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ORIGINAL ARTICLE

LncRNA *HOTTIP* and *HOTTIP* SNP rs1859168 in Colorectal Cancer

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ABSTRACT

Background: *Long non-coding RNA HOXA transcript at the distal tip (lncRNA HOTTIP) has been suggested as a tumor promoter in colorectal cancer (CRC). The emergence and spread of CRC have been associated with aberrant lncRNA expression. Single nucleotide polymorphisms (SNPs) are proven to be linked to cancer susceptibility and may influence the expression and function of lncRNAs.* ***Objectives:*** *This study aimed to evaluate the diagnostic and prognostic roles of lncRNA HOTTIP in CRC patients and to investigate the risk association between CRC susceptibility and HOTTIP SNP rs1859168.* ***Methodology:*** *This study included 30 CRC patients and 30 healthy controls. Gene expression of lncRNA HOTTIP in peripheral blood mononuclear cells (PBMCs) and tissue using real-time PCR (RT-PCR) was done. SNP genotyping of HOTTIP SNP rs1859168 was also done.* ***Results:*** *PBMCs and tissue expression levels of HOTTIP were upregulated in CRC cases with higher tissue expression than that of PBMCs in CRC cases. The frequency of the homozygous CC and the C allele of HOTTIP SNP rs1859168 was increased in CRC cases.* ***Conclusion****: The tissue and PBMCs expression levels of HOTTIP were able to diagnose CRC and differentiate between grade II and III CRC. Regarding HOTTIP SNP rs1859168, the homozygous CC and the C allele were associated with an increased risk of developing CRC.*

INTRODUCTION

Colorectal cancer (CRC) is considered the second deadliest and the third most prevalent malignancy globally [1]. The American Cancer Society projected that in 2024, new cases (106,590 colon cancer and 46,220 rectal cancer) could be diagnosed. Incidence rates decreased by approximately 1% year between 2011 and 2019. However, elderly individuals are the main demographically affected by this declining tendency [2]. In industrialized nations, the prevalence of CRC is comparatively high and is still rising [3, 4].

Cancer patients' peripheral circulations emit soluble circulating cancer-associated nucleic acids, including DNA, RNA, micro RNA, and long non-coding RNA, which enable non-invasive gene expression detection. Tumor biomarkers, such as lncRNAs, are useful in diagnosing and treating various malignancies, including CRC. The emergence and spread of CRC are associated with aberrant lncRNA expression [5].

Since lncRNAs are strongly linked to carcinogenesis and significantly influence cancer development, they were identified as possible new molecular targets for the fight against malignant tumors [6].

There aren't many lncRNAs that are found to be linked to CRC. To better realize the possible mechanisms and find useful lncRNAs that could aid in CRC diagnosis, prognosis, and therapy, the involvement of many lncRNAs must be investigated [5]. LncRNAs have been described in various cancer research as trustworthy diagnostic, prognostic, and key regulatory biomarkers for several cancer biological behaviors. Although many studies have suggested using serum lncRNA expression levels as a diagnostic CRC marker, whether lncRNA expression levels in PBMCs, as a circulating component, might be used as a CRC marker remains largely uncharacterized [7].

LncRNA *HOTTIP* guides the expression of several genes and proteins that affect migration, invasion, apoptosis, adhesion, proliferation, chemosensitivity, and tumor differentiation[6]. It boosts the expression of interleukin 6 (IL-6), which can break immune tolerance to malignant cells via upregulating the expression of PD-L1(programmed cell death ligand-1) - a crucial checkpoint of immunity- on neutrophils, which binds to the T lymphocyte surface receptor PD-1 causing suppression of host immune response and promoting the cancer cells' immune escape [8]. A strong relationship has been suggested between *HOTTIP* expression and the overall survival (OS), distant metastasis (DM), lymph node metastasis (LNM), tumor stage, and prognosis of several human malignancies [9].

Single nucleotide polymorphisms (SNPs) are considered the most common genetic variation in human genomes, proven to be linked to cancer prognosis and susceptibility, and may influence the expression and function of lncRNAs. SNP rs1859168 may be a functional polymorphism on *HOTTIP* that modifies its regulative function [10]. Research has revealed a major association between the *HOTTIP* SNPs rs1859168 CC genotype and the allele C with the increased risk of breast cancer[11]. However, little research has been done on the connection between *HOTTIP* SNPs rs1859168 and CRC.

METHODOLOGY

Study design

This case-control study was conducted in the Medical Microbiology and Immunology Department, South Egypt Cancer Institute and Medical Research Center, Faculty of Medicine, Assiut University.

The study included 30 CRC patients admitted to the Surgical Oncology Department, South Egypt Cancer Institute, from July 2021 to October 2023, and 30 healthy persons matched with the cases were included in the study as controls.

Clinical assessment of the included patients:

Newly diagnosed CRC patients aged 25-65 years were confirmed by histopathological examination and did not undergo any lines of treatment.

Samples collection and processing

Five milliliter fresh venous blood was withdrawn from each participant in EDTA blood collection tubes using a 5 ml sterile disposable plastic syringe. Regarding the blood sample, it was taken fresh, and the separation of PBMCs was done within 2 hours for best results [12].

Small-sized paired tissue samples were taken: one from the cancer tissue and another from the safety margin (at least 3 cm from the edge of the tumor) as a normal control tissue sample. The tissue sample was collected in a 1ml TRIzol™ reagent containing Eppendorf tube and stored at -80 °C [13].

Separation of PBMCs using Ficoll Histopaque-1077 (Biowest, France) (LOT: MS008Q1017) and RNA extraction from the whole blood using Invitrogen™ TRIzol™ reagent USA (Catalog Number: 15596026) was done.

Conversion of RNA into cDNA using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Applied Biosystems, USA) (Catalog. number #K1622) was done.

Gene expression by quantitative real-time PCR (q RT-PCR) was done as follows:

- Primers used for *HOTTIP*[14], and *GAPDH* (as housekeeping gene) [15] (Invitrogen, USA).

HOTTIP:
Forward:5′-AGCTCTCAGGGAAACGAAGC-3′,
Reverse:5′ TTTCCGGCAAACTCCCTCTC-3′
GAPDH: Forward:
5′-CCCTTCATTGACCTCAACTA-3′
Reverse: 5′-TGGAAGATGGTGATGGGATT-3′

The PCR condition for all genes: Denaturation at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and finally annealing and extension at 60°C for 60 seconds. The relative gene expression was normalized to the level of *GAPDH* transcript, and relative quantification was performed using the $2^{-\Delta\Delta CT}$ method [10].

TaqMan® SNP genotyping assay

-DNA was extracted from whole blood using (Thermo Scientific GeneJET Genomic DNA Purification Kit, #K0721, USA).

One SNP genotyping assay was selected in this study:
-SNP ID: rs 1859168.

- Polymorphism: transversion substitution (A/C). Context sequence (VIC/FAM):
5'GCTCTCGCCAAGAAAAAGCTTGGGG
(A/C)CTGAATTCTTGAGATTTATGGTGCA 3'.

-TaqMan® SNP genotyping assay using RT-PCR [ThermoFisher Scientific (USA, catalog number:4351379)] and TaqMan™ genotyping master mix (catalog number:4371353) was done.

RT-PCR conditions for SNP genotyping: The reaction was performed in a 7500 Real-Time PCR system with the following amplification protocol: denaturation at 95˚C for 10 min, followed by 45 cycles of denaturation at 92 ˚C for 15 seconds and annealing and extension at 60˚C for 90 seconds.

Statistical Analysis:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. Data was statistically described in terms of mean ± standard deviation (±SD), or median and range when not normally distributed, frequencies (number of cases), and relative frequencies (percentages) when appropriate. Comparison of quantitative variables was done using the Student t-test for normally distributed data and Mann Whitney U test as the data was not normally distributed. Wilcoxon sign rank test was used to compare the paired quantitative data. For comparing categorical data, Chi-square ($\chi 2$) was used or the Fisher Exact test instead of Chi-square ($\chi 2$) when the expected frequency was less than 5. A receiver operating characteristic curve (ROC) analysis was used to find the best cut-off values to validate the prediction of CRC and its grade using studied biomarkers. The risk association of *HOTTIP* SNP with CRC was assessed via logistic regression analysis. The ***p-value*** is always a 2-tailed set significant at 0.05 level.

RESULTS

The study included 30 CRC cases and 30 healthy-matched controls.

Demographic data of the studied participants

Studied groups are matched in age and gender, with no statistically significant difference, as shown in Table (1).

Table 1: Demographic data of studied groups

Demographic data	Cases (n=30)		Controls (n=30)		*p*-value
Age (years)					0.917
Mean ± SD	48.87 ± 6.94		49.03 ± 5.28		
Median (range)	50 (32 – 64)		49 (38 – 61)		
Gender					0.766
Male	23	(76.7%)	22	(73.3%)	
Female	7	(23.3%)	8	(26.7%)	
Family history					
Negative	10	(33.3%)			
Positive	20	(66.7%)			

Data is presented as mean ± SD and median (range) or number (percentage). Significance is defined by ***p-value*** < 0.05.

Clinical and pathological data of the studied CRC cases

Bleeding per rectum was the most common clinical presentation and recto-sigmoid cancer was the most common tumor site in 80%, 10% had ascending, and another 10% had descending colon tumors. More than half of the studied cases (60%) had a tumor size ≥ 3 cm. And 56.7% had tumor grade III. Almost all studied cases (93.3%) had adenocarcinoma.

PBMCs and tissue expression levels of *HOTTIP* among studied participants

Table (2) shows that *HOTTIP*, both PBMCs and tissue levels, have statistically significant upregulation among the CRC cases compared to controls (***p*** < 0.001) and the tissue expression was significantly higher than that of PBMCs (***p*** < 0.001).

Relationship between expression levels of *HOTTIP* as regards different patients' and tumor characteristics are shown in Table (3).

Table 2: Expression levels of *HOTTIP* in PBMCs versus tissue among the studied participants

Variable name	Cases (n=30)	Controls (n=30)	*p* value[1]
***HOTTIP* in PBMCs**			
Mean ± SD	1.10 ± 1.09	0.59 0.40	
Median (range)	1.02 (0.01 – 4.73)	0.81 (0.00 – 0.97)	**0.010**
***HOTTIP* in Tissue**			
Mean ± SD	3.67 ± 5.27	0.62 ± 0.33	
Median (range)	2.06 (0.91 – 27.89)	0.60 (0.06 – 1.65)	**<0.001**
***p* value[2]**	**<0.001**	0.813	

Data is presented as mean ± SD and median (range). Significance is defined by ***p-value*** < 0.05.
p value[1]: comparing both studied groups.
p value[2]: comparing PBMCs versus tissue samples in the same group separately.

Table 3: Relationship between expression levels of *HOTTIP* as regards different patients' and tumor characteristics

Demographic data	*HOTTIP* in PBMCs	*HOTTIP* in tissue	*p*-value[1]
Age (years)			
< 50 years (n=15)	1.01 (0.01 – 3.48)	2.04 (0.91 – 9.79)	**0.005**
≥ 50 years (n=15)	1.28 (0.04 – 4.73)	2.21 (1.06 – 27.89)	**0.027**
***p* value[2]**	0.202	0.870	
Gender			
Male (n=23)	1.02 (0.01 – 2.43)	2.21 (1.06 – 27.89)	**<0.001**
Female (n=7)	1.71 (0.04 – 4.34)	1.11 (0.91 – 4.34)	1
***p* value[2]**	0.131	0.069	
Family history			
Negative (n=10)	1.14 (0.04 – 4.73)	2.35 (1.06 – 11.94)	0.059
Positive (n=20)	1.02 (0.01 – 3.48)	1.91 (0.91 – 27.89)	**0.002**
***p* value[2]**	0.914	0.530	
Tumor characteristics			
Tumor site			
Colon	1.01 (0.01 – 3.48)	1.11 (0.91 – 2.50)	0.600
Recto-sigmoid	1.02 (0.04 – 4.73)	2.21 (1.06 – 27.89)	**<0.001**
***p value*[2]**	0.820	**0.025**	
Pathological type			
Adenocarcinoma	1.14 (0.04 – 4.73)	2.06 (0.91 – 27.89)	**0.001**
Mucinous	0.16 (0.01 – 0.31)	1.82 (1.14 – 2.50)	0.180
***p value*[2]**	0.138	0.966	
Tumor size			
< 3 cm (n=12)	0.53 (0.01 – 3.48)	1.13 (0.91 – 9.79)	**0.041**
≥ 3 cm (n=18)	1.28 (0.07 – 4.73)	2.34 (1.06 – 27.89)	**0.003**
***p value*[2]**	0.065	**0.012**	
Tumor grade			
GradeII (n=13)	0.31 (0.01 – 3.48)	1.49 (0.91 – 9.79)	**0.033**
GradeIII (n=17)	1.28 (0.07 – 4.73)	2.35 (1.06 – 27.89)	**0.004**
***p value*[2]**	0.145	**0.039**	

Data is presented as median (range). Significance is defined by ***p-value*** < 0.05.
p value[1]: comparing PBMCs versus tissue samples. ***p*** value[2]: comparing the expression of the studied gene according to different patients' and tumor characteristics.

The diagnostic and predictive ability of *HOTTIP* to detect CRC cases and differentiate CRC grades, as shown in Table (4)

HOTTIP PBMCs expression levels were able to diagnose CRC by using the ROC curve analysis (***p*** = 0.01), While *HOTTIP* tissue expression levels showed much more diagnostic ability to detect CRC (***p*** < 0.001) as shown in figure [1 (a and b)].

HOTTIP PBMCs expression levels failed to differentiate CRC grades (***p*** = 0.137), While tissue expression levels were able to differentiate CRC grades (***p*** = 0.04) as shown in figure [1(c)].

Table 4: The best cut-off, sensitivity, and specificity for prediction of CRC and differentiating CRC grades by *HOTTIP* in PBMCs and tissue

Markers	Cut off	95%CI	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	*p-value*
HOTTIP in PBMCs	≥ 0.84	0.552 – 0.836	56.7%	56.7%	56.7%	56.7%	56.7%	0.694	**0.01 ***
HOTTIP in tissue	≥ 0.90	0.953 – 1.0	100.0%	93.3%	93.8%	100.0%	96.7%	0.982	**<0.001***
HOTTIP in PBMCs Grade III vs Grade II	≥ 0.92	0.454 – 0.867	70.6%	61.5%	70.6%	61.5%	66.7%	0.661	0.137
HOTTIP in tissue Grade III vs Grade II	≥ 2.1	0.535 – 0.908	64.7%	76.9%	78.6%	62.5%	70.0%	0.772	**0.04**

PPV: positive predictive value; **NPV**: negative predictive value; **AUC**: area under the curve; **CI**: confidence interval. *Significance is defined by ***p-value*** < 0.05.

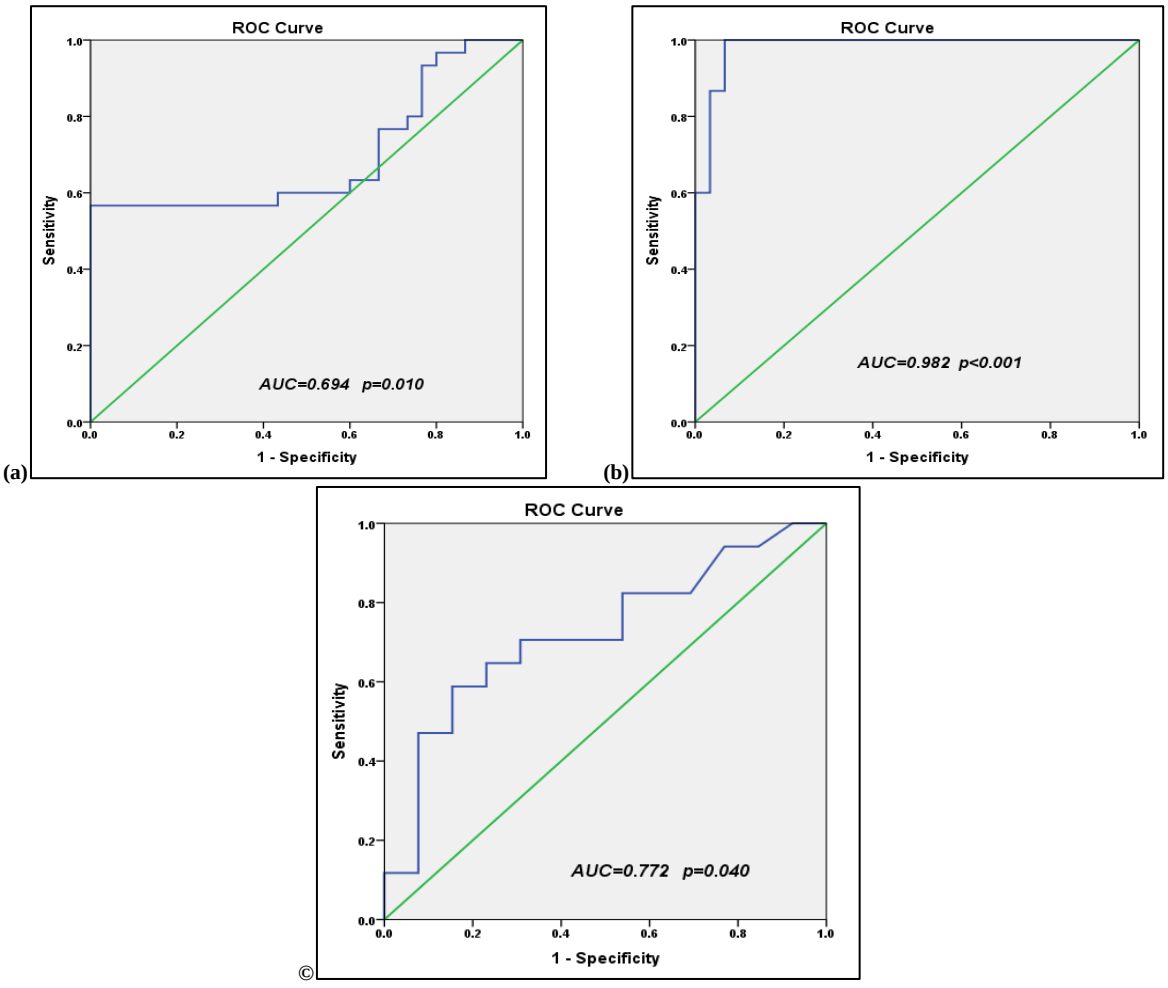

Fig. 1: ROC curve for (a) CRC detection in studied participants as regards expression levels of *HOTTIP* in PBMCs. (b) CRC detection in studied participants as regards expression levels of *HOTTIP* in tissues. (c) Prediction of CRC grades in studied cases regarding expression levels of *HOTTIP* in tissues. *HOTTIP* in PBMCs, tissue (blue), and reference line (green).

The genotype distribution of lncRNA *HOTTIP* SNP rs1859168 among the studied participants

The CC genotype frequency was significantly higher among the studied CRC cases than matched controls (***p*** < 0.001). Also, the dominant allele "C" was significantly higher among the studied CRC cases compared to matched controls (***p*** < 0.001).

As regards the risk association of *HOTTIP* SNP rs1859168 with CRC, the homozygous CC was associated with three times more increased risk of developing CRC than the heterozygous AC but with no significance (***p*** = 0.065). Furthermore, the C allele was associated with five times significantly increased risk of CRC (***p*** < 0.001**)** when compared with controls, as shown in Table (5).

Table 5: The genotype distribution of *HOTTIP* SNP rs1859168 in CRC patients and controls.

Genotype distribution	Cases (n=30)		Controls (n=30)		P value	OR	95% CI OR	*p*-value
Genotype					**<0.001**			
AA	0	(0.0%)	9	(30 %)		-----		
AC	12	(40 %)	14	(46.7%)		Ref		
CC	18	(60 %)	7	(23.3%)		3	0.936 – 9.616	0.065
Dominant Allele					**<0.001**			
A	12	(20 %)	32	(53.3%)		Ref		
C	48	(80 %)	28	(46.7%)		4.571	2.032 – 10.284	**<0.001**

Data is presented as numbers (percentages). Significance is defined by ***p-value*** < 0.05, OR: odds ratio, and CI: confidence interval. Significant at $p < 0.05$.

The genotype distribution of selected lncRNA *HOTTIP* SNP rs1859168 among the studied CRC cases according to patients' characteristics, clinical presentation, and tumor characteristics

No significant difference was observed between the AC and the CC genotypes lncRNA *HOTTIP* SNP according to CRC patients' age, sex, positive family history of CRC patients' clinical presentation, tumor size, and tumor grade among the studied CRC cases (***p*** > 0.05 for all).

Relationship between the genotypes distribution of *HOTTIP* SNP rs1859168 and expression levels of *HOTTIP* in CRC cases is shown in Table (6).

Table 6: Relationship between genotypes distribution of selected lncRNA *HOTTIP* SNP rs1859168 and expression levels of *HOTTIP* in CRC cases

HOTTIP	AC (n=12)	CC (n=18)	*p* value[1]
In PBMCs	1.14 (0.04 – 2.05)	1.02 (0.01–4.73)	0.723
In tissue	3.42 (1.06–27.89)	1.63 (0.91–4.92)	0.065
***p* value[2]**	**0.005**	**0.028**	

Data is presented as median (range). Significance is defined by ***p-value*** < 0.05.

p value[1]: comparing the expression of the studied genes in different lncRNA *HOTTIP* SNP.

p value[2]: comparing the expression of the studied genes in blood versus tissue samples.

DISCUSSION

Understanding the roles of lncRNAs implicated in colon tumorigenesis may eventually accelerate their clinical application in the diagnosis, treatment, and prognostic evaluation of various types of cancer [16].

The present study investigated the possible diagnostic and prognostic role of lncRNA *HOTTIP* in CRC. This was done by comparing the expression levels of *HOTTIP* in PBMCs and tissue in CRC cases and healthy controls. Another target of our study was to realize the association between rs1859168 *HOTTIP* SNP and CRC susceptibility.

The lncRNA expression in PBMC and its clinical relevance in CRC remains largely uncharacterized. To the best of our knowledge, this is the first study to estimate the expression level of *HOTTIP* lncRNA in PBMCs vs tissue in CRC cases compared to healthy matched controls. Recent studies have tried to discover novel PBMC lncRNA signatures as diagnostic biomarkers for CRC.PBMCs from CRC cases showed significantly variable expression profiles of lncRNAs, and it was concluded that detecting these variably expressed lncRNAs may offer useful data for basic and clinical research [7].

In the current study, *HOTTIP* expression levels in PBMCs showed high significant upregulation among the CRC cases compared to controls. This was consistent with *Ali Akbar-Esfahani et al.* [17], who reported upregulation of *HOTTIP* in plasma samples of CRC patients compared to the normal group ($p < 0.001$), and *Ali et al.* [10], who reported similar upregulation of serum *HOTTIP* in CRC group compared

to those with adenomatous polyposis or controls ($p < 0.0001$).

Upregulation of *HOTTIP* was reported by several previous studies as a cause of influencing cancer susceptibility via regulating cancer-promoting genes [18, 19]. However, *Oehme et al.* [20], reported that lncRNA *HOTTIP* expression was significantly decreased in CRC patients' serum-derived exosomes compared to healthy controls.

In our study, *HOTTIP* tissue expression levels showed high statistically significant upregulation among CRC cases compared to controls ($p < 0.001$). These results were consistent with *those of Liu et al.* [21] and *Liu et al.* [22].

Various studies revealed the role of *HOTTIP* in almost all types of human cancers. For example, in renal cell carcinoma, the binding of *HOTTIP* with lysine-specific demethylase 1 (LSD1) and EZH2 suppresses the expression of large tumor suppressor kinase 2 (LATS2). Down-regulation of this tumor suppressor gene enhances the proliferation of cancer cells [23].

As regards the association of *HOTTIP* expression and different patients' characteristics, no significant difference according to age, gender, and family history of the studied CRC cases was detected and this was consistent with *Ren et al.* [24], and *Ali Akbar-Esfahani et al.* [17].

According to *Mueller et al.* [25], younger patients are more frequently presented with more advanced tumors compared to older ones. Furthermore, *Liu et al.* [21], stated that *HOTTIP* tissue expression was positively correlated with late-stage tumors. These findings were consistent with our study as tissue expression level was significantly higher than that of PBMCs in younger patients than older ones.

On the other hand, *HOTTIP* tissue expression levels were significantly higher among patients with tumor size ≥ 3 cm compared to those with tumor size < 3 cm ($p = 0.012$), and among patients with tumor grade III compared to those with tumor grade II ($p = 0.039$). This was consistent with *Liu et al.* [21], who stated that *HOTTIP* expression level was positively correlated with tumor size and largely increased in late-stage tumor tissues and *Ren et al.* [26] , whose study showed a significant positive association of *HOTTIP* tissue expression levels with clinical stage. The role of *HOTTIP* in developing more aggressive and advanced tumors is probably due to the promotion of cell proliferation, migration, and inhibition of cell apoptosis in various types of cancers. An in vitro assay suggested the role of *HOTTIP* inhibition in G0/G1 arrest and enhancement of colon cancer growth via silencing the expression of *p21* [27].

As regards the diagnostic ability of *HOTTIP* in PBMCs, ROC curve analysis showed that the expression level of *HOTTIP* in PBMCs was able to differentiate CRC cases from controls ($p = 0.01$). This was consistent with *Ali et al.* [10], who reported a diagnostic ability of serum *HOTTIP* to differentiate CRC cases from healthy controls (*p-value* < 0.0001). Also, *Ali Akbar-Esfahani et al.* [17], reported consistent results for plasma *HOTTIP*. However, this was inconsistent with *Oehme et al.* [20], who stated a significantly decreased *HOTTIP* expression in serum-derived exosomes in CRC cases compared to controls.

Furthermore, the tissue expression level of *HOTTIP* was more accurate in differentiating CRC cases from healthy ones ($p < 0.001$). Consistent with our result, *Gharib et al.* [28], reported similar results for tissue *HOTTIP* expression ($p < 0.0001$).

HOTTIP in tissue was able to predict grade III CRC at a cut-off value of ≥ 2.1 ($p = 0.04$). Consistent with our result, *Ali et al.* [10], reported a prognostic ability of serum *HOTTIP* to differentiate CRC patients with grade III from those with lower grades ($p < 0.0001$).

From the above findings, PBMCs from CRC patients show significantly variable lncRNA expression profiles. Detecting these differentially expressed lncRNAs may provide novel diagnostic and prognostic markers for different cancers and offer useful information for basic and clinical research [7].

The SNPs are the most common genetic variants of concern in lncRNA genes. It is suggested that lncRNA expression and role are affected by them [29]. Many studies have suggested a critical role of lncRNA polymorphism in splicing and modulating the stability of mRNA, resulting in the modification of their related partners. Several studies have assessed the associations between cancer susceptibility and about 20 lncRNA SNPs, but the results are widely variable [30].

In the present study, regarding *HOTTIP* SNP rs1859168 genotype distribution, the wild genotype AA was not present in CRC cases but represented 30 % of controls. Furthermore, by comparing the frequency of the homozygous CC vs the heterozygous AC genotype, the CC genotype was significantly higher among the studied CRC cases compared to matched controls (60% vs. 23.3%, $p < 0.001$), also the dominant allele "C" was significantly higher among the studied CRC cases compared to matched controls (80 % vs. 46.7%, respectively, $p < 0.001$).

On the other hand, analysis of the risk association of *HOTTIP* SNP rs1859168 with CRC showed that the homozygous CC was associated with three times increased risk of developing CRC than the heterozygous AC genotype. Although CC risk association with CRC yielded a non-significant *p*-value (0.06), it still was close enough to 0.05 to be worth commenting on.

In addition, the C allele was associated with a five times significantly increased risk of CRC than that of the A allele ($p < 0.001$) compared to controls. Consistent with our results, *Ali et al.* [10], reported similar findings with the absence of the wild AA genotype in CRC cases but occurred in 20% of healthy controls.

Also, they found that by comparing the abundance of the two genotypes AC with CC, the CC homozygous genotype was significantly higher in CRC cases than in matched controls (53.6% versus 26.7%, respectively, $p = 0.001$). Also, the mutant C allele was significantly higher in CRC cases than in controls ($P < 0.0001$), with about three times significantly increased risk of CRC in the CC genotype and the dominant C allele ($p < 0.001$) when compared with controls.

The genotype distribution of *HOTTIP* SNP rs1859168 among the studied CRC cases according to patients' characteristics, clinical presentation, and tumor characteristics revealed no significant difference between the AC and the CC genotypes. Inconsistent with our results, *Ali et al* [10], reported that the CC genotype was associated with a higher risk in male patients and all age groups ($p < 0.0001$). Also, they reported a significantly higher percentage of CRC patients with the AC genotype experienced abdominal pain ($p = 0.002$) and weight loss ($p = 0.009$) but with less constipation ($p < 0.0001$).

In the present study, in both AC and CC genotypes, the tissue expression level of *HOTTIP* was significantly higher than that of PBMCs (p = 0.005 and 0.028, respectively), *Ali et al.* [10], also reported decreased serum *HOTTIP* with the CC genotype than that with the AC genotype in CRC patients but with high significance ($p < 0.0001$). Briefly, different rs1859168 genotypes were associated with different *HOTTIP* expression levels in CRC cases.

CONCLUSION

LncRNA *HOTTIP* expression levels in both PBMCs and tissue showed significant upregulation among the CRC cases compared to healthy matched controls. Also, tissue expression levels were able to differentiate CRC cases from healthy controls and predict higher tumor grade. Furthermore, assessment of the risk association of *HOTTIP* SNP rs1859168 and CRC susceptibility revealed that the homozygous CC genotype was significantly higher than the heterozygous AC genotype among CRC cases compared to matched controls with higher dominant allele "C" among the studied CRC cases compared to matched controls. The tissue expression level of *HOTTIP* was significantly higher with the AC than the CC genotype. So, the AC genotype appears to be associated with more tissue expression levels of *HOTTIP*, which may link it to the severity of the disease.

Statements and Declarations

Competing Interests

The authors declare no competing interests.

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Ethical Considerations

Ethics approval

The study protocol was accepted by the local Ethical Committee of the Faculty of Medicine, Assiut University (**IRB No.: 17200584**), registered on Clinical Trials.gov **(ID: NCT04729855)**, and was conducted following the principles embodied in the Declaration of Helsinki.

Consent to participate

All patients provided written informed consent.

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Contributions

Esraa Hassan: Practical part, formal analysis, investigation, writing original draft, visualization, and funding acquisition.

Dr. Aliaa Mahmoud: Editing, supervision, project administration, and funding acquisition.

Dr. Mohamed Ali Mohamed Elfeky and Dr. Salwa Sayed Ahmed Hassan Seif Eldin: Supervision, conceptualization, project administration, and funding acquisition.

Dr. Mohamed Abouelmagd Salem Mahmoud: Obtaining specimens, supervision, project administration, and funding acquisition.

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