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Evaluation of Methyl Transferase-like 3 (METTL3) expression in Pancreatic Adenocarcinoma: an immunohistochemical study

Abstract

Background: Pancreatic carcinoma is the 12th most common cancer worldwide. Pancreatic cancer is one of the leading causes of cancer death worldwide. **Aim:** Evaluation Of the role of Methyltransferase like 3 (METTL3) expression by immunohistochemistry in pancreatic ductal adenocarcinoma and its relation with clinicopathological data. **Material and methods:** This retrospective study analyzed 50 cases of pancreatic ductal adenocarcinoma (PDAC) and 15 normal pancreatic tissue samples from Egyptian patients, collected between 2019 and 2023 using formalin-fixed paraffin-embedded (FFPE) samples. METTL3 proteins was assessed by Immunohistochemistry in these cases. **Results:** METTL3 expression was negative in 26% of cases, low in 36%, and high in 38% of PDAC cases. A significant positive relationship was found between METTL3 expression and histologic grade, primary tumor extent, regional lymph node involvement, and pathological Tumor-Node-Metastasis (TNM) staging ($p<0.05$). Additionally, METTL3 expression showed significant negative correlation with tumor infiltrating lymphocytes**. Conclusion:** The study reveals that METTL3, an N6-methyladenosine RNA methyltransferase, is upregulated in pancreatic ductal adenocarcinoma (PDAC) tissues, correlated with tumor size, grade, and advanced staging, suggesting potential therapeutic target.

Key words: Pancreatic ductal adenocarcinoma; Methyl Transferase-like 3 (METTL3).

Introduction

Pancreatic carcinoma continues to represent a significant global health burden. It is the 12th most prevalent malignancy, accounting for 2.6% of all cancer cases, and ranks as the 7th leading cause of cancer-related deaths, contributing to 4.7% of all cancer mortality. This cancer is the 12th most common globally, being the 12th most frequent in men and the 11th most frequent in women [(1)]. The overall 5-year survival rate for pancreatic carcinoma remains low, as over half of cases are diagnosed at advanced stages [(2)]. In Egypt Pancreatic cancer is the 11th most common cancer type with an incidence rate of about 2.2% among cancer patients [(3)].

Roles of RNA modifications in cancer are garnering increasing interest. The research on m6A RNA methylation in cancer is in full swing at present. Among these RNA modifications, various methylations account for two-thirds of total cases and exist on almost all RNAs. However, there are still many other popular RNA modifications involved in the regulation of gene expression post-transcriptionally besides m6A RNA methylation. Currently, more than 170 modifications have been identified on RNA [(4)].

The role of RNA modifications in cancer is attracting growing attention, with research on m6A RNA methylation currently at the forefront. Among these modifications, various methylations make up two-thirds of all cases and are present on nearly all RNA types. However, m6A methylation is just one of many RNA modifications that play a role in the

post-transcriptional regulation of gene expression. To date, over 170 distinct RNA modifications have been identified [4].

N6-Methyladenosine (m6A), the most prevalent internal modification in mRNA, has been extensively and increasingly studied over the past decade. Dysregulation of RNA m6A modification and its associated machinery, including writers, erasers and readers- is frequently observed in various cancer types and are critical for malignant tumor initiation, progression, metastasis, as well as drug resistance and cancer relapse. The dysregulation profiles might serve as diagnostic, prognostic and/or predictive biomarkers [5].

The majority of m6A in mRNA is deposited co-transcriptionally by a writer called the m6A methyltransferase complex (MTC) 9–11 [6].

The MTC includes three core components, METTL3, METTL14 and WTAP10–12. Heterodimerization with METTL14 facilitates the allosteric activation of METTL3, the only catalytically active component of the MTC [5].

Methyltransferase-like 3 (METTL3) is a methyltransferase which has been identified to promote m6A modification [7]. The connection between m6A and cellular physiology was revealed by the discovery and cloning of METTL3 as the m6A-forming enzyme in mRNA. METTL3 is known to play a crucial role in promoting cancer cell proliferation in several human cancers, including breast cancer, colorectal carcinoma, lung cancer, ovarian carcinoma, and bladder cancer. However, the specific role of METTL3 in pancreatic cancer remains unclear and is still under investigation [8].

The aim of this study was evaluation of immunohistochemical (METTL3) expression in pancreatic ductal adenocarcinoma and its relation with clinicopathological data.

Material and methods

This was a retrospective study including 50 cases of pancreatic ductal carcinoma to evaluate the role of (METTL3) expression in pancreatic ductal adenocarcinoma. This study examined pancreatic ductal adenocarcinoma (PDAC) cases and normal pancreatic tissue using FFPE samples from Egyptian patients collected between 2019 and 2023, aiming to identify molecular and histopathological differences. The study was approved by the Research Ethical Committee of Faculty of Medicine, Benha University, Egypt No. **{M.S.25.7.2023}.** The studied groups were categorized into two groups: (I) the Pancreatic Ductal Adenocarcinoma (PDAC) group, which included 50 cases of pancreatic ductal adenocarcinoma obtained surgically through Whipple operations, and (II) the control group, which consisted of 15 normal pancreatic tissue samples obtained surgically from non-neoplastic pancreatic cases, providing a baseline for comparison in the study. Additionally, the study was conducted in collaboration with Menoufia University and the National Liver Institute.

Inclusion criteria: Cases were selected based on the availability of formalin-fixed paraffin-embedded (FFPE) tissue blocks suitable for serial cutting and histological examination, with inclusion criteria requiring complete clinicopathological data such as age, tumor size, histologic grade, and stage, and only cases of pancreatic ductal adenocarcinoma (PDAC), Not Otherwise Specified (NOS), were included to ensure a homogeneous sample population for analysis.

Exclusion criteria: Any PDAC cases that received neo-adjuvant chemotherapy prior to surgical resection, as well as PDAC cases of special subtypes or mixed PDAC with other tumors, to maintain a homogeneous study population. Additionally, cases with unavailable clinical data or insufficient paraffin blocks for analysis- were also excluded, ensuring the reliability and consistency of the findings.

Methods

Clinicopathological data, including; age, tumor size, histologic grade, lymph node (LN) status, lymphovascular invasion (LVI), perineural invasion, TNM stage, and tumor-infiltrating lymphocytes (TILs)- were collected from patient files. Hematoxylin and eosin (H&E) slides were reviewed to confirm pancreatic ductal adenocarcinoma (PDAC), Not Otherwise Specified (NOS), and assess histologic grade using the College of American Pathologists (CAP) system: grade I (well-differentiated), grade II (moderately differentiated), and grade III (poorly differentiated) [9,10]. For statistical analysis, grades were grouped into low (GI and GII) and high (GIII). Lymph node status followed AJCC 8th edition staging: N0 (no metastasis), N1 (1-3 LNs involved), and N2 (≥4 LNs involved), with regional LNs including peripancreatic, pancreaticoduodenal, and porta-hepatis LNs [11].

Tissue microarrays (TMAs) were constructed by selecting representative areas from H&E slides, punching 1.5μ cores from donor blocks, and arraying them in triplicate on recipient blocks. A TMA map was created to track core positions, and 4-5 μm sections were cut for immunohistochemical (IHC) analysis [12].

METTL3 immunohistochemical study:

For immunohistochemical staining, three positive slides were prepared:

Slides were immunostained according to manufacturer's instructions with Methyltransferase Like 3 (METTL3)antibody **(Rabbit polyclonal antibody, 0.1mg/ml concentration, AB clonal company, Cat. #A8370, Wuhan, China)** at 1:25 dilution.

The staining process included deparaffinization, antigen retrieval in citrate buffer (pH 6.0) via microwave heating, peroxidase blocking with hydrogen peroxide, and overnight incubation with the primary antibody at 4°C. Binding was detected using a streptavidin-biotin system (Dako Cytomation) and diaminobenzidine (DAB), followed by hematoxylin counterstaining and DPX mounting. Negative controls omitted the primary antibody, while normal testicular tissue served as positive controls.

Interpretation of METTL3 expression:

Positivity was considered as brownish homogenous nuclear staining of tumor cells. METTL3 expression was scored based on staining intensity (0–3) and proportion of positive cells (0–4), yielding a staining index (0–12). Scores of 0–6 indicated low expression, while 8–12 indicated high expression [13].

Ethical consideration

Being a retrospective study, a written informed consent was not needed. The study was approved by the Research Ethical Committee of Faculty of Medicine, Benha University, Egypt No. **{M.S.25.7.2023}**.

Statistical analysis

Statistical analysis was conducted using SPSS version 26 (SPSS Inc., PASW Statistics for Windows version 26. Chicago: SPSS Inc_), employing descriptive statistics (mean, standard deviation, median, and percentages) and analytic tests such as Chi-Square, Monte Carlo, t-test, and ANOVA with Tukey post hoc. Spearman's correlation assessed relationships between non-normally distributed variables, with significance set at $p < 0.05$, and highly significant at $p \leq 0.01$.

Results

Clinicopathological parameters of studied pancreatic ductal adenocarcinoma cases:

The mean age of pancreatic ductal adenocarcinoma cases was 58.78 years, with 80% being male. The mean tumor size was 4.30 cm, and 54% had tumors ≥4 cm. Regarding grade, 64%

were low grade. Tumor classification showed 56% were at tumor stage 2, and 60% had lymph node stage 1. Most cases (58%) were stage IIB, and 98% had perineural invasion as shown in **Table (1)**.

Table (1): Demographic and Clinical pathological Data among studied group

	Range / years		**Mean ± SD**
Total cases age (years)	36-75		58.78±9.99
Age groups	Number		Percentage (%)
<60	27		54.0%
≥60	23		46.0%
Sex	**Number**		**Percentage (%)**
Male	40		80.0 %
Female	10		20.0 %
Total	50		100.0 %
Tumor size (cm)	Range	Mean ± SD	Median
	2-11	4.30±1.93	4.0
<4	23(46%)		
≥4	27(54%)		
Total	50(100%)		
Histologic grading system of College of American Pathologists (CAP)	Number=50		Percentage (%)
1	10		20.0 %
2	22		44.0 %
3	18		36.0 %
Histologic Grade			
Low	32		64.0
High	18		36.0
Primary tumor extent	Number=50		Percentage (%)
T1	1		2.0 %
T2	28		56.0 %
T3	21		42.0 %
regional LN metastasis	Number=50		Percentage (%)
Negative	11		22.0 %
N1	30		60.0 %
N2	9		18.0 %
LN lumbing	**Number=50**		**Percentage (%)**
Negative	12		24.0 %
Positive	38		76.0 %
Pathological TNM staging (AJCC 8th edition)	Number		Percentage %
IA	1		2.0 %
IB	5		10.0 %
IIA	5		10.0 %
IIB	29		58.0 %
III	10		20.0 %
Lymphovascular invasion	Number=50		Percentage (%)
Absent	30		60.0 %
Present	16		32.0 %
Not identified	4		8.0 %
Perineural invasion	**Number=50**		**Percentage (%)**
Negative	1		2.0 %
Positive	49		98.0 %
Percentage of tumor infiltrating lymphocytes (TIL) (%) / 10 HPF	Range	Mean ± SD	Median
	1-45	20.1±11.91	20
<20	19(38.0%)		
≥20	31(62.0%)		

METTL3 immunohistochemical results among studied cases:

The total staining score of methyltransferase-like 3)METTL3) was 4.92±3.95, ranging from 0 to 12. Expression levels were negative in 26% of cases, low in 36%, and high in 38% of cases. All normal pancreatic tissues showed no expression of methyltransferase-like 3, with a highly significant difference in expression between normal and pancreatic carcinoma tissues ($P < 0.01$) as shown in **Table (2) and Figure 1(a, b, c and d).**

Table (2): METTL3 expression and total staining score of METTL3 expression

		Number=50	**Percentage (%)**
Total staining score		4.92±3.95 4(0-12)	
METTL3 expression			
Negative		13	26.0 %
Positive	Low	18	36.0 %
	High	19	38.0 %

Relation between METTL3 expression and clinicopathological features among studied cases

METTL3 expression is significantly positively correlated with larger tumor size (p=0.024), higher histologic grade (p=0.009), primary tumor extend (P=0.024) advanced TNM stage (p=0.012), and lymph node metastasis (p=0.001), lymphovascular invasion (P=0.018) suggesting a more aggressive tumor behavior. Additionally, METTL3 expression is significantly negatively correlated with percentage of tumor-infiltrating lymphocytes (TILs), METTL3-negative tumors had a higher percentage of tumor-infiltrating lymphocytes (TILs) (p=0.001), indicating a possible role of METTL3 in immune evasion. A non-statistically significant relation was detected between METTL3 expression and age, sex or perineural invasion of studied cases as shown in **Table (3)**.

Table (3): Relation between METTL3 expression grouping and all clinicopathological features

	METTL3 expression			**Test of significance**	**P value Within group significance**
	Negative Number=13	Low Number=18	High Number=19		
Age groups					P1=0.982
<60	8(61.5%)	11(61.1%)	8(42.1%)	χ^2=1.75	P2=0.290
≥60	5(38.5%)	7(38.9%)	11(57.9%)	Pvalue=0.418	P3=0.258
Sex					P1=0.650
Male	11(84.6%)	14(77.8%)	15(78.9%)	χ^2=0.242	P2=0.704
Female	2(15.4%)	4(22.2%)	4(21.1%)	Pvalue =0.886	P3=0.931
Tumor size (cm)	3.35±1.26	4.09±1.65	5.16±2.25		P1=0.269
<4	8(34.8%)	9(39.1%)	6(26.1%)	F=4.02	P2=0.008*
≥4	5(18.5%)	9(33.3%)	13(48.2%)	Pvalue=0.024*	P3=0.08
Histologic grading system of College of American Pathologists (CAP)					
1	6(46.2%)	4(22.2%)	0	χ^2=13.45	P1=0.347
2	4(30.8%)	10(55.6%)	8(42.1%)	Pvalue	P2=0.002*
3	3(23.1%)	4(22.2%)	11(57.9%)	=0.009*	P3=0.01*
Histologic Grade 2					P1=0.960
Low	10(76.9%)	14(77.8%)	8(42.1%)	χ^2=6.38	P2=0.04*
High	3(23.1%)	4(22.2%)	11(57.9%)	P value =0.04*	P3=0.023*
Primary tumor extent					
T1	1(100%)	0	0		P1=0.493
T2	9(32.1%)	13(46.4%)	6(21.4%)	χ^2=11.26	P2=0.004*
T3	3(14.3%)	5(23.8%)	13(61.9%)	Pvalue=0.024*	P3=0.016*
regional LN metastasis					
Negative	7(53.8%)	4(22.2%)	0	χ^2=21.07	P1=0.052
N1	6(46.2%)	13(72.2%)	11(57.9%)	Pvalue	P2=0.001*
N2	0	1(5.6%)	8(42.1%)	=0.001*	P3=0.001*
LN lumbing					P1=0.032*
Negative	7(53.8%)	4(22.2%)	1(5.3%)	χ^2=10.04	P2=0.001*
Positive	6(46.2%)	14(77.8%)	18(94.7%)	Pvalue=0.007*	P3=0.197
Pathological TNM staging (AJCC 8th edition)					
IA	1(7.7%)	0	0		
IB	3(23.1%)	2(11.1%)	0	χ^2=19.64	P1=0.017*
IIA	3(23.1%)	2(11.1%)	0	Pvalue=0.012*	P2=0.001*
IIB	6(46.2%)	12(66.7%)	11(57.9%)		P3=0.016*
III	0	2(11.1%)	8(42.1%)		
Lymphovascular invasion					P1=0.201
Absent	12(40%)	10(33.3%)	8(26.7%)	χ^2=11.86	P2=0.01*
Present	0(0%)	8(50%)	8(50%)	Pvalue=0.018*	P3=0.155
Not identified	1(25%)	0(0%)	3(75%)		
Perineural invasion					P1=0.138
Negative	1(7.7%)	0	0	χ^2=2.90	P2=0.134
Positive	12(92.3%)	18(100%)	19(100%)	Pvalue =0.234	P3=1.0
Percentage of tumor infiltrating lymphocytes (TIL) (%)	32.69±5.63	24.72±5.55	7.11±4.72	F =101.73 Pvalue=0.001*	P1=0.001* P2=0.001* P3=0.001*
<20	0	0	19(100%)		
≥20	13(41.9%)	18(58.1%)	0		

χ^2=Chi-Square test,

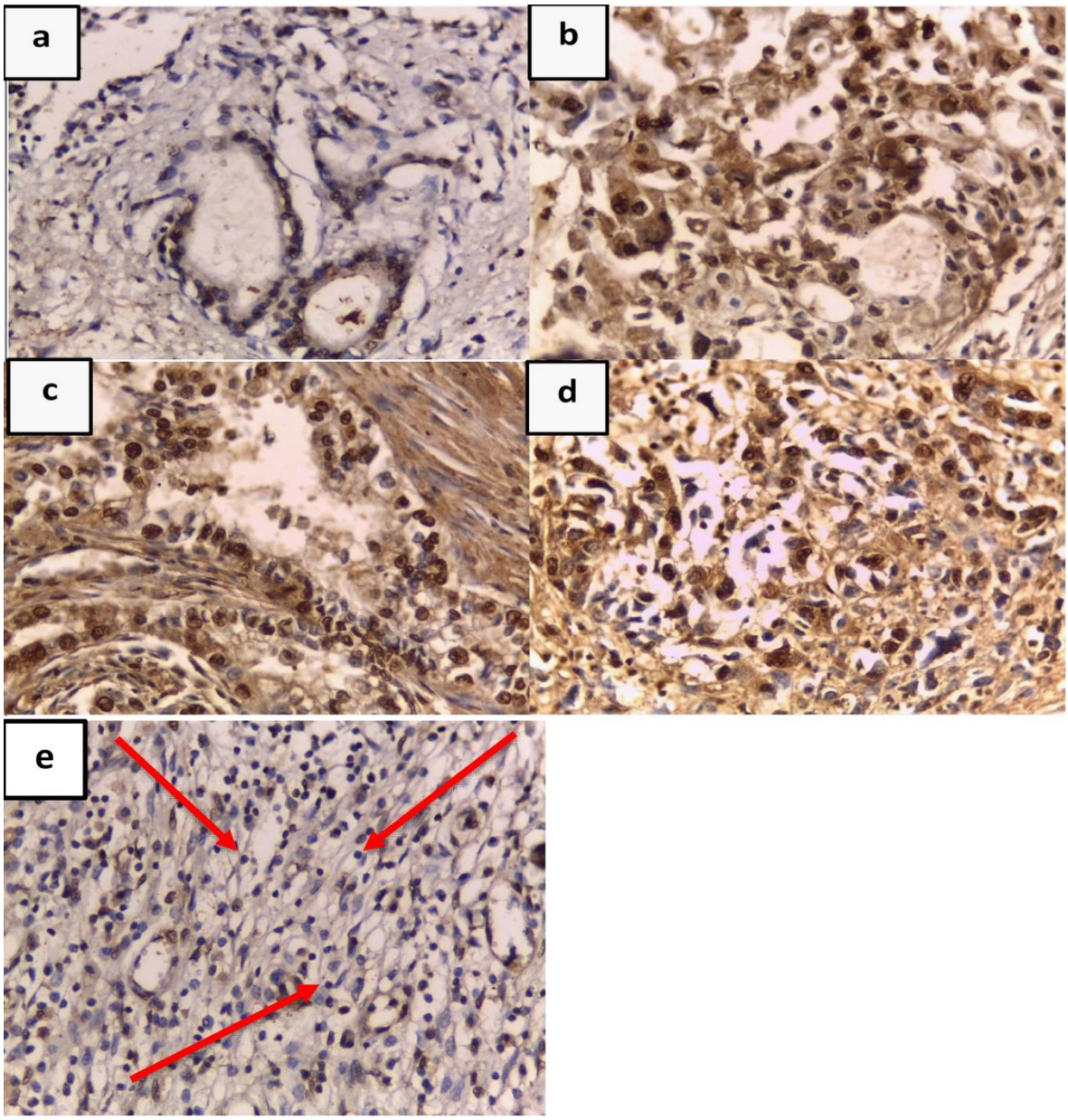

Figure (1) as showing:

a. PDAC, Grade 1 showing low nuclear METTL3 expression. (ABC, x400)
b. PDAC, Grade 2 showing high nuclear METTL3 expression. (ABC,x400)
c. PDAC, Grade 2 showing high nuclear METTL3 expression. (ABC, x400)
d. PDAC, Grade 3 showing high nuclear METTL3 expression. (ABC, x400)
e. A case of PDAC, grade 2 showing infiltration by tumor infiltrating lymphocytes showing low nuclear METTL3 expression (red arrows), (ABC, x400).

Discussion

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers, ranking as the 12th most common cancer and the 7th leading cause of cancer-related deaths globally [(1)].

Among the key regulators in cancer, RNA modifications, particularly N6-methyladenosine (m6A), play a vital role in post-transcriptional gene regulation, tumor progression, and drug resistance [(5)]**.** METTL3, the core catalytic component of the m6A methyltransferase complex, has been implicated in the progression of several cancers, including breast, lung, colorectal, and ovarian carcinomas [(8)]**.**

This study aimed to evaluate METTL3 expression in PDAC and assess its correlation with clinicopathological features to explore its potential role as a prognostic marker.

The current study showed that, the mean age of patients in this study was 58.78 years (range 36–75 years). This finding was in line with **Lyu et al., [14]** who reported a mean age of 58.2 years.

The current study showed that- regarding gender distribution in the current study- PDAC cases showed a male predominance (eighty percent). This finding was in line with most literatures including [15, 16, 17] who reported a male predominance in PDAC cases.

In this study, the IHC expression of METTL3 was evaluated in PDAC and its relation to different clinicopathological variables was also assessed. Seventy-four percent of studied PDAC cases were positive for METTL3 IHC expression with highly significant difference concerning METTL3 expression in pancreatic carcinoma tissue (P value < 0.01). All control groups showed complete negative staining for METTL3 IHC expression.

This finding was in line with most literatures including **Xia, et al., [17]** who reported METTL3 positivity in 79% of studied PDAC cases and **Li, et al., [18]** who reported that the METTL3 expression was significantly increased in Pancreatic carcinoma tissues; with 54 cases showed high expression and 37 cases showed low expression.

In the present study, there was a significant statistical relation between METTL3 IHC expression with tumor size (P=0.024) and the histologic grade (P=0.009) in studied PDAC cases.

This run parallel to **Ge, et al., [19]** who found that METTL3 IHC expression was highly significantly related to Mean tumor size (P=0.019) in studied PDAC cases. Also, **Lin, et al., [20]** reported that METTL3 was upregulated in GEM-resistant pancreatic ductal adenocarcinoma and its knockdown suppressed cancer progression. Similarly **Li, et al., [18]** demonstrated that METTL3 expression was upregulated in hepatocellular carcinoma and stronger METTL3 staining was correlated with higher histological grading (P= 0.02). In contrast with our results, **Zhang, et al., [21]** reported that METTL3 expression showed no significant correlation with tumor size (P= 0.43) in gastric adenocarcinoma cases but significantly related to higher pathologic stage (P= 0.002) and distant metastasis (P = 0.016). This discrepancy could be attributed to different sample sizes.

In our study, there was a significant statistical relation between METTL3 IHC expression and primary tumor extent (p=0.024), Regional LN Metastasis (P=0.001) and the AJCC stage group (P=0.012) in studied PDAC cases.

These results run parallel to previous studies such as **Xia, et al.**, [17] reported that METTL3 expression was significantly related to high pathological stage (P=0.016) and positive nodal metastasis (P=0.017). Also, **Wei, et al., [22]** demonstrated that elevated levels of METTL3 expression are correlated with advanced pathological stages in studied PDAC cases. Similarly, **Tang, et al., [23]** reported that METTL3 was abundantly expressed in PDAC and played an oncogenic role in the development of pancreatic carcinoma via promoting m6A-mediated E2F5 stability, enhancing pancreatic carcinoma growth and metastasis.

These results support the hypothesis that METTL3 has a role in the progression of PDAC. Also, **Li, et al., [24]** reported that METTL3 expression was significantly up-regulated in colorectal cancer (CRC) tissues, and was significantly related to lymph node metastasis (P=0.043), and TNM staging (P=0.032).

In the current work, there was a significant statistical relation between METTL3 IHC expression and lymphovascular invasion (p=0.018) but no significant statistical relation was

found between METTL3 IHC expression and perineural invasion (p=0.088) in studied PDAC cases.

This was in line with **Okugawa, et al.,** [25] who reported statistically significant correlation between METTL3 expression and lymphovascular invasion (P=0.011) in studied gastric carcinoma cases. However in study of **Li , et al.,** [24] **Liu, et al.,** [26] they found non statistical significant relation between METTL3 expression and lymphovascular invasion (P value = 0.173 and 0.175 respectively) in studied hepatocellular carcinoma and colorectal carcinoma cases. This difference may be explained by different sample size.

The study of pancreatic ductal adenocarcinoma (PDAC) continues to be a critical area of research, given its aggressive nature and poor prognosis. This research has shed light on the role of METTL3 in PDAC, highlighting its potential as a biomarker and therapeutic target. The findings underscore the importance of understanding the molecular mechanisms that drive cancer progression, particularly in the context of m6A modifications and their impact on tumor biology.

Conclusion

This study highlights key clinicopathological features of PDAC, including male predominance. METTL3- an m6A RNA methyltransferase- was significantly upregulated in PDAC tissues and correlated with tumor size, grade, lymph node metastasis, staging, and lymphovascular invasion- suggesting its oncogenic role. Additionally, METTL3 expression negatively correlated with tumor-infiltrating lymphocytes, contributing to an immunosuppressive microenvironment. These findings indicate METTL3's involvement in PDAC progression through m6A regulation of oncogenic pathways, cell cycle, and EMT-making it a potential therapeutic target. Further research is needed to explore its mechanisms and potential as a biomarker.

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