan probe was labeled with FAM at 5'-end and non-fluorescent quencher at 3'-end. The quantitative RT-PCR reaction mixture consisted of TaqMan Gene Expression Master Mix (Applied Biosystems), 0.9 μM of each primer for gene *NMT1* and the gene encoding β-actin, and 0.9 μM of the TaqMan probe. 30μL of PCR reaction mixture was added to each well of the 96-smaple qPCR Cancer Survey Panel. The amplification was carried out under the following conditions: 2 min at 50°C, 10 min at 95°C, 60 cycles of 15 s at 95°C, and finally 1 min at 60°C. The expression level of hNMT-1 was averaged in each disease stage and normalized to β-actin. The fold-difference in mRNA expression at each disease stage was determined by comparison to expression levels in normal patients (stage 0, expression level set as 1). Unpaired t-test with Welch's correction between the hNMT-1 expression levels in normal and cancer patients for each cancer type was performed with GraphPad Prism 4.0 (GraphPad Software, San Diego, California, USA) when possible, with $\alpha = 0.05$.

RESULTS AND DISCUSSION

To evaluate whether hNMT-1 could be used as a biomarker for human cancers, the mRNA expression level of hNMT-1 was quantitatively analyzed in eight different cancer types. As shown in Table **2**, hNMT-1 mRNA expression levels were significantly elevated by an average of 3.7, 3.1, 2.3 and 1.8 fold in breast, colon, lung and ovarian cancers, respectively, (P=0.032, P=0.001, P=0.003 and P=0.012 for breast, colon, lung and ovarian cancer, respectively) but not in kidney, liver, prostate and thyroid cancers. These data implicate a role for hNMT-1 as a biomarker for early detection of human breast, colon, lung and ovarian cancers and suggest that hNMT-1 inhibitors could be used as potential therapeutic agents against such cancers.

Table 2. The Average Fold Difference (FD) in hNMT-1 mRNA Expression Levels in Patients with Cancer Relative to Patients Without Cancer

Cancer Type	FD	P-value
Breast	3.7	0.032
Colon	3.1	0.001
Kidney	1.0	0.986
Liver	0.9	0.742
Lung	2.3	0.003
Ovarian	1.8	0.012
Prostate	1.2	0.253
Thyroid	1.3	0.225

To obtain a preliminary impression on whether the expression of hNMT-1 varies with cancer progression, we also wished to compare mRNA expression levels at each cancer stage (Fig. **2**). However, the small sample size associated with the panel precluded a complete analysis, one that must await a larger scale screening study. Nonetheless, we have gleaned valuable information from our limited analysis, which will provide an important reference for the design of future screenings. In breast and lung cancers, we noted that hNMT-1 expression generally increases with disease progression, such that by stage IV hNMT-1 expression was 4.5- and 3.5-fold higher than normal subjects for breast and lung cancer, respectively. These data suggest hNMT-1 may contribute to the development and progression of breast and lung cancer, and further identified potential role of hNMT-1 inhibitors as effective therapeutics against breast and lung cancer throughout disease progression.

In colon cancer, upregulation of hNMT-1 was greatest in early stage cancer (~6.5-fold higher in stage I) (only one 93-year old patient; larger scale screening needed) relative to late stage cancer (~3.5-fold increase in stage IV). These data suggest use of hNMT-1 inhibitors as potential therapeutic agents against colon cancer, particularly during early stage progression. This conclusion is consistent with the previous studies that showed N-myristoyltransferase inhibitors depressed the colony formation of colonic cancer cell lines [29]. In ovarian cancer, hNMT-1 expression demonstrated a general increase with disease progression until stage IIIa (only one patient; larger scale screening needed) and returned to normal expression levels by stage IV. Although the reason for this return to normal expression levels in late stage ovarian cancer is not known, the data does indicate that hNMT-1 inhibitors might be effective for treatment of early stage ovarian cancer. Furthermore, as a biomarker, early detection of hNMT-1 expression might be necessary for effective hNMT-1 inhibitor based therapy. Interestingly, we noticed a 2-fold decrease and a 3-fold increase in the hNMT-1 expression at stage IV liver cancer and stage III thyroid cancer, respectively, without changes at other stages of diseases' progression. The panel was based on two stage IV liver cancer and two stage-III thyroid cancer patient samples, and this observation could simply represent a spurious result due to the low sample number. Further studies are required to clarify this outcome.

CONCLUSION

In this study, we showed that hNMT-1 expression was up-regulated in human breast, colon, lung and ovarian cancers, which suggests its use as a potential biomarker for these cancers. Furthermore, the available hNMT-1 inhibitors may be potential therapeutic agents against breast and lung cancers through all stages of cancer progression, while their

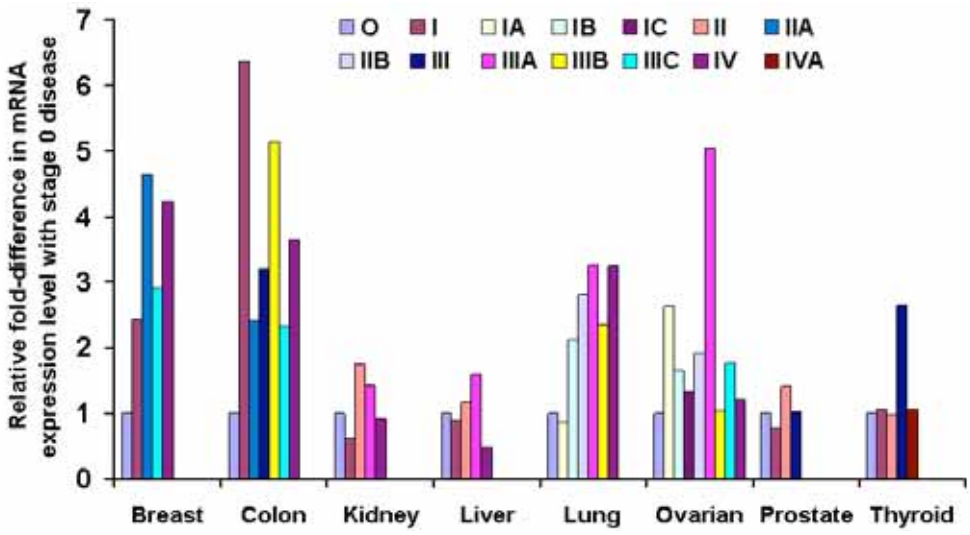

Fig. (2). Relative fold-difference in hNMT-1 mRNA expression at different stages of human cancer progression (hNMT-1 mRNA expression was screened in the Origene 96-patient-sample TissueScan Oncology qPCR Cancer Survey Panel).

use would likely be limited to early stage colon and ovarian cancers.

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