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Original Article

Prognostic Significance of HMGA1, c-CBL and TNFRSF11B Expression in Gastric Adenocarcinoma

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ABSTRACT

Background: The signaling pathway of Wnt/β-catenin is considered a hallmark of cancer development in gastric cancer. It facilitates cancer stem progression, thus leading to metastasis and therapy response resistance. Therefore, the therapeutic prospect of agents that target this signaling pathway in carcinoma is a challenge. This study aimed to **e**valuate the relationships between clinicopathological characteristics of gastric cancer patients, disease progression and therapy response with HMGA1, c-CBL and TNFRSF11B expressions.

Methods:

Results: The expressions of HMGA1, in gastric cancer were correlated with the tumor size, LN metastases, stage, mortality and recurrence P (0.038, <0.001,0.006, 0.006 and 0.004) respectively ,the expression of TNFRSF11B was significantly correlated with age, grade, stage ,LN metastases, stage AJCC, mortality and recurrence P (0.024, <0.001, 0.01, <0.001, 0.003, 0.002 and 0.003) respectively, low expression of c-CBL was significantly linked with size of tumor, grade, stage, LN metastasis and AJCC staging. Moreover, the OS of patients with high HMGA1 and TNFRSF11B strong tumors was significantly lower than that of patients with negative tumors (p =, 005 Vs p=0.004) respectively. However, the expression of low c-CBL in GC was correlated with de-differentiation, advanced stage, lymph nodes metastasis, and distant metastases (p=0.004). However, High c-CBL expression was linked with no tumor recurrence (p=0.002) and favorable survival rates (p <0.001). Up-regulation of HMGA1 and TNFRSF11B had a significant association with poor clinical response to the therapy respectively. However, that high c-CBL expression is related to good response to therapy (p=0.002).

Conclusions: The HMGA1 and TNFRSF11B over expression had an essential role in gastric cancer development and progression. c-CBL expression negatively correlates with tumor infiltration and metastasis in lymph node, so they play role in prognosis and clinical response prediction in GC patients.

Keywords: Gastric carcinoma; HMGA1; c-CBL; TNFRSF11B, IHC.

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INTRODUCTION

Gastric carcinoma is considered one of the most frequent diagnosed cancers and the 4th causing of worldwide cancer mortality worldwide [1].

Metastatic cancer patients still have more treatment resistance, poor response to drug therapy and low survival rate [2].

Abnormal activation signaling pathway of Wnt/β-catenin promotes cancer stem tumorigenesis and cancer progression, so leads to metastasis of cancer and therapy response resistance [3]. The therapeutic potential of agents that target this signaling pathway in cancer is a challenge [4].

Wnt signaling consists of two intracellular pathways: canonical and noncanonical pathways. The activation of canonical pathway leads to catenin and transcription of target genes nuclear accumulation [5]. Defects in this pathway are linked with human malignancies development [6].

The signaling pathway of Wnt/β-cateninis is regarded as a hallmark in the development of gastric cancer and is primarily mediated by nuclear b-catenin [7]. High-mobility group AT-hook 1 (HMGA1) is a non-histone, chromatin-binding protein that has been found overexpressed in many tumor types. It has contributed to drug resistance, invasion and metastasis leading to worse patient survival [8, 9,10].

Akaboshi *et al*. demonstrated that the Wnt/β-catenin pathway is associated with induction of*HMGA1*, leading to induction and promotion of gastric cancer and its Overexpression was constantly linked to β-catenin nuclear accumulation in human gastric carcinoma tissues [11].

The Casitas B-lineage lymphoma (c-CBL) proteins have different roles as signal transductionregulators. The defects in CBL proteins can lead to cancer development and/or to immune dysfunction. It was identified as a unique E3 ubiquitin ligase targeting the active nuclear β-catenin. Emerging data indicate c-CBL as a suppressor of Wntsignaling [12].

TNF receptor superfamily member 11b (TNFRSF11B) is a member of the tumor necrosis factor that is also called Osteoprotegerin (OPG) [13].

TNFRSF11B has two TNF family ligands: TNF related apoptosis-inducing ligand (TRAIL) and receptor activator of nuclear factor (NF)-kB ligand. TNFRSF11B is thought to have a protective anti-apoptotic action as it can overcome the tumor surveillance exerted by TRAIL. Thus, its expression could be a proper approach for avoidance TRAIL induced apoptosis [14].

In our study, the expression level of HMGA1, c-CBL and TNFRSF11B was investigated for assessment of their prognostic value in gastric cancer to help in improving survival of GC patients. Up to our knowledge, little is unveiled regarding their impact on GC prognosis and response to adjuvant treatment.

METHODS

This retrospective study was conducted on 40 GC patients. Patients with GC were presented to Surgery Department, Zagazig University, Egypt from 2020 till 2023 and underwent radical gastrectomy. All clinicopathological, follow-up, recurrence, and survival data were obtained from their files at Clinical Oncology and Nuclear Medicine Department, Zagazig University. Paraffin blocks from all specimens were prepared at Pathology Department, Faculty of Medicine, Zagazig University for a histopathological examination.

The histological type was assessed according to WHO and Lauren' classification, the final stage was assessed according to the International Cancer Control League (UICC) classification system [15].

Informed consents were obtained from patients according to Helsinki declaration, and the study was approved by the Ethical Committee (ethics code101039-27-8-2023: Zag).

Adjuvant treatment was received as 3D conformal radiotherapy, chemo-radiotherapy or only adjuvant chemotherapy according to tumor characteristics and its risk factors in Clinical Oncology Department, Zagazig University Hospitals then stratification occurred regarding immunohistochemical assay of various prognostic markers.

PFS and median survival were investigated in this study. PFS was defined as the length of time between diagnosis till progression. Median survival was defined as time length at which 50% of the patients have died and 50% have survived.

Response to treatment was assessed according to revised RECIST guidelines (version 1.1)

IHC performance

The sections were incubated with HMGA1(1:200, Abcam, ab4078), anti-c-CBL antibody (LS C358440, 1:50), and anti

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TNFRSF11B monoclonal antibody (ab9986, Abcam, UK; 1:200) at 4°C overnight.

Analysis of IHC expression for HMGA1, c-CBL and TNFRSF11B

HMGA1 nuclear expressions are observed. Low HMGA1 was considered when there were no expressions in less than 20% of the malignant cells [16].

c-CBL IHC expression was detected in cytoplasm of malignant cells. The intensity of immunostaining was evaluated by a numeric score ranging from zero to three, expressing the intensity as follows: zero, no staining; one, weak staining (light yellow); two, moderate staining (brown, yellow); and three, intense staining (tan). The scoring of the expression range was as follows: less than 20% (0 points), 20% to 50% (1 point), 51% to 75% (2 points), and >75% (3 points). The overall score is obtained by multiplication of the value from dye intensity and the value of the expression range. The score was categorized as ≤2 points, low expression and more than 2 points, high expression [17].

TNFRSF11B expression was demonstrated and evaluated in the cytoplasm. The intensity of staining was assessed as follows: 0, no staining; 1, light yellow staining; 2, yellow-brown staining; and 3, deep brown staining. The scoring of percentage of positive cells was as follows: 0, 0–5%; 1, 6–25%; 2, 26–50%; 3, 51–75%; and 4, >75%. The calculation of final score was staining intensity score × positive cell score. Then, patients were arranged into less than 10 points "weak expression" and more than or equal 10 points "strong expression". [18]

Follow up was done at Clinical Oncology Department every 3-6 months in the first 2 years then every 6 months in the next year. Follow up was done by history and physical examination, endoscopic surveillance, imaging, and laboratory tests.

The importance of this follow-up was to detect any complications from surgery, radiotherapy, or chemotherapy and to manage them. Also, this follow up was for detecting the occurrence of recurrences or any progression in the case as recurrences were more frequent in the first 3 years.

Statistical analysis

The software SPSS version 20 was used for Data analysis. Quantitative variables were illustrated via using their means and standard deviations. The Chi square test and Fisher exact test were used for description of Categorical variables. Chi square for trend test was used for comparing two groups concerning ordinal categorical data. Phi correlation coefficient was used for assessment the strength and direction of association between two dichotomous categorical variables. Survival analysis and Kaplan Meire plot was used for measuring the fraction of subjects living for a certain amount of time after treatment and for assessing the expected duration of time till occurring of one event, either death or recurrence. The overall survival time was defined as the period between operation and the death time or the last follow-up, and the DFS time " disease free survival" was defined as the time from operation till recurrence. The level of statistical significance was set at 5% (P<0.05).

RESULTS

Clinicopathological results

The age of the 40 patients ranged from (41-60) years (Mean: 52.57 ± 5.25 years), There were 30 (75%) males and 10 (25%) females, most cases 35 (87.5%) were of the intestinal type, and only 5 (12.5%) were of diffuse-type adenocarcinoma.

Results of HMGA-1 expression

HMGA1 was expressed in 28 cases (70%), HMGA1was significantly correlated with tumor size, LN metastases, stage AJCC, mortality and recurrence P (0.038, <0.001,0.006,0.006 and 0.004) respectively. Out of 40 patients, 22 (55%) were dead and 18 (45%) were living at the last follow-up. Among twenty-two dead patients, 20 expressed high HMGA1, while 8 of 18 living patients expressed high HMGA1. On follow up of cases, 52.5% showed relapse, among those, 19 patients showed high HMGA1. However, patients didn't show relapses (47.5%), 9 of them showed high HMGA1. Low expression of HMGA1 was significantly associated with disease-free survival (DFS) and overall survival (OS) (p<0.001 for both).

Results of c-CBL expression

c-CBL was highly expressed in 16 cases (40%); low c-CBL expression was significantly correlated with tumor size, grade, stage, LN metastasis and AJCC staging, (P values 0.036, <0.001, 0.001, 0.042, 0.012) respectively. Low c- CBL expression was observed in 21/22 of dead patients. On the other hand, 3/18 living patients expressed low c- CBL expression.

The expression of c- CBL was low in 17/21 patients with relapses, and in 7/19 patients without relapse. c- CBL high expression was

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significantly associated with DFS and OS (p<0.001 for both).

Results of TNFRSF11B expression

TNFRSF11B was expressed in 30 cases (75%), it was significantly correlated with age, grade, stage, LN metastases, mortality, and recurrence, (P values 0.024, <0.001, 0.01, <0.001, 0.003, 0.002 and 0.003) respectively.

Table 1

Characteristics	All (N=40)			Characteristics	All (N=40)	
	No.	%			No.	%
Sex				LN metastasis		
Male	30	75%		Negative	8	20%
Female	10	25%		Positive	32	80%
				N		
				N0	8	20%
Age				N1	12	30%
≤ 55 years	25	62.5%		N2	9	22.5%
> 55 years	15	37.5%		N3	11	27.5%
Site				AJCC stage		
Fundus	5	12.5%		Stage I	5	12.5%
Body	15	37.5%		Stage II	11	27.5%
Distal & pylorus	20	50%		Stage III	24	60%
Size				HMGA1		
				Low	12	30%
				High	28	70%
<5 cm	10	25%		TNFRSF11B		
= 5 cm	6	15%		-ve	10	25%
>5 cm	24	60%		+ve	30	75%
				c- CBL -ve +ve 26	14	35% 65%
Histological type				Follow-up (months)		
Intestinal	35	87.5%		Mean ± 23.01 ±2.22		
Diffuse	5	12.5%		Median (Range) 13(8-36)		
Grade				Relapse		
Well-differentiated	12	30%		Absent 21 (52.5%)		
Moderately differentiated	20	50%		Present 19 (47.5%)		
Poorly differentiated	8	20%				
T				Death		
T1	7	17.5%		Alive 18 (45%)		
T2	14	35%		Died 22(55%)		
T3	13	32.5%				
T4	6	15 %				

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Table (2): Relation between HMGA-1 expression and clinicopathological criteria of studied patients

Characteristics	Total N=40 (%)	HMGA1 – low 12 (30%)	HMGA1-high 28 (70%)	p
Sex				
Male	30 (75%)	8(26.7%)	22(73.3%)	0.451
Female	10 (25%)	4(40%)	6(60%)	
Age				
years ٥٥ ≥	25(62.5%)	10(40%)	15(60%)	0.152
years ٥٥ <	15(37.5%)	2(13.3%)	13(86.7%)	
Site				
Fundus	5 (12.5%)	1(20%)	4(80%)	0.758
Body	15 (37.5%)	4(26.7%)	11(73.3%)	
Distal & pylorus	20 (50%)	7(35%)	13(65%)	
Size				
cm ٥ >	10 (25%)	4(40%)	6(60%)	0.038*
cm ٥ =	6(15%)	5(83.3%)	1(16.7%)	
cm ٥ <	24(60%)	3(12.5%)	21(87.5%)	
Histological type				
Intestinal	35 (87.5%)	11(31.4%)	24(68.6%)	>0.999
Diffuse	5 (12.5%)	1(20%)	4(80%)	
Grade				
Well differentiated	12 (30%)	4(33.3%)	8(66.7%)	
Moderately differentiated	20 (50%)	2(10%)	18(90%)	0.119
Poorly differentiated	8 (20%)	6(75%)	2(25%)	
T				
T1	7 (17.5%)	1(14.3%)	6(85.7%)	
T2	14 (35%)	2(14.3%)	12(85.7%)	0.35
T3	13 (32.5%)	9(69.2%	4(30.8%)	
T4	6 (15%)	0(0%)	6(100%)	
LN metastasis				
Negative	8 (20%)	7(87.5%)	1(12.5%)	<0.001**
Positive	32 (80%)	5(15.6%)	27(84.4%)	
N				
N0	8 (20%)	7(87.5%)	1(12.5%	
N1	12 (30%)	3(25%)	9(75%)	<0.001**
N2	9 (22.5%)	2(22.2%)	7(77.8%)	
N3	11 (27.5%)	0(0%)	11(100%)	
AJCC stage				
Stage I	5(12.5%)	4(80%)	1(20%)	
Stage II	11(27.5%)	4(36.4%)	7(63.6%)	0.006*
Stage III	24(37.5%)	4(16.7%)	20(83.3%)	
Mortality:				
No	22 (55%)	2 (9.1%)	20 (90.9%)	0.002*
Yes	18 (45%)	10 (55.6%)	8 (44.4%)	
Recurrence				
No	21 (52.5%)	2 (9.5%)	19 (90.5%)	0.004*
Yes	19 (47.5%)	10 (52.6%)	9 (47.4%)	

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Table (3): Relation between C-Cbl expression and clinicopathological criteria of studied patients.

Characteristics	Total N=40 (%)	C- cbl low expression 24 (60%)	C-cbl high expression 16 (40 %)	p
Sex				
Male	30 (75%)	18(75%)	12(25%)	>0.999
Female	10 (25%)	6(60%)	4(40%)	
Age				
years ° ° ≥	25(62.5%)	16(64%)	9(36%)	0.505
years ° ° <	15(37.5%)	8(53.3%)	7(46.7%)	
Site				
Fundus	5 (12.5%)	2(40%)	3(60%)	0.574
Body	15 (37.5%)	10(66.7%)	5(33.3%)	
Distal & pylorus	20 (50%)	12(60%)	8(40%	
Size				
cm ° >	10 (25%)	4(40%)	6(60%)	0.036*
cm ° =	6(15%)	2(33.3%)	4(66.7%)	
cm ° <	24(60%)	18(75%)	6(25%)	
Histological type				
Intestinal	35 (87.5%)	19(54.3%)	16(54.7%)	0.051
Diffuse	5 (12.5%)	5(100%)	0(0%)	
Grade				
Well differentiated	12 (30%)	1(8.3%)	11(91.7%)	
Moderately differentiated	20 (50%)	15(75%)	5(25%)	<0.001**
Poorly differentiated	8 (20%)	8(100%)	0(0%)	
T				
T1	7 (17.5%)	2(28.6%)	5(71.4%)	0.001**
T2	14 (35%)	5(35.7%)	9(64.3%)	
T3	13 (32.5%)	11(84.6%)	2(15.4%)	
T4	6 (15%)	6(100%)	0(0%)	
LN metastasis				
Negative	8 (20%)	2(25%)	6(75%)	0.042*
Positive	32 (80%)	22(68.8%)	10(31.2%)	
N				
N0	8 (20%)	2 (25%)	6(75%)	
N1	12 (30%)	8(66.7%)	4(33.3%)	0.017*
N2	9 (22.5%)	4(44.4%)	5(55.6%)	
N3	11 (27.5%)	10(90.9%)	1(9.1%)	
AJCC stage				
Stage I	5(12.5%)	1 (20%)	4 (80%)	
Stage II	11(27.5%)	5 (45.5%)	6 (54.5%)	0.012*
Stage III	24(37.5%)	18 (75%)	6 (25%)	
Mortality:				
No	**22 (55%)**	1 (4.5%)	21 (95.5%)	<0.001*
Yes	**18 (45%)**	15 (83.3%)	3 (16.7%)	
Recurrence				
No	**21 (52.5%)**	4 (19%)	17 (81%)	0.004*
Yes	**19 (47.5%)**	12 (63.2%)	7 (36.8%)	

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Table (4): Relation between TNFRSF11B expression and clinicopathological criteria of studied patients.

Characteristics	Total N=40 (%)	TNFRSF11B--ve 10 (25)%	TNFRSF11B +ve 30 (75%)	p
Sex				
Male	**30 (75%)**	9(30%)	21(70%)	0.401
Female	**10 (25%)**	1(10%)	9(90%)	
Age				
years ٥٥ ≥	**25(62.5%)**	3(12%)	22(88%)	0.024*
years ٥٥ <	**15(37.5%)**	7(46.7%)	8(53.3%)	
Site				
Fundus	**5 (12.5%)**	1(20%)	4(80%)	
Body	**15 (37.5%)**	6(40%)	9(60%)	0.365
Distal & pylorus	**20 (50%)**	3(15%)	17(85%)	
Size				
cm ٥ >	**10 (25%)**	2(20%)	8(80%)	0.526
cm ٥ =	**6(15%)**	1(16.7%)	5(83.3%)	
cm ٥ <	**24(60%)**	7(29.2%)	17(70.8%)	
Histological type				
Intestinal	**35 (87.5%)**	8(22.9%)	27(77.1%)	0.584
Diffuse	**5 (12.5%)**	2(40%)	3(60%)	
Grade				
Well differentiated	**12 (30%)**	8(66.7%)	4(33.3%)	<0.001**
Moderately differentiated	**20 (50%)**	2(10%)	18(90%)	
Poorly differentiated	**8 (20%)**	0(0%)	8(100%)	
T				
T1	**7 (17.5%)**	6(85.7%)	1(14.3%)	
T2	**14 (35%)**	2(14.3%)	12(85.7%)	0.01*
T3	**13 (32.5%)**	1(7.7%)	12(92.3%)	
T4	**6 (15%)**	1(16.7%)	5(83.3%)	
LN metastasis				
Negative	8 (20%)	7(87.5%)	1(12.5%)	<0.001**
Positive	32 (80%)	3(9.4%)	29(90.6%)	
N				
N0	**8 (20%)**	7(87.5%)	1(12.5)	
N1	**12 (30%)**	2(16.7%)	10(83.3%)	0.001**
N2	**9 (22.5%)**	1(11.1%)	8(88.9%)	
N3	**11 (27.5%)**	0(0%)	11(100%)	
AJCC stage				
Stage I	**5(12.5%)**	4(80%)	1(20%)	0.003*
Stage II	**11(27.5%)**	3(27.3%)	8(72.7%)	
Stage III	**24(37.5%)**	3(12.5%)	21(87.5%)	
Mortality:				
No	**22 (55%)**	1 (4.5%)	21 (95.5%)	0.002*
Yes	**18 (45%)**	9 (50%)	9 (50%)	
Recurrence				
No	**21 (52.5%)**	1 (4.8%)	20 (95.2%)	0.003*
Yes	**19 (47.5%)**	9 (47.4%)	10 (52.6%)	

Recurrence				
No	**21 (52.5%)**	4 (19%)	17 (81%)	Results of C-cbl expression (T4)
Yes	**19 (47.5%)**	12 (63.2%)	7 (36.8%)	0.004*

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Table (5): Relation between recurrence free survival and expression of HMGA1, TNFRSF11B and C-Cbl among studied patients.

	Total	Events	Censored	Mean ± SE	95% CI		p
HMGA-1							
Low	12	2	10(83.3%)	31.75 ± 3.39	25.11	38.39	0.008*
High	28	19	9(32.1%)	17.95 ± 2.19	13.66	22.24	
TNFRSF11B							
Negative	10	1	9(90%)	33.8 ± 3.04	27.85	39.57	0.006*
Positive	30	20	10(30%)	18.08 ± 2.14	13.87	22.28	
C-Cbl							
High	16	4	12(75%)	31.81 ± 2.39	27.12	36.5	0.002*
Low	24	17	7 (29.2%)	16.27 ± 2.49	11.4	21.14	
Overall	40	21	19(47.5%)	23.01 ± 2.22	18.65	27.36	

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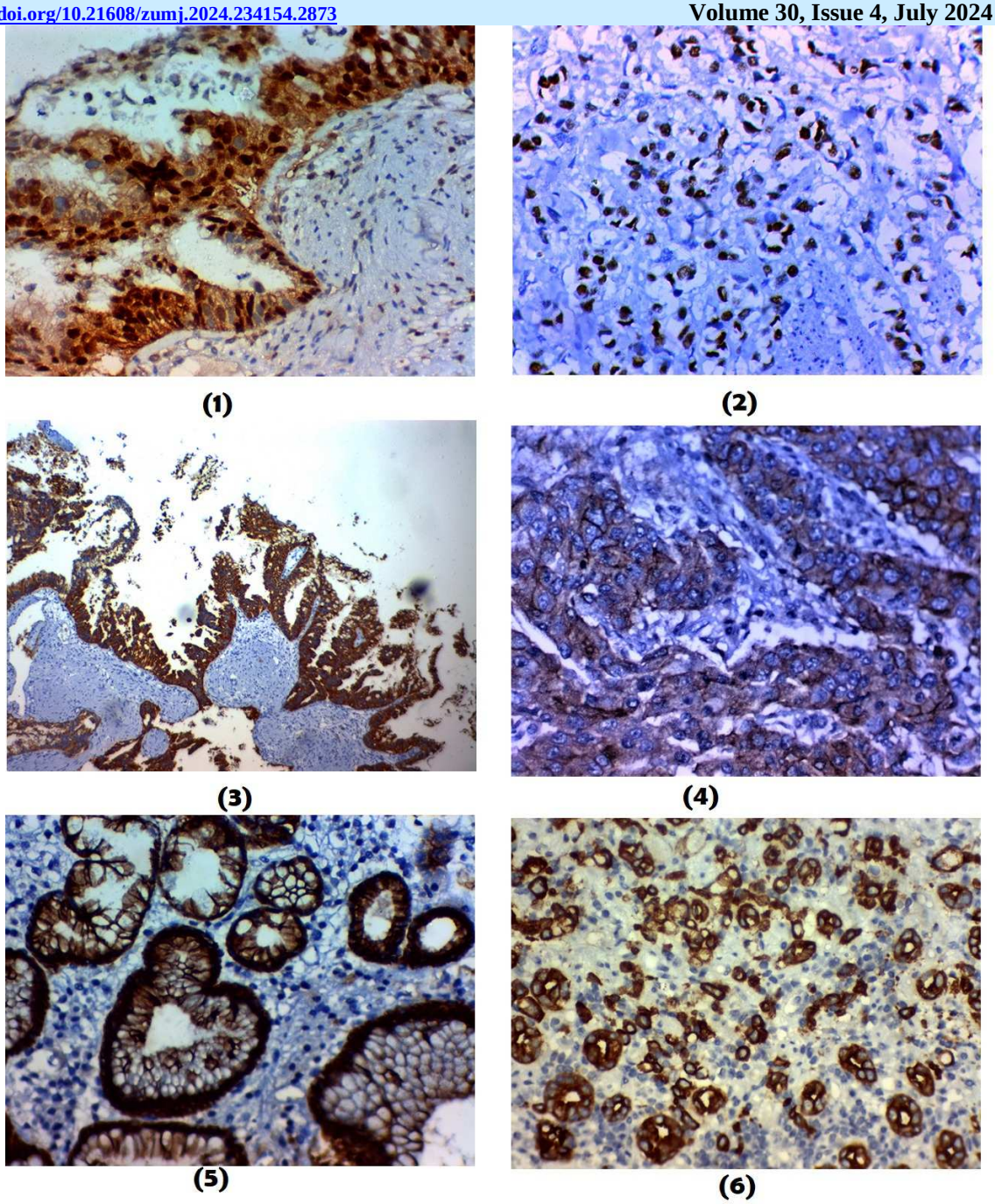

Figure 1: Immunostaining of Gastric adenocarcinoma specimens showing:
1- GC G II with strong HMGA1 immunostaining (ABC x 400)
2- GC G III with strong HMGA1 immunostaining (ABC x 400)
3- GC G II with strong TNFRSF11B immunostaining (ABC x 200)
4- GC G III with strong TNFRSF11B immunostaining (ABC x 400)
5- GC G I with strong c- CBL immunostaining (ABC x400)
6- GC G II with moderate c- CBL immunostaining (ABC 200)

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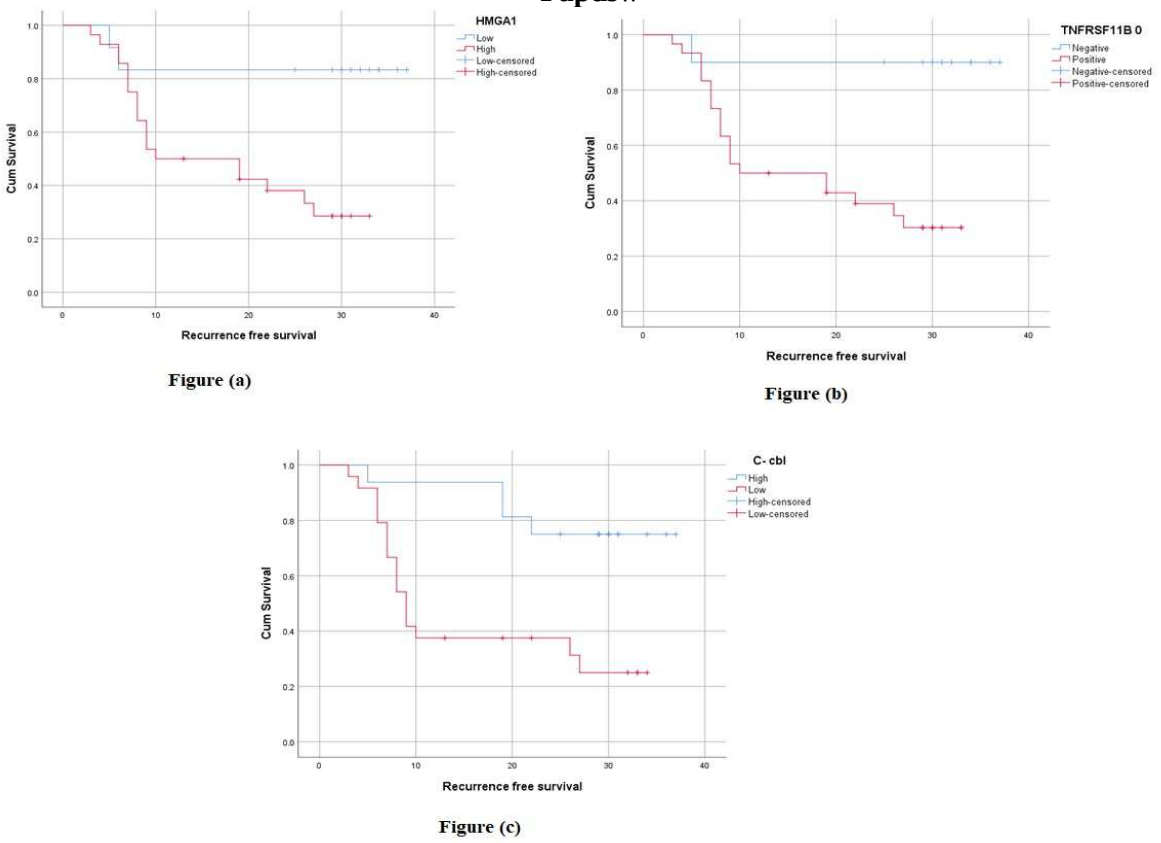

Figure 2: Kaplan Meier plot showing:
A- Significant relation between HMG1 and recurrence free survival among studied patients
B- Significant relation between TNFRSF11B 0 and recurrence free survival among studied patients

C- Significant relation between c- CBL and recurrence free survival among studied patients

DISCUSSION

Gastric cancer ranks among the top ten causes of cancer-related deaths [1]. HMGA1 is one of the target genes of the Wnt signaling in GC [11]. We found higher expression levels of HMGA1 in tumor tissues compared to adjacent normal tissues.

With the organ development, HMGA1 level is gradually decreased in mature cells [21]. Its high expression was noted in (70%) of the tumors, which is analogous to that observed in Pádua *et al*. report in the series of GC cases (69%) [16].

Our study revealed that upregulation of HMGA1 was closely related to tumors with high-grade, metastasis to lymph nodes, tumor size, and high tumor stages. Our findings supported the previous data that the capability of HMGA1 to enhance tumor invasiveness by promoting epithelial-to-mesenchymal transition [21, 22].

In GC, the overexpression of HMGA1 downregulated E-cadherin, while silence of HMAG1 yielded the opposite results [21]. Besides, Yang *et al*. reported that upregulation of HMGA1 increases the migratory capacity of GC cell by transactivating SUZ12 which presents one of EMT promotors. Inhibition of HMGA1 can regulate EMT and affect the metastasis and prognosis of non-small cell lung cancer [22, 23].

Furthermore, HMGA1 suppression can decrease the expression of the pluripotency

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genes [24] and promotes matrix metalloproteinase 2 (MMP2) transcriptions [25]. Therefore, targeting HMGA1-mediated tumorigenic activity could be useful for the treatment of GC.

Disturbance of glucose metabolism has evolved as a feature of cancer cells. HMGA1 is as a glycolysis regulator in GC which promotes gastric cancer tumorigenic and glycolytic phenotypes via regulating the expression of c-myc. Low levels of HMGA1 reduces the uptake of glucose, lactic acid production, and inhibits the invasion of tumor cells. Therefore, its high expression indicates a poor clinical outcome, tumor relapse and poor OS and DFS and occurrence of distant metastases which were in accordance with previous studies [26]. Our study is the second one that identified the expression of HMGA1 as a valuable marker for prediction chemotherapy response in GC. [27]

Based on D'Angelo *et al* findings, proteins of HMGA increase malignant cell resistance to chemotherapeutic agents by enhancing Ataxia telangiectasia mutated expression, shifting the signaling of ATM from cell fatality to its survival. Gemcitabine resistance in cancer pancreas is due to HMGA1 over expression depending on an Akt mechanism [28, 29].

Therefore, the targeted suppression of HMGA1 could be a prospective therapeutic approach for increasing chemosensitivity in malignant cells [9].

On the other hand, Pádua *et al* who studied 323 cases of GC did not observe a prognostic value for HMGA1; reporting a considerable superior overall survival in cases who presented with HMGA1 overexpression when they received chemotherapy. Therefore, further studies with a larger number of GC cases are recommended [16].

We found that c- CBL showed low expression in 60% with significant correlation with tumor size, grade, stage, LN metastasis and AJCC staging. In agreement with previous investigations, it demonstrated that reduced expression of c- CBL was more in the malignant gastric tissues in comparison with that in the normal tissue, confirming the loss role of c- CBL in progression in GC [30].

CBL family proteins have a definite expression affinity in various tumors as was shown in previous studies. Its expression was up-regulated in glioma with closely association with tumor progression, worse overall and progression-free survival [31].

On the other hand, CBL could alleviate immunosuppression and inhibit tumor proliferation and invasion in breast cancer. This discrepancy in CBL proteins roles in diverse tumors could be because they target different substrates and so they activate different downstream signaling pathways [32].

Kumaradevan *et al*. demonstrated that over expression of c- CBL in Colorectal cancer associated with an improved overall survival of patients; confirming its role as a negative regulator of Wnt/b-catenin signaling and an inhibitor to CRC tumor growth. Therefore, the underlying mechanisms of its action in cancer require more investigations [33].

In this study, low c- CBL expression correlates with de-differentiation, higher tumor stage, lymph nodes presence, and distant metastases; confirming that its over expression is associated with gastric tumor suppression. Similarly, these results were in accordance with previous studies that showed that the high expression of c- CBL has a protective function against tumor progression in gastric and colon cancer [17, 30, and 33].

According to the survival analysis here, higher expression of c- CBL in GC tumors is associated with better overall survival of patients, indicating their prognostic value in patients with GC. c- CBL plays a tumor suppressive effect through binding to the PD-1 tail in the cytoplasm and targets it for its proteasomal degradation in macrophages, leading to PD-1 downregulation and reduction of its surface expression resulting in more phagocytosis of tumor and malignant tumor suppression.

Furthermore c- CBL can downregulate oncoproteins as Wnt/β-catenin receptor tyrosine kinases in malignant cells [12, 34].

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Besides, c- CBL has also been shown to slow down tumor-associated angiogenesis [12].

Regarding the response to therapy in our study, among the chemotherapy-treated group, the patients with high c- CBL expression showed a better response rate towards "taxol+carboplatin "regimen than patients with low c- CBL expression. Ma *et al* [35]. reported that inhibition of c- CBL linked with reduction of lapatinib resistance via degradation of HER2 in GC cells. So, patients with low c-CBL expression can benefit from lapatinib treatment in GC. Therefore, our results suggested that c- CBL may provide new strategies for GC therapy.

In cancer therapy, it is essential to identify the tumor microenvironment as membrane-bound or secreted ligand-receptor systems as these factors aid in tumor existence, growth, and progression [36,37, 38].

In gastric carcinoma, TNFRSF11B expression contributes to tumor genesis, survival of cancer cells and therapy resistance via Wnt/ β-catenin pathway; that is considered a hallmark of this cancer [18].

We noted that TNFRSF11B was over expressed in the cytoplasm of malignant gastric tissues. It was expressed in 30 cases (75%) of the gastric cancer tissues studied. These results are near to Luan 2020 who reported its expression in 74.3% (52/70) [18]. So, TNFRSF11B in GC cells significantly promoted cell proliferation and tumorigenesis.

Our study concluded that high expression of TNFRSF11B was closely related with high-grade tumors, high tumor stages and node metastasis. As previously, we confirmed the ability of TNFRSF11B to increase the invasiveness of GC cells [18].

TNFRSF11B can activate GSK-3b phosphorylation and increase b-catenin expression and promote the invasiveness of gastric cancer [39].

In cancer colon, it has a potential role in the regulation of the immune response. Zhang *et al.* examined the expression of TNFRSF11B in cancer colon and related Osteoprotegerin expression to clinicopathological information such as tumor stage, presence of lymph node, invasion depth and prognosis [19]. Furthermore, concentration of serum TNFRSF11B is linked with both high tumor grade and tumor stage colon cancer and could result in a poorer survival rate [40].

In addition, Mizutani *et al.* demonstrated that high serum TNFRSF11B levels are prognostic of early recurrence in bladder cancer patients. Therefore, serum TNFRSF11B concentration may have utility as a prognostic parameter [41].

Regarding TNFRSF11B's impact on survival in the studied GC cases, it was correlated with poor prognosis and chemo-resistance. The Kaplan Meier survival curve showed that the OS rate of patients with positive expression of TNFRSF11B was lower than that of patients with negative expression of TNFRSF11B. These data are in accordance with tendency that have been reported in GC and colorectum [18, 19].

Our study is consistent with previous findings, showing that Gastric carcinoma is associated with high HMGA1, TNFRSF11B-positive, and c-CBL low expression, that are all related to poor prognosis.

CONCLUSIONS

As HMGA1, TNFRSF11B & c- CBL markers may be involved in tumor start, metastasis, and chemo-radio resistance, they include an attractive target for anti-cancer drug therapy. These markers will help in tailoring the treatment modality and chemotherapy regimen for each patient as patients with low HMGA1, TNFRSF11B and high c- CBL will be a candidate for taxol+carboplatin regimen.

However, with high HMGA1, TNFRSF11B and low c- CBL will benefit from more aggressive chemotherapy regimens such as FOLFOX or XELOX rather than taxol+carboplatin regimen. So, they help in gastric cancer prognosis prediction, treatment modality choice and GC clinical outcome.

Conflict of interest: The authors declared that they have no conflicts of interest with respect to the authorship and publication of this article.

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REFERENCES

1. **Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al**. Global Cancer Statistics 2020: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021; 71: 209–49.
2. **Feng W, Ding Y, Zong W, Ju S**. Non-coding RNAs in regulating gastric cancer metastasis. Clin Chim Acta. 2019; 496:125–33.
3. **Jiang J, Wang K, Chen Y, Chen H, Nice EC, Huang C.** Redox regulation in tumor cell epithelial—Mesenchymal transition: Molecular basis and therapeutic strategy. Signal Transduct. Target. 2017; 2: 17036.
4. **Zhang Y, Wang X**. Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol. 2020; 13: 165.
5. **Anastas JN, Moon RT.** WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer. 2013; 13: 11–26.
6. **Fu Y, Chen Y, Huang J, Cai Z, Wang Y. RYK,** a receptor of noncanonical Wnt ligand Wnt5a, is positively correlated with gastric cancer tumorigenesis and potential of liver metastasis. Am. J. PhysiolGastrointest. Liver Physiol. 2020; 318: 352–60
7. **Koushyar S, Powell AG, Vincan E, PhesseT J.** Targeting Wnt signaling for the treatment of gastric cancer. International journal of molecular sciences 2020; 21(11): 3927.
8. **Sumter TF. Xian L, Huso T, Koo M, Chang,Y.T. Almasri TN, et al**. The High Mobility Group A1 (HMGA1) Transcriptome in Cancer and Development. Curr. Mol. Med. 2016; 16: 353–93.
9. **Wang CQ.** MiR-195 reverses 5-FU resistance through targeting HMGA1 in gastric cancer cells. Eur. Rev. Med. Pharmacol. Sci. 2019; 23: 3771–8.
10. **Fu F, Wang T, Wu Z, Feng Y, Wang W, Zhou S, et al.** HMGA1 exacerbates tumor growth through regulating the cell cycle and accelerates migration/invasion via targeting miR-221/222 in cervical cancer. Cell Death Dis. 2018; 9; 594.
11. **Akaboshi S, Watanabe S, Hino Y, Sekita Y, Xi Y, Araki K, et al**. HMGA1 is induced by Wnt/beta-catenin pathway and maintains cell proliferation in gastric cancer. Am. J Pathol. 2009; 175(4):1675-85.
12. **Shivanna S, Harrold I, Shashar M, Meyer R, Kiang C, Francis J, et al.** The c-Cbl ubiquitin ligase regulates nuclear β-catenin and angiogenesis by its tyrosine phosphorylation mediated through the Wnt signaling pathway. J. Biol. Chem. 2015; 290(20): 12537-46.
13. **Goswami S,** Sharma-Walia N. Osteoprotegerin rich tumor microenvironment: implications in breast cancer. Oncotarget. 2016; 7: 42777-91.
14. **Dostert C, Grusdat M, Letellier E, Brenner D.** The TNF family of ligands and receptors: communication modules in the immune system and beyond. Physiol. Rev. 2019; 99(1): 115-160.
15. **Gunduz I, Acehan T, Alemdar A, Surel AA, Coskun N**. Comparison of staging systems in gastric carcinoma. Int J Clin Pract. 2021; 75(11): 14703.
16. **Pádua D, Pinto DF, Figueira P, Pereira CF, Almeida R, Mesquita P**. HMGA1 has predictive value in response to chemotherapy in gastric cancer. Current Oncology 2021; 29(1): 56-67.
17. **Chen C, Hui Y, Chen Y, Qian C, Sun M**. Loss of c-Cbl expression correlates with de-differentiation status and lymphatic metastasis in gastric cancer. Indian J. Pathol. Microbiol. 2019; 62(4): 549.
18. **Luan F, Li X., Cheng X, Huangfu L, Han J, Guo T, et al**. TNFRSF11B activates Wnt/β-catenin signaling and promotes gastric cancer progression. Inter. J. Biol. Sci. 2020; 16(11):1956.
19. **Zhang JR, Hou P, Wang XJ, Weng Z, Shan-guan X, Wang H, et al.** TNFRSF11B suppresses memory CD4+ T cell infiltration in the colon cancer microenvironment: a multiomics integrative analysis. Front Immunol. 2021; 12.
20. **Gregorieff A, Clevers H.** Wnt signaling in the intestinal epithelium: from endoderm to cancer. Genes Dev. 2005; 19:877–90.
21. **Jin G-H, Shi Y, Tian Y, Cao T-T, Mao y, Tang T-Y.** HMGA1 accelerates the malignant progression of gastric cancer through stimulating EMT. Eur. Rev. Med. Pharmacol. Sci. 2020; 24: 3642–7.
22. **Yang Q, Wang Y, Li M, Wang Z, Zhang J, Dai W, et al**. HMGA1 promotes gastric cancer growth and metastasis by transactivating SUZ12 and CCDC43 expression. Aging (Albany NY). 2021; 13(12):16043-61.
23. **Ma Y, Li X, Chen S, Du B, Yaming L**. MicroRNA-4458 suppresses migration and epithelial-mesenchymal transition via targeting HMGA1 in non-small-cell lung cancer cells. Cancer Manag. Res. 2019; 11: 637–49.
24. **Shah SN, Kerr C, Cope L, Zambidis E, Liu C, Hillion J, et al**. HMGA1 Reprograms Somatic Cells into Pluripotent Stem Cells by

https://doi.org/10.21608/zumj.2024.234154.2873 Volume 30, Issue 4, July 2024

Inducing Stem Cell Transcriptional Networks. PLoS ONE 2012; 7: e48533.

25. **Hillion J, Roy S, Heydarian M, Cope L, Xian L, Koo M, et al**. The High Mobility Group A1 (HMGA1) gene is highly overexpressed in human uterine serous carcinomas and carcinosarcomas and drives Matrix Metalloproteinase-2 (MMP-2) in a subset of tumors. Gynecol Oncol. 2016; 141:580–87.
26. **Cao XP, Cao Y, Zhao H, Yin J, Hou P.** HMGA1 promoting gastric cancer oncogenic and glycolytic phenotypes by regulating c-myc expression. BBRC. 2019; 516(2): 457-65.
27. **Yang M, Guo Y, Liu X, Liu N.** HMGA1 Promotes Hepatic Metastasis of Colorectal Cancer by Inducing Expression of Glucose Transporter 3 (GLUT3). Med Sci Monit. 2020; 26: e924975.
28. **D'Angelo D, Mussnich P, Rosa R, Bianco R, Tortora G, Fusco A.** High mobility group A1 protein expression reduces the sensitivity of colon and thyroid cancer cells to antineoplastic drugs. BMC cancer 2014; 14(1): 1-9.
29. **Liau SS, Jazag A, Ito K, Whang EE.** Overexpression of HMGA1 promotes anoikis resistance and constitutive Akt activation in pancreatic adenocarcinoma cells. Br J Cancer 2007; 96: 993–1000
30. **Mohamed A H, Harb OA, Hemeda R, Sharafedeen M, Abd-Elhady WA, Ali RM**. Prognostic Performance of High Expression of KIF23 and Loss of c-CBL in Gastric Cancer Patients: An Immunohistochemical Study. MEJS. 2022; 13(3): 393-403.
31. **Jing Z, Li L, Wang X, Wang M, Cai Y, Jin ZI, et al.** High c-Cbl expression in gliomas is associated with tumor progression and poor prognosis. Oncol Lett. 2016; 11(4):2787-91
32. **Li W, Lei T, Song X, Deng C, Lu J, Zhang W, et al**. CBLC inhibits the proliferation and metastasis of breast cancer cells via ubiquitination and degradation of CTTN. J Recept Signal Transduct Res. 2022 Dec;42(6):588-598
33. **Kumaradevan S, Lee SY, Richards S, Lyle C, Zhao Q, Tapan U, et al**. c-Cbl Expression Correlates with Human Colorectal Cancer Survival and Its Wnt/β-Catenin Suppressor Function Is Regulated by Tyr371 Phosphorylation. Am J Pathol. 2018;188(8):1921-33.
34. **Shashar M, Siwak J, Tapan U, Lee SY, Meyer RD, Parrack P, et al**. c-Cbl mediates the degradation of tumorigenic nuclear β-catenin contributing to the heterogeneity in Wnt activity in colorectal tumors. Oncotarget. 2016; 7(44):71136-50
35. **Ma L, Zhu W, Wang Q, Yang F, Qian J, Xu T. et al**. JWA down-regulates HER2 expression via c-Cbl and induces lapatinib resistance in human gastric cancer cells. Onco target. 2016; 7(44):71790-801.
36. **Bissell MJ, Hines WC.** Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011; 17(3): 320-9.
37. **Faget J, Contat C, Zangger N, Peters S, Meylan E.** RANKL signaling sustains primary tumor growth in genetically engineered mouse models of lung adenocarcinoma. JTO. 2018; 13(3): 387-98.
38. **Goswami S, Sharma-Walia N**. Osteoprotegerin secreted by inflammatory and invasive breast cancer cells induces aneuploidy, cell proliferation and angiogenesis. BMC Cancer. 2015; 15: 935.
39. **Birgisson H, Tsimogiannis K, Freyhult E**, Kamali-Moghaddam M. Plasma Protein Profiling Reveal Osteoprotegerin as a Marker of Prognostic Impact for Colorectal Cancer. Transl. Oncol. 2018; 11:1034–43
40. **Mizutani Y, Matsubara H, Yamamoto K, Nan Li Y, Mikami K, Okihara K**. Prognostic significance of serum osteoprotegerin levels in patients with bladder carcinoma. Cancer 2004; 101(8): 1794-802.

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