N-myristoyltransferase: A Novel Molecular Marker for Colorectal Adenomatous Polyps and Cancer

Tharmini Rathinagopal (1,2), Shiv Bhanot (2), Sergey Yegrov (1), Shiby Kuriakose (1,2), Nan Hu (3), Shailly Varma Shrivastav (1), Harminder Singh (4,5) and Anuraag Shrivastav (2, 5)

(1) Oncodrex Inc, Winnipeg, MB, Canada
(2) Department of Biology, University of Winnipeg, Winnipeg, MB, Canada
(3) Division of Biostatistics, University of Utah, Salt Lake City, UT, USA
(4) Division of Gastroenterology, College of Medicine, University of Manitoba, Winnipeg, MB, Canada
(5) Research Institute of Oncology and Hematology, Cancer Care Manitoba, Winnipeg, Canada.

Background

Protein N-myristoylation refers to the covalent attachment of myristate, a 14-carbon saturated fatty acid, to the N-terminal glycine residue of proteins. The recent discovery that lysine residues of proteins can also be myristoylated further emphasized the significance of myristoylation. Existing as two isoforms that share 77% homology, myristoylation of proteins is catalyzed by an enzyme N-myristoyltransferase (NMT). Many oncoproteins such as c-Abl, ARF, cAMP dependent protein kinase, and various tyrosine kinases (pp60src, pp60Yes, pp56lck, pp59fyn/syn) that are crucial for the onset and progression of cancer are myristoylated.
There are various pathways like mTOR, PI3K-Akt, Ras/Raf/MEK/ERK, Notch pathway, and others, which play a significant role in the proliferation of neuroendocrine cancers. The dysregulation of these pathways presents themselves as molecular signatures that are potential therapeutic targets and diagnostic markers in managing neuroendocrine tumors. We recently demonstrated that the expression of NMT1 increased with rapamycin treatment over the period with a concomitant decrease in mTOR phosphorylation.

Significance to NET-GI

We observed overexpression of NMT2 in PBMC of subjects with colorectal adenomatous polyps and cancers, which agrees with previously reported observation of high expression of NMT2 in colorectal polyps and cancer tissues. Our results from breast cancer and colorectal cancer (CRC) studies suggest that NMT is a crucial regulator of onco-pathways, especially PI3K/mTOR pathway. The expression profile of NMT in the neuroendocrine tumor in the gastrointestinal tract (NET-GI) could result in the identification of a novel therapeutic target. The inhibitors of NMT in clinical trials could potentially benefit in the management of NET-GI. In this prospective study, we demonstrated that NMT2 is a molecular marker for detecting colorectal polyps and cancer.

Experimental Approach

Ethics

The ethics approval for the study was obtained from the Human Research Ethics Board, University of Manitoba.

Recruitment
A total of 74 subjects were recruited prospectively (no indication of disease (NED) with a normal lower gastrointestinal tract endoscopy = 24; adenomatous polyps (AP) = 19; non-adenomatous polyps (NAP) = 12; CRC= 15); 4 of the subjects had polyps confirmed by colonoscopy.

Immunohistochemistry (IHC)
Cytospin slides of PBMCs were prepared in duplicate using cytospin centrifuge and sent for IHC at the Manitoba Tumor Bank in CancerCare Manitoba, Winnipeg. The PBMC Cytospin slides were stained for NMT2 on a Ventanna autostainer. An H-scores derived from assessing both staining intensity (scale 0-3) and the percentage of positive cells (0-100%) was given to each sample. These two scores were multiplied to generate an H-score of 0-300.

Statistical analysis.
Analysis of covariance (ANCOVA) was performed after confirming the assumptions of normality and heteroscedasticity. Multivariable models were constructed using the CRC status as the fixed grouping effect, adjusting for age, family history of polyps/cancer, and diverticulosis. All tests are two-sided.

Results
We report for the first time that N-myristoyltransferase 2 is significantly upregulated in peripheral blood mononuclear cells (PBMC) of subjects with colorectal adenomatous polyps and cancer compared to individuals with non-adenomatous polyps or no evidence of disease. Collectively, our findings provide evidence that NMT2 could be a promising biomarker for the detection of pre-malignant adenomatous polyps as well as CRC and can serve as a potential screening test.
The performance of NMT2 in the detection of pre-malignant lesions was further evaluated. In subjects with NED, NMT2 expression in PBMC ranged from negative to weak positivity except for 4 cases. The H score ranged from 0-240, with an average score of 70.9. In cases where the polyps were characterized as NAP, the H score ranged from 2-210 with an average score of 70.1. In contrast, CRC patients and AP subjects displayed intense NMT2 staining and a high percentage of positive cells. The H score for colorectal AP and cancer ranged from 90-240 and 90-270, respectively, with an average score of 162.6 for AP and 196.7 for CRC. In the univariate analysis, compared to the NED, subjects with CRC and AP had significantly elevated NMT-2 H scores (median values 195 and 150, respectively, $p<0.001$ for both).

Conclusions/Future Studies
Here we longitudinally examined the utility of blood NMT expression as a biomarker for colorectal cancer. Our analysis demonstrated that the NMT2 H-scores perform best for distinguishing CRC/AP from NED/NAP. The levels of NMT-2 protein in PBMC appear to gradually increase along with the natural progression of polyp development from non-adenomatous to adenomatous to CRC. In our future study, we plan to assess the NMT1/NMT2 expression in subjects with NET and investigate its utility as a diagnostic marker and therapeutic target.

Lay Abstract

We have developed a prototype blood test based on the overexpression of an enzyme called N-myristoyltransferase 2 (NMT 2) in the white blood cells of colorectal cancer (CRC) patients. In this study and previous studies using breast cancer models, we have discovered that NMT is a crucial molecule that regulates signaling pathways that are crucial for the development and progression of neuroendocrine tumors of the gastrointestinal tract (NET-GI). Although the current study demonstrates the utility of the biomarker in CRC, it is highly likely that it plays a vital role in NET-GI. NMT could potentially serve as a novel therapeutic target, and NMT inhibitors currently undergoing clinical trials could potentially be used for the treatment of NET-GI.