2024 Joint Usage and Research Report

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<tr><td colspan="2">Title of Research Project</td><td colspan="3">Unraveling the impact of methyl transferase like 3 (Mettl3) on immune responses in triple negative breast cancer.</td></tr>
<tr><td colspan="2">Status</td><td colspan="3">√ New / Continued</td></tr>
<tr><td rowspan="2">Applicant</td><td>Institution</td><td>University of Chittagong, Bangladesh.</td><td>Circle if under 40</td><td>Circle if under 35</td></tr>
<tr><td>Job title and Name</td><td>Associate Professor
Dr. Sunanda Baidya</td><td>◯</td><td></td></tr>
<tr><td rowspan="4">Research collaborators
(Please add lines as appropriate)</td><td>Institution</td><td>Hokkaido University</td><td></td><td></td></tr>
<tr><td>Job title and Name</td><td>Professor Akinori Takaoka</td><td></td><td></td></tr>
<tr><td>Institution</td><td></td><td></td><td></td></tr>
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<tr><td colspan="2">Host researcher at IGM</td><td colspan="3">Professor Akinori Takaoka</td></tr>
<tr><td colspan="2">Purpose of the Research Project (within 200 words)</td><td colspan="3">Breast cancer (BC) is the second leading cause of cancer death in women, with approximately 2.26 million new cases and 685,000 deaths globally each year [GLOBOCAN, 2020]. It is classified into three main types based on molecular patterns: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Triple-negative breast cancer (TNBC), lacking ER, PR, and HER2, constitutes 15% to 20% of BC cases and remains difficult to treat due to limited therapeutic targets [Noman AS et al., Scientific Reports, 2016]. The incidence of TNBC is alarmingly increasing, contributing to rising mortality rates. RNA methylation, particularly via the enzyme Methyltransferase-like 3 (Mettl3), has emerged as an important factor in cancer development. Lower Mettl3 expression is linked to metastasis in TNBC, while higher levels are seen in other subtypes, indicating potential for targeted therapies [Shi Y et al., Front. Oncol., 2020]. Immunotherapy shows promise for treating TNBC, but the role of Mettl3 in regulating the immune landscape is not yet fully understood. This study aims to investigate the role of Mettl3 in TNBC development and its impact on immune evasion.</td></tr>
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Results (including the number of web meetings; within 1,000 words)	We carefully collected tumor tissue alongside adjacent control tissue from an intriguing cohort of 36 breast cancer patients, including 15 individuals diagnosed with triple-negative breast cancer (TNBC) at Chittagong Medical Hospital in Bangladesh. In addition to these vital specimens, we collected blood samples from patients after they underwent between one to six cycles of intensive chemotherapeutic treatment. Delving into the expansive TCGA database, which encompassed data from 1,082 breast cancer patients, we uncovered that Mettl3 expression remained largely unchanged in luminal and HER2-positive subtypes. However, a stark contrast emerged in the TNBC subtype, where Mettl3 exhibited a significant downregulation. In fact, a compelling analysis revealed that Mettl3 was markedly upregulated in breast cancer tissues compared to normal breast tissues within luminal and HER2-positive groups, while TNBC samples displayed alarmingly low levels of Mettl3. The Kaplan–Meier (KM) Plotter analysis painted a grim picture—lower expression levels of Mettl3 in TNBC patients correlated with poorer overall survival outcomes. Furthermore, our qRT-PCR data demonstrated a progressive rise in Mettl3 expression, achieving statistical significance by the fifth and sixth chemotherapy cycles when juxtaposed against TNBC patients who received no such treatment. This experimental evidence pointed to the hypothesis that diminished Mettl3 expression leads to a reduction in m6A levels, facilitating the advancement of TNBC, thereby positioning Mettl3 as a potential tumor suppressor gene in this aggressive cancer subtype. Next, we investigated the expression of interferons (IFNs) within the TNBC landscape, revealing a concerning downregulation of IFN-β1 and IFN-γ levels among TNBC patients. Notably, enhanced production of these key immune molecules increased following each cycle of chemotherapy. A positive correlation emerged, linking both IFN-β1 and IFN-γ with Mettl3, suggesting that the lowered expression of Mettl3 could lead to decreased promoter methylation of IFN-β1 and IFN-γ, potentially stifling the immune response in TNBC. This sequence of events implies that methylation of the promoter regions of these interferons may silence their activity, culminating in genomic instability and mutations that pave the

	way for TNBC carcinogenesis. Moreover, our analysis of the TCGA and qRT-PCR data unveiled an intriguing increase in FOXP3 expression within TNBC tumors, with significant changes manifesting after each chemotherapy cycle. A concerning negative correlation has been observed between FOXP3 and Mettl3 expressions. We also documented elevated levels of CTLA-4 in TNBC tissues compared to their healthy counterparts. Following chemotherapy, a gradual decline in CTLA-4 expression was noted, revealing a negative correlation between CTLA-4 and Mettl3 methylation status. This implies a suppression of immune function due to their overexpression in TNBC, which further declines immune surveillance. In our investigation of regulatory T cells in TNBC, we focused on the expression of interferon-stimulated genes such as CXCL10, MX1, and OAS1. We observed a pronounced overexpression of these immune markers in TNBC tissues compared to normal samples, accompanied by a gradual decrease after each chemotherapy cycle. Correlational analysis revealed an inverse relationship between Mettl3 and these immune markers, hinting at a loss of immune-suppressive cells and an encouraging rise in immune effector cells. These multifaceted results suggest that Mettl3 may represent a promising therapeutic target in the battle against TNBC. Our findings indicate that chemotherapy plays a pivotal role in altering the methylation landscape during TNBC progression. Consequently, Mettl3 could emerge not only as a valuable prognostic biomarker but also as a potential therapeutic avenue for patients grappling with TNBC. To substantiate these findings, further validation with larger sample sizes will be essential. To advance our research, we have procured the TNBC cell line MDA-MB-231 along with the control cell line MCF-10A, intending to conduct crucial experiments to validate the role of Mettl3 in the realm of immunomodulation. If our in vitro studies yield positive results, our current work could establish a robust foundation for a novel understanding of the METTL3/IFN-axis-mediated N6-methyladenosine modification, illustrating its function as an immunomodulator in TNBC, in addition to its role in tumor suppression. As breast cancer treatment modalities continue to

	advance, METTL3 may emerge as a clinically viable prognostic biomarker and a crucial therapeutic target for patients battling TNBC.
Publication *Provide the details of the conferences or journals where the above work was presented or published.	Baidya, Sunanda; et al. "Methyltransferase like-3 in immunomodulation of triple-negative breast cancer" in 3rd International Symposium on Integrated Management and Treatment of Cancers, University of Dhaka, Bangladesh, 26th May, 2024.
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