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# POTENTIAL ROLES OF METHYLTRANSFERASE FAMILY 16 IN BREAST CANCER

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***Abstract***

*Three out of ten members of methyltransferase family 16 (Mtase16) have been separately demonstrated involves in cancer regulation. However, no study has been conducted to elucidate the potential roles of other Mtase16 family members in other types of cancer. This study aims to comprehensively identify the potential cancer regulator of Mtase16 family members in breast cancer adenocarcinoma (BRCA). To investigate the regulatory roles of Mtase16 family members in breast cancer, we employ bioinformatics analysis particularly by looking at the transcriptomic level of these methyltransferases in patients with breast cancer. The clinical relevancies of these methyltransferases were analyzed by survival analysis. Among Mtase16 family members, METTL18 showed upregulated in tumors compared to normal tissues in the TCGA-BRCA cohort. Lower survival probability is also being shown in breast cancer patients with high expression of METTL18. These results delineate METTL18 as a potential oncogene in breast cancer and further exploration of the mechanism study will hasten the therapeutic potential of targeting this protein in breast cancer.*

***Keywords:*** *methyltransferase; breast cancer; bioinformatics*

## Introduction

Protein methylation is a transfer process of methyl from methyl donor S-Adenosyl methionine (SAM) to the substrates resulting in methylated substrates and conversion from SAM into S-adenosylhomocysteine (SAH) which is mediated by enzymes called methyltransferase (Rudenko et al. 2022). This post-translational modification has been known particularly involved in transcription regulation, signal-transduction modulation, mRNA processing, protein translocation, and metabolism (Bhat et al. 2021). Dysregulation of expression or activity of these enzymes has been associated with multiple diseases such as metabolic disorders or cancer (Hamamoto and Nakamura 2016).

Breast cancer is the most common type of cancer in women worldwide and it is recognized that malignant cells arise from breast tissues (Sharma et al. 2010) ; (Siegal, Miller, and Jemal 2014). As a heterogeneous disease, breast cancer is divided into three clinically therapeutic groups: the estrogen receptor (ER) positive group, HER2 amplified

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group, and triple-negative breast cancers (TNBCs: lacking ER, HER2 and progesterone receptor (PR) (Rodenhiser et al. 2011). This clinical categorization rises from the understanding of the molecular mechanism of this disease. Additionally, at the molecular level, The Cancer Genome Atlas (TCGA) network has also identified PI3KCA, PTEN, AKT1, TP53, GATA3, CDH1, RB1, MLL3, MAP3K1, CDKN1B, TBX3, RUNX1, CBFB, AFF2, PIK3R1, PTPNN22, NF1, SF3B1, and CCND3 as significantly mutated genes in breast cancer (Rodenhiser et al. 2011). Furthermore, PRR14 was found overexpressed in human breast cancer and characterized as an oncogene (Ren et al. 2020). Although several key players in breast tumorigenesis have been found, additional identification of target protein will provide broaden understanding of this disease and alternative therapeutic options for patients with breast cancer.

Mtase16 is a family of methyltransferases that preferentially interact with molecular chaperones (Cloutier et al. 2013). There are 10 members of enzymes in this family: CAMKMT, FAM86A (EEF2KMT), METTL18, METTL20 (ETFBKMT), METTL21A, METTL21B (EEF1AKMT3), METTL21D (VCPKMT), METTL22, and METTL23. So far only METTL18, METTL21B, and METTL21D have been reported to be involved in cancer regulation (Hong et al. 2022); Li et al., 2021; (Thiele et al. 2011).

In this study, I sought to identify potential cancer regulators in the Mtase16 family in breast cancer by exploration on the transcriptomic data of patients with breast adenocarcinoma. Following survival analysis in breast cancer patient's dataset, I found that among MTase16 genes, METTL18 showed to be the most promising candidate for oncogene in breast cancer. Understanding the potential roles of METTL18 as an oncogene followed by its mechanism might open up an opportunity to target this protein for therapeutic options for breast cancer.

## Research Methods

### 1. Transcriptomics Analysis Of TCGA-BRCA

R version 4.2.1 in Rstudio was used to analyze transcriptomics data. TCGA biolinks packages were employed to retrieve and process the gene expression data from Genomic Data Commons (GDC) (Colaprico et al. 2016). Gene expression quantifications from TCGA-BRCA downloaded from GDC databases with the genome of reference hg19 with GDC query, pre-processed with TCG Aanalyze Preprocessing, and normalized with TCG Aanalyze Normalization modules within TCG Abiolinks. Subsequently, TCG Aanalyze_Filtering was performed to filter and returned the genes higher than the quantile mean across all samples (qnt.cut = 0.25).

### 2. Survival Analysis

Kaplan-Meier survival analysis was performed using Kaplan-Meier plotter (www.kmplot.com) with Affymetrix ID corresponding to Mtase16 genes. Overall survival was chosen in the survival options and patients were split by using auto-select best cutoff. The analysis was performed with defaults parameters in the breast cancer dataset (Győrffy 2021).

### 3. Statistical Analyses And Data Visualization

Statistical analyses and data visualization were performed using R version 4.2.1 in Rstudio. Wilcoxon rank sum test (unpaired) was used to compare the expression of Mtase16 between normal and tumor tissues from the TCGA-BRCA dataset. *P* value < 0.05 were considered statistically significant.

## Results and Discussion

To identify Mtase16 members with potential regulatory roles in breast cancer, transcriptomic data from the TCGA-BRCA dataset were analyzed. The total sample retrieved and analyzed were as follows: Solid Tissue Normal = 113, Primary Tumour = 1098. After gene queries, normalization, and filtering, among 10 members of Mtase16, METTL21C was filtered out due to a lower level of expression across samples. Hence, nine out of ten methyltransferases expression were compared between normal and tumor tissues.

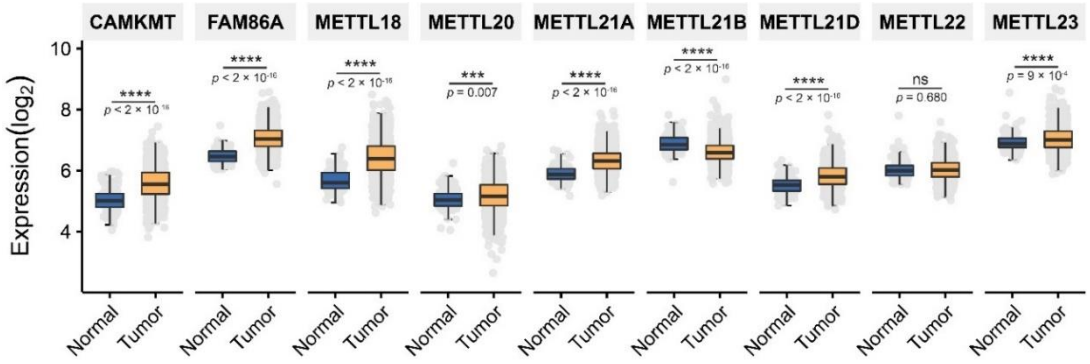


Figure 1
Mtase16 genes expression between solid tissue normal and primary tumor in breast adenocarcinoma (BRCA-TCGA). ** p-value < 0.01, **** p-value < 0.0001, ns = not significant

As shown in Figure 1, CAMKMT, FAM86A, METTL18, METTL20, METTL21A, METTL21D, and METTL23 gene expressions were upregulated in the tumor compared to normal tissues. METTL21B showed lower gene expression in tumor compared to normal tissues. Meanwhile, there are no statistical differences in METTL22 gene expression between normal and tumor tissues. These results indicate that the gene expressions of members of the Mtase16 family were highly altered in breast cancer patients.

Clinical relevancies of these genes were also assessed by comparing the overall survival probability of patients with low versus high expression in the breast cancer cohort. Interestingly, overall survival probability was significantly different between patients with low versus high expression of METTL18, METTL20, and METTL21A (Figure 2). High expression of METTL18 showed a lower survival probability in patients with breast cancer compared with patients with low expression of METTL18 with a

hazard ratio (HR) = 1.29. Meanwhile high expression of METTL20 and METT21A correlated with higher survival probability compared to breast cancer patients with low expression of these genes.

METTL18 is a histidine methyltransferase that catalyzes the methylation of H225 in 60S ribosomal protein L3 (RPL3) affecting ribosome biogenesis and its function (J. M. Małecki et al. 2021). Recently, METTL18 has also been reported as a potential prognosis biomarker in hepatocellular carcinoma (Li et al., 2021). Meanwhile, METTL21D has been reported to promote tumor metastasis however it is unclear whether these effects are dependent or independent of its enzymatic activity. METTL21D catalyzes trimethylation at K315 of valosin-containing protein (VCP) (Kernstock et al. 2012). In addition to these genes, I recently found that METTL21B act as a tumor suppressor in gastric cancer (Hong et al. 2022).

Other Mtase16 family members have been reported to regulate a variety of cellular processes. CAMKMT mediates the trimethylation of K115 in calmodulin, however, the biological function of this modification has not been reported (Magnani, Dirk, Trievel, & Houtz, 2010). FAM86A mediates trimethylation at K525 of eukaryotic elongation factor (EEF2) (Davydova et al. 2014). Increases in sensitivity toward sordarin were observed in yeast lacking EEF2 methylation, however, the biological importance of EEF2 methylation by FAM86A has not been addressed in humans. METTL20 catalyzes the methylation of the beta subunit of electron transfer flavoprotein (ETFB) at K200 and K203, reducing the ability of ETFB to receive electrons from acyl-CoA dehydrogenase and the glutaryl-CoA dehydrogenase (J. Małecki et al. 2015).

METTL21A mediates methylation at K561 of heat shock protein of ~70 kDa (Hsp70) affecting its ability to interact with client proteins (Jakobsson et al. 2013). METTL21B methylates translation elongation factor EEF1A at K165 which is suggested to be important for translation optimization (Hamey et al. 2017).

METTL21C mediates trimethylation at K943 of alanine rRNA synthetase 1 (AARS1) which influences protein synthesis in muscle tissue (Zoabi et al. 2020). METTL22 catalyzes methylation of Kin17 at K135 which possibly affects chromatin association (Cloutier et al. 2014). METTL23 catalyzes asymmetric dimethylation of H3 R17 which is proposed to be a key regulator of paternal genome reprogramming (Hatanaka et al. 2017).

The results demonstrated the potential regulatory roles of METTL18 as an oncogene in breast adenocarcinoma. METTL18 gene expression was found to be highly upregulated in tumor tissues compared to normal tissues and patients with higher expression of METTL18 showed a lower survival probability. Although METTL20 and METTL21A showed significant differences in survival probability between patients with low vs high expression group, it does not show to be correlated with the gene expression data in the comparison with normal tissues. Thus, these genes are unlikely to have regulatory roles in breast cancer.

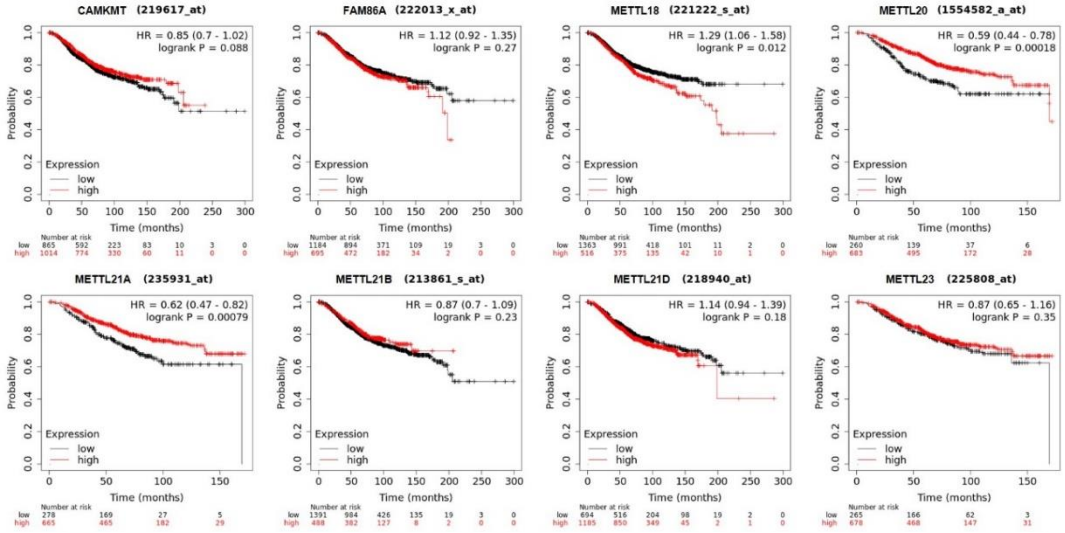


Figure 2
Survival Analysis Of Mtase16 Family Members In
Breast Cancer Patients. HR = Hazard Ratio

## Conclusion

Taken together, this study demonstrated that among Mtase16 family members, METTL18 is a potential oncogene in breast cancer.

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