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Role of Human Pituitary Tumor-Transforming Gene1 (HPTTG1) as a prognostic biomarker for metastasis in Egyptian patients breast, colon and Liver cancer

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Abstract

HPTTG1 is an oncogene that overexpressed in most human carcinomas. It was reported to be involved in cell cycle regulation and sister chromatid separation. PTTG expression level has been associated with tumor progression, invasion, and metastasis. In this study, HPTTG1 expression was analyzed in three cancer patients' groups including; breast cancer, colorectal cancer, and hepatocellular carcinoma patients using RT-PCR. Our data revealed that the expression of PTTG1 was high in three groups; however, the metastatic cases reported higher expression levels of PTTG1 compared to non-metastatic groups. Thus, PTTG1 could be a prognostic marker in different types of cancer patients, and targeting PTTG1 might be a good strategy against metastasis.

Keywords: hPTTG1**,** Human Pituitary Tumor-Transforming Gene1, metastasis, breast cancer, colorectal cancer, hepatocellular carcinoma

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1. Introduction

The protein product of pituitary tumor-transforming gene-1 (*PTTG1*) was first isolated from GH4 rat pituitary tumor cells [1]. PTTG1 was known as human securin, a critical regulator of sister chromatid separation in late-stage mitosis [2]. The level of PTTG1 is very low or undetectable levels in most normal human cells however, its expression is so high in malignant cell lines and pituitary tumors [3]. Accumulating evidence reported that PTTG1 was directly regulated by estrogen, insulin, basic fibroblast growth factor, epidermal growth factor, β-catenin/transcription factor, Rb/E2F1 pathways, STAT3, and consequently involved in multiple steps of tumor progression including tumorigenesis, invasiveness, metastasis, and angiogenesis [4, 5].

In breast cancer, the PTTG1 expression level is higher in patient-derived breast cancer tissues than the normal samples and associated with poor prognosis [6]**.** In malignant breast cancer cell lines, down-regulation of PTTG1 expression decreases migratory and invasive properties. PTTG1 was found to enhance EMT by stimulating in Snail but not Slug, Twist, and Zeb1 transcription factors [7]**.** Additionally, the PI3K/AKT pathway is activated by PTTG1 expression which suggested its role in the maintenance of self-renewing and tumorigenic cancer stem cells [6].

In hepatocellular carcinoma (HCC), PTTG1 has been reported to overexpress and correlated with tumor angiogenesis and is significantly associated with disease-free and overall survival rates [8]**.**

In a colorectal cancer cells, a previous study found a correlation between FoxM1 and PTTG1. They provided evidence showing that FoxM1 activates PTTG1 transcription which caused activation to the cell migration and invasion [9].

In the current study, due to the essential role of PTTG1 expression in cancer progression and metastasis, PTTG1 expression was analyzed in breast cancer, colorectal cancer, and hepatocellular carcinoma patients. Moreover, PTTG1 expression was evaluated to compare between metastatic and non-metastatic cases in these patients' groups.

2. Materials and methods

Patients

This is a prospective study that included 60 patients diagnosed with Breast cancer (20 patients) & colorectal cancer (20 patients) and Liver cancer (20 patients) in addition to control samples at least 20 samples in Banha University hospital. The study was approved by the institutional Research Ethics Committee of Banha University, number RC 4-2-2020.

Inclusion criteria were 1- Breast, Colon, and Liver cancer proven by histopathology (stage I, II, III, IV), 2- Age 18 to 70 years old, and Available Clinical Data of the patients. Exclusion criteria were 1- Cardiac patients, 2- Terminal cases, 3- Past history of malignancy.

RT PCR

The mononucleated lymphocytic cell pellet was prepared according to Dagur and McCoy (2015) [10] in which the sequence of the following steps was conducted: Haemolysin buffer was added to the whole blood in 10 times of blood volume in a sterile falcon tube and left for 30 minutes' incubation with an occasional vigorous vortex. Lesser volumes of haemolysin buffer were used till complete removal of red blood cells from the cell pellet. Phosphate buffered saline (PBS) was used to wash the cell pellet several times till it becomes clear & white. The lymphocytic cell pellet was suspended in 100 µl PBS and then the sample was subjected to RNA extraction for determination of RNA expression by real-time PCR of the following genes: PTTG1 and ACTB. Total RNA was extracted from the lymphocytic cell pellet with a total RNA purification kit (Qiagen, Hilden, Germany) in which Isopropanol (300 µl) was added to the cell lysate to extract the nuclear RNA. After several steps of centrifugations and washings of the spin column placed in a 2 ml collection tube, pure RNA was separated and eluted with 40 µl of elution buffer into an RNase-free microcentrifuge tube. The produced RNA was converted into cDNA by a ready-made kit from Thermo Fisher, UK in which reverse transcription enzyme was added to the template of RNA in the presence of nucleotides and the primers of target genes. Real-time PCR was done according to manufacturer's instructions by using Qiagen syber green PCR master mix using primers mentioned in table 1). The analysis of data was performed by using the ΔΔ Ct method [11]. Transcript values were normalized to those obtained from the amplification of the internal control Beta Actin.

Primer sequences for qPCR:

Gene	Forward primer	Revers primer
PTTG1	5'AAGCCTATGAAGACTGGCAAACC3'	5'GCAGGAACAGAGCTTTGTGTCTTA3'
Beta-Actin (ACTB)	5' CACCATTGGCAATGAGCGGTTC3'	5' AGGTCTTTGCGGATGTCCACGT3'

Statistical Methods

Statistical analysis was done using IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Pearson's Chi-square test or Fisher's exact test was used to examining the relation between qualitative variables. Quantitative variables were tested for normality using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. For quantitative data, a comparison between the two groups was done using the Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using either analysis of variance (ANOVA) for normally distributed quantitative variables or Kruskal-Wallis test (non-parametric ANOVA) for not normally distributed numeric variables then post-Hoc test" was used for pair-wise comparison based on Kruskal-Wallis distribution. Spearman-rho method was used to test the correlation between numerical variables. All tests were two-tailed. A p-value < 0.05 was considered significant.

3. Results

Patients` characteristics:

Gender *difference between the studied cancer groups.*

All group have 20 patients, all patients in breast cancer (BC) group were females. On the other hand, colorectal cancer (CRC) group had 30% (6 patients) males and 70% (14 patients) females and hepatocellular carcinoma (HCC) had 95% (19 patients) males and 5% (1 patient) female. In all groups, males represented 41.7% (25 patients) and females represented 58.3% (35 patients) as revealed in table (1).

Table (1) *: Patients gender in each group.

			Group1			
			BC	CRC	HCC	Total
Gender	Male	Count	0	6	19	25
		% within Group1	0%	30%	95%	41.7%
	Female	Count	20	14	1	35
		% within Group1	100%	70%	5%	58.3%
Total		Count	20	20	20	60
		% within Group1	100%	100%	100%	100%

Chi-Square Tests

	Value	p-value
Pearson Chi-Square	38.811	**<0.001**

*Data presented as counted number of patients and percentage N (%) to the total number of patients (60). Pearson's Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. P-value < 0.05 was considered significant. Abbreviations: BC: Breast Cancer; CRC: Colorectal Cancer; HCC: Hepatocellular Carcinoma.

The Gender difference between metastatic and non-metastatic cases within the studied cancer patients' groups.

In the breast cancer group, ten females were metastatic (Mets) cases while the other ten were non-metastatic (Non-Mets). For the colorectal carcinoma group, 10 patients were non-metastatic [Non-Mets] (4 males and 6 females) and 10 patients were metastatic [Mets] (2 males and 8 females). Additionally, hepatocellular carcinoma group included 10 patients (9 males and 1 female) were non-metastatic [Non-Mets] and 10 patients all of them are males metastatic (Mets) cases as shown in table (2).

Table (2) *: Gender differences between metastatic and metastatic cases within the studied groups

			Group						Total
			Non-Mets BC	Mets BC	Non-Mets CRC	Mets CRC	Non-mets HCC	Mets HCC	
Gender	Male	Count	0	0	4	2	9	10	25
		% within Group	0%	0%	40%	20%	90%	100%	41.7%
	Female	Count	10	10	6	8	1	0	35
		% within Group	100%	100%	60%	80%	10%	0%	58.3%
Total		Count	10	10	10	10	10	10	60
		% within Group	100%	100%	100%	100%	100%	100%	100%

Chi-Square Tests

	Value	p-value
Fisher's Exact Test	42.226	<0.001

*Data presented as counted number of patients and percentage N (%) to the total number of patients (60). Pearson's Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. P-value < 0.05 was considered significant. Abbreviations: BC: Breast Cancer; CRC: Colorectal Cancer; HCC: Hepatocellular Carcinoma.

The Age difference between the studied cancer patients' groups.

As revealed in the table (3), the average age in breast cancer and colorectal carcinoma groups was almost 45 while the hepatocellular carcinoma group has an average age of 54 approximately. Using one-way ANOVA, no significant difference was found either between or within groups.

Table (3)[#]: Age difference between the studied cancer patients' groups

	N	Mean	SD
BC	20	45.500	10.5307
CRC	20	46.700	18.2875
HCC	20	53.450	9.3442
Total	60	48.550	13.5639

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	734.700	2	367.350	2.069	0.136
Within Groups	10120.150	57	177.546		
Total	10854.850	59			

* Data presented as counted number of patients and percentage N (%) to the total number of patients (60 Statistical significance was done using one-way ANOVA, then post-Hoc test" was used for pair-wise comparison based on Kruskal-Wallis distribution. P-value < 0.05 was considered significant. Abbreviations: BC: Breast Cancer; CRC: Colorectal Cancer; HCC: Hepatocellular Carcinoma.

The Age difference between metastatic (METS) and non-metastatic (non-METS) cases within cancer patients 'groups

Table (4) shows the difference in age in metastatic cases and non- metastatic in all studied groups. First, breast cancer patients, the average age in the non-metastatic group was 48 while in the metastatic group was 43. On the other hand, the average age was the same in both metastatic and non-metastatic cases in colorectal cancer patients. In the hepatocellular carcinoma group, the average age was 58 in the non-metastatic group and 49 in metastatic cases. However, this difference between metastatic and non-metastatic cases between or within the studied groups was not significant.

Table (4)[#]: Age difference between metastatic (METS) and non-metastatic (non-METS) cases within the studied groups

	N	Mean	SD
Non-Mets BC	10	47.600	10.3086
Mets BC	10	43.400	10.8648
Non-Mets CRC	10	46.900	22.1030
Mets CRC	10	46.500	14.7441
Non-Mets HCC	10	57.700	7.5873
Mets HCC	10	49.200	9.3071
Total	60	48.550	13.5639

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	1184.950	5	236.990	1.323	.268
Within Groups	9669.900	54	179.072		
Total	10854.850	59			

#Data presented as counted number of patients (N), mean and standard deviation (SD). Statistical significance was done using one-way ANOVA, then post-Hoc test" was used for pair-wise comparison based on Kruskal-Wallis distribution. P-value < 0.05 was considered significant. **Abbreviations**: BC: Breast Cancer; CRC: Colorectal Cancer; HCC: Hepatocellular Carcinoma

Pituitary tumor transforming gene (PTTG1) expression in breast cancer BC, CRC and HCC patients' groups

Pituitary tumor transforming gene (PTTG1) is a recently discovered oncogene and has been implicated in the development and progression of many malignancies ***(Fujii et al., 2006).*** The upregulation of PTTG1 has been correlated with aggressive disease and poor prognosis in many cancer types ***(Minematsu et al., 2006).*** As revealed in a table (5), *PTTG*1 expression was high in breast cancer, colorectal cancer, and hepatocellular carcinoma groups with no significant difference between groups.

Table (5) #: PTTG1 expression in the studied groups

			Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	Group1	BC	20	21.82	10.65	**21.82**	**6.26**	**40.15**
		CRC	20	24.64	13.04	**21.22**	**10.36**	**60.71**
		HCC	20	30.90	20.07	**28.02**	**10.15**	**75.24**

	Chi-Square	p-value
PTTG1	1.359	0.507

#Data presented as counted number of patients (N), mean and standard deviation (SD). Statistical significance was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc test" was used for pair-wise comparison based on Kruskal-Wallis distribution. P-value < 0.05 was considered significant.

Difference expression of PTTG1 in non-metastatic (non-mets) cases in breast cancer BC, CRC and HCC patients' groups

Table (6) shows the difference in PTTG1 expression between non-metastatic cases in breast cancer, colorectal, and hepatocellular carcinoma groups. PTTG1 expression was investigated in all metastatic studied groups. PTTG1 expression was significantly higher in hepatocellular carcinoma metastatic than colorectal cancer metastatic groups.

Table (6): PTTG1 expression difference in all non-metastatic studied groups

			Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	Non-mets	**BC**	**10**	**14.36**	**9.03**	**10.36**	**6.26**	**34.42**
		CRC	**10**	**15.88**	**3.86**	**15.40**	**10.36**	**21.28**
		HCC	**10**	**14.99**	**5.23**	**13.72**	**10.15**	**25.81**

	Chi-Square	p-value
CXCR2	18.705	**<0.001**
PTTG1	2.054	0.358

Data presented as counted number of patients (N), mean and standard deviation (SD). Statistical significance was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc test" was used for pair-wise comparison based on Kruskal-Wallis distribution. P-value < 0.05 was considered significant.

PTTG1 expression in metastatic (Mets) cases in breast cancer BC, CRC and HCC patients' groups

PTTG1 expression was investigated in all metastatic studied groups. PTTG1 expression was significantly higher in hepatocellular carcinoma metastatic than colorectal cancer metastatic groups table (7).

Table (7) [#]: PTTG1 expression difference in all metastatic studied groups

			Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	Mets	**BC**	**10**	**29.29**	**5.84**	**30.40**	**20.02**	**40.15**
		CRC	**10**	**33.39**	**13.17**	**28.82**	**20.37**	**60.71**
		HCC	**10**	**46.82**	**16.14**	**45.25**	**30.23**	**75.24**

	Chi-Square	p-value
PTTG1	7.760	**0.021**

Post-Hoc of PTTG1

Groups	**p-value**
Mets BC-Mets CRC	1.000
Mets BC-Mets HCC	**0.033**
Mets CRC-Mets HCC	0.071

Comparison between CXCR2, PTTG1, RUNX1, and STAT3 expression in metastatic and non-metastatic breast cancer patients.

After that, each group was studied individually to explore the difference between metastatic and non-metastatic cases. As well as PTTG1 expression was double in metastatic breast cancer patients in comparison with non-metastatic breast cancer cases (Table 8).

Table (8)[#]: Comparison of PTTG1 level in metastatic and non-metastatic breast cancer patients.

		Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	**Non-mets**	**10**	**14.36**	**9.03**	**10.36**	**6.26**	**34.42**
	Mets	**10**	**29.29**	**5.84**	**30.40**	**20.02**	**40.15**

	Mann-Whitney U	p-value
PTTG1	11.000	**0.002**

Data presented as counted number of patients (N), mean and standard deviation (SD). Statistical significance was done using Mann-Whitney test (non-parametric t-test). P-value < 0.05 was considered significant.

C***omparison between PTTG1 expression in metastatic and non-metastatic colorectal cancer patients.***

Table (9) represents the genes expression differences in colorectal cancer metastatic and non-metastatic cases. In agreement with breast cancer results, PTTG1 expression was 1.5-fold higher in metastatic colorectal cancer than non-metastatic patients.

Table (9): Comparison of PTTG1 level in metastatic and non-metastatic colorectal cancer patients.

		Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	**Non-Mets**	**10**	**15.88**	**3.86**	**15.40**	**10.36**	**21.28**
	Mets	**10**	**33.39**	**13.17**	**28.82**	**20.37**	**60.71**

	Mann-Whitney U	p-value
PTTG1	2.000	**<0.001**

Comparison between PTTG1 expression in metastatic and non-metastatic hepatocellular carcinoma patients.

Table (10) shows the genes expression differences in hepatocellular carcinoma metastatic and non-metastatic cases. PTTG1 expression was 15 in non-metastatic hepatocellular carcinoma while its expression was 47 in metastatic hepatocellular carcinoma patients.

Table (10)[#]: Comparison of PTTG1 level in metastatic and non-metastatic hepatocellular carcinoma patients.

		Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	**Non-Mets**	**10**	**14.99**	**5.23**	**13.72**	**10.15**	**25.81**
	Mets	**10**	**46.82**	**16.14**	**45.25**	**30.23**	**75.24**

	Mann-Whitney U	Wilcoxon W	p-value
PTTG1	0.000	55.000	**<0.001**

#Data presented as counted number of patients (N), mean and standard deviation (SD). Statistical significance was done using Mann-Whitney test ow Wilcoxon test (non-parametric t-tests). P-value < 0.05 was considered significant.

Comparison between all studied groups in PTTG1 expression

After that PTTG1 expression was compared between breast cancer, colorectal and hepatocellular carcinoma in metastatic and non-metastatic cases. Table (11), PTTG1 expression was lower in non-metastatic than metastatic breast cancer patients. Similarly, PTTG1 expression in both colorectal and hepatocellular carcinoma patients was much higher in metastatic cases than non-metastatic patients. Also, metastatic hepatocellular carcinoma had elevated expression of PTTG1 compared to other patients' groups.

Table (11)[#]: Comparison between all studied groups in PTTG1 expression

		Valid N	Mean	SD	Median	Minimum	Maximum
PTTG1	**Non-Mets BC**	**10**	**14.36**	**9.03**	**10.36**	**6.26**	**34.42**
	Mets BC	**10**	**29.29**	**5.84**	**30.40**	**20.02**	**40.15**
	Non-Mets CRC	**10**	**15.88**	**3.86**	**15.40**	**10.36**	**21.28**
	Mets CRC	**10**	**33.39**	**13.17**	**28.82**	**20.37**	**60.71**
	Non-Mets HCC	**10**	**14.99**	**5.23**	**13.72**	**10.15**	**25.81**
	Mets HCC	**10**	**66.82**	**16.14**	**45.25**	**30.23**	**75.24**

	Chi-Square	p-value
PTTG1	40.373	**<0.001**

[#]Data presented as counted number of patients (N), mean and standard deviation (SD). Statistical significance was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc test" was used for pair-wise comparison based on Kruskal-Wallis distribution. P-value < 0.05 was considered significant.

Groups	**p-value**
Non-mets BC- Mets BC	**0.019**
Non-mets BC-Non-Mets CRC	1.000
Non-mets BC-Mets CRC	**0.010**
Non-mets BC-Non-mets HCC	1.000
Non-mets BC-Mets HCC	< **0.001**
Mets BC- Non-Mets CRC	0.092
Mets BC- Mets CRC	1.000
Mets BC- Non-Mets HCC	**0.036**
Mets BC- Mets HCC	1.000
Non-Mets CRC- Mets CRC	0.053
Non-Mets CRC- Non-Mets HCC	1.000
Non-Mets CRC- Mets HCC	< **0.001**
Mets CRC - Mets HCC	**0.021**
Mets CRC - Non-Mets HCC	1.000
Non-Mets HCC - Mets HCC	< **0.001**

Discussion

Cancer is the ultimate consequence of uncontrollable cell growth that illustrates an enormous group of associated diseases. It is the most common cause of death worldwide, with 12.7 million cases in 2008, and it is expected that 27 million new cases will be diagnosed in 2030 [12]. Metastasis is the real cause of cancer malignancy. Its targeting is much more challenging than that of cell proliferation because of the complex interactions between the tumor and the stroma; the contribution of the pathways of adhesion and motility is added to that of the pathways of cell proliferation and survival [13].

Based on the previous study, it was found that PTTG1-driven acquisition of migratory and invasive properties was followed by suppression of E-cadherin, a marker of epithelial cells, and an increase in N-cadherin and vimentin, markers of mesenchymal cells [14]. These changes in expression profile were accompanied by morphological changes to a more spindle-shape and a less compact growth pattern, implying that PTTG1 promotes EMT in breast cancer cells. These results agree with our findings, PTTG1 was overexpressed in metastatic breast cancer cases compared to non-metastatic patients. In colorectal cancer, an early report from The Lancet journal showed PTTG1 was

overexpressed in all of 48 colon cancer and corrected with Dukes' stage and lymph-node invasion [15]**.** As well as, Wang MN et al. noticed the expression of PTTG1 mRNA and protein was elevated in tumor samples, blood, and stool of patients with colorectal neuroendocrine tumors and positively correlated with a differentiated degree, clinical stage, and lymph node metastasis [16]. However, the prognostic significance and biological function of PTTG1 in colorectal cancer are still unknown. Moreover, PTTG1 is a key player associated with tumor metastasis via activation of c-Myc and cyclin D3 (CCND3) to facilitate cell proliferation, increase basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and matrix metalloproteinase 2 (MMP2) expressions to induce angiogenesis, which plays an important role in tumor development and cancer metastasis, and induction interleukin-8 to function in metastasis [17]. In harmony with previous findings; our data revealed that PTTG1 was overexpressed in metastatic colorectal cases compared to non-metastatic ones. On the other hand, a previous study reported a significant relationship between PTTG1 expression and intratumoral microvessel density and its role in the upregulation of fibroblast growth factor (FGF)–2, one of angiogenesis and modulation of tumor progression, in hepatocarcinogenesis [8]. In this study, a high level of PTTG1 in metastatic hepatocellular carcinoma patients was noticed in the comparison to non-metastatic patients. These findings confirm the essential role of PTTG1 as a prognostic marker in many types of cancer.

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