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A correlation between BCL-2 modifying factor, p53 and livin gene expressions in cancer colon patients

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ABSTRACT

Accumulating evidence has revealed that livin gene and BCL-2 modifying factor (BMF) gene are closely associated with the initiation and progression of colon carcinoma by activating or suppressing multiple malignant processes. Those genes that can detect colon - cancer are a promising approach for cancer screening and diagnosis. This study aimed to evaluate correlation between livin, BMF and p53 genes expression in colon cancer tissues of patients included in the study, and their relationship with clinicopathological features and survival outcome in those patients. In this study, 50 pathologically diagnosed early cancer colon patients included and their tissue biopsy with 50 matched adjacent normal tissue, and 50 adenoma tissue specimens were analyzed for livin gene and BMF gene expressions using real time PCR. The relationship of those genes expressions with clinicopathological features, tumor markers, Time to Progression and overall survival for those patients were correlated in cancer colon group. In this study, there was a significant a reciprocal relationship between over expression of livin gene and down regulation of BMF and p53 genes in colon cancer cells. Livin mRNA was significantly higher, while BMF and p53 mRNA were significantly lower in colorectal cancer tissue compared to benign and normal colon tissue specimens ($P < 0.001$), however, this finding was absent between colon adenomas and normal mucosa. There was a significant association between up regulation of livin and down regulation of BMF and p53 expressions with more aggressive tumor (advanced TNM stage), rapid progression with metastasis and decreased overall survival in cancer colon patients, hence these genes can serve as significant prognostic markers of poor outcome in colon cancer patients. This work highlights the role of livin, BMF and p53 genes in colorectal tumorigenesis and the applicability of using those genes as a diagnostic and prognostic markers in patients with colon carcinoma and as a good target for cancer colon treatment in the future.

1. Introduction

Human cancer colon is a common malignancy worldwide. It is the second most common diagnosed cancer in females and the third in males, with almost 835,000 deaths in 2015 [1]. In Egypt, There is high incidence of cancer colon among the young Egyptian population under 40 years. So, awareness must be present about the potential for cancer colon [2].

A subset of hyperplastic polyps, especially micro-vesicular hyperplastic polyps, progress to serrated neoplasms (SSP or TSA) and a fraction of these serrated neoplasms progress to Cancer colon. There are a large number of factors that play a direct role in driving the polyp → cancer colon sequence, including gene mutations, epigenetic alterations, and local inflammatory changes [3].

Alterations in key pathways that directly or indirectly regulate apoptosis may affect tumor cell survival. So, apoptosis regulators play a role in colon cancer treatment and prevention, by selective induction of apoptosis in cancer cells [4].

Livin is novel member of the inhibitor of apoptosis protein family, that is considered a new anti-apoptotic oncogene due to its unique structure, ability to protect cells against a series of specific allergen-induced stimulation and inhibition of apoptosis [5]. It is a new biomarker that is able to accurately and reliably detect cancer colon at its earliest stages representing a promising alternative approach for its

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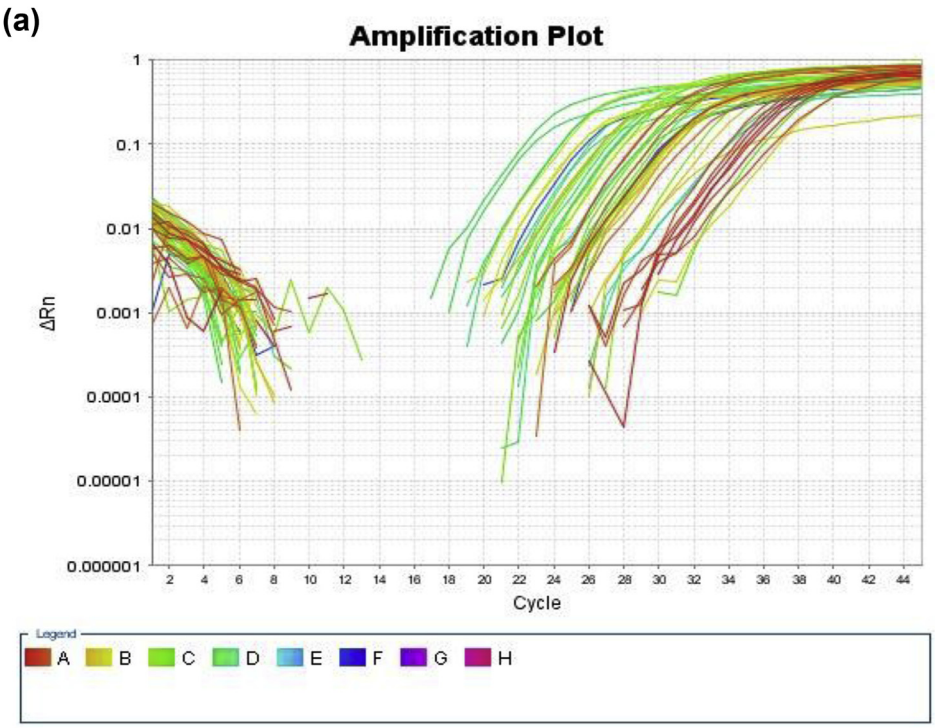

Fig. 1a. the amplification plot of Livin gene expression.

screening [6].

The protein encoded by proapoptotic BCL-2 modifying factor (BMF) gene belongs to the BCL2 (B-cell lymphoma 2) family. This BCL-2 family proteins regulate activation of the intrinsic apoptotic pathway. BCL-2 family includes anti-apoptotic members (Bcl-2L, Bcl-extra-large and Mcl1) and other pro-apoptotic. The pro-apoptotic BCL-2 family members can be further divided into the multi-BH-domain (containing BH1, BH2 and BH3 domains) and the BH3-only proteins (only region of homology to BCL-2 is BH3). Among BH3-only proteins extensively studied up till now, PUMA, NOXA and BIM (Bcl-2 interacting mediator of cell death) whereas little is known about their closest relative, BMF [7].

In this study we attempt to evaluate livin and BMF genes expression in colon cancer tissues for patients included in the study, and investigate the correlation between them, and with clinicopathological features and survival outcome in those patients.

2. Patients and methods

This study was carried out on 50 pathologically proven cancer colon patients between June 2017 and August 2019; diagnosed at Surgery Departments while treated & followed up at Clinical Oncology Department, Menoufia University. In addition 50 patients with colorectal adenoma were included in the study. An informed written consent was obtained from all participants. The protocol was approved by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University.

All participants were subjected to full history taking, clinical examination, complete laboratory investigations included: liver and kidney function tests, determination of serum carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were determined.

Adequate imaging of the chest and abdomen should be obtained for staging purposes, ideally preoperatively. Abdominal/pelvic computed tomography (CT), and abdominal/pelvic magnetic resonance imaging (MRI) scans are appropriate for imaging the abdomen and liver, for the purpose of staging. Imaging studies may also include a chest radiograph or chest CT scan, an abdominal barium study to better delineate the primary lesion preoperatively, and bone scan. Positron emission tomography (PET) scanning is emerging as a very useful modality for staging and assessment of colorectal cancers. The newest addition, a fusion PET-CT scan, allows for detection of metastatic deposits and has the added tissue-based resolution of CT.

Cancer colon patients were staged based on clinical TNM classification and early & advanced stage were included. The pathological grading was based on the world health organization (WHO) criteria, and performance status was estimated based on ECOG classification [8]. Demographic data of the patients, Body mass index (BMI), tumor characteristics (site, side, regional node status, histological characteristics, and grade) were reported.

All patients with stage III and high-risk stage II colon cancer received adjuvant FOLFOX chemotherapy regimen which is given over 2 days. The regimen is repeated for 12 cycles, every 2 weeks. All cases of metastatic disease, FOLFOX regimen was the first line regimen and evaluated by CTs and tumor markers (CEA & CA 19.9) every 2–3 months. Patients finished chemotherapy were followed by CTs and tumor markers every 3 months and colonoscopy on annual bases. Response assessment was done based on RECIST criteria. Follow-up was completed for all patients until August 2019.

The study used 150 tissue specimens collected from 100 patients, underwent surgery or colonoscopic biopsy; for Livin and BMF gene expressions estimation. They were categorized according to histopathological diagnosis as follows: Group I: 50 tissue specimens for patients with colon adenocarcinoma, Group II: 50 tissue specimens for patients with colon adenoma, and Group III: 50 tissue specimens of accompanying normal colon mucosa from same patients in group I for comparison.

Tissue samples from the cancerous tissue, adjacent healthy marginal and colon polyp were taken. The tissue was homogenized in trizol reagent using a Qiagen Tissuelyzer, with a stainless steel bead added to the sample to beat the tissue into solution. Total RNA was extracted from tissue by Direct-zol™ RNA MiniPrep kit, Zymo Research.

Two-step RT–PCR was done, for reverse transcription step, samples were prepared in a final volume of 20 μl containing RT buffer, Multi scribe reverse transcriptase (PE Applied Biosystems), and 20 ng total

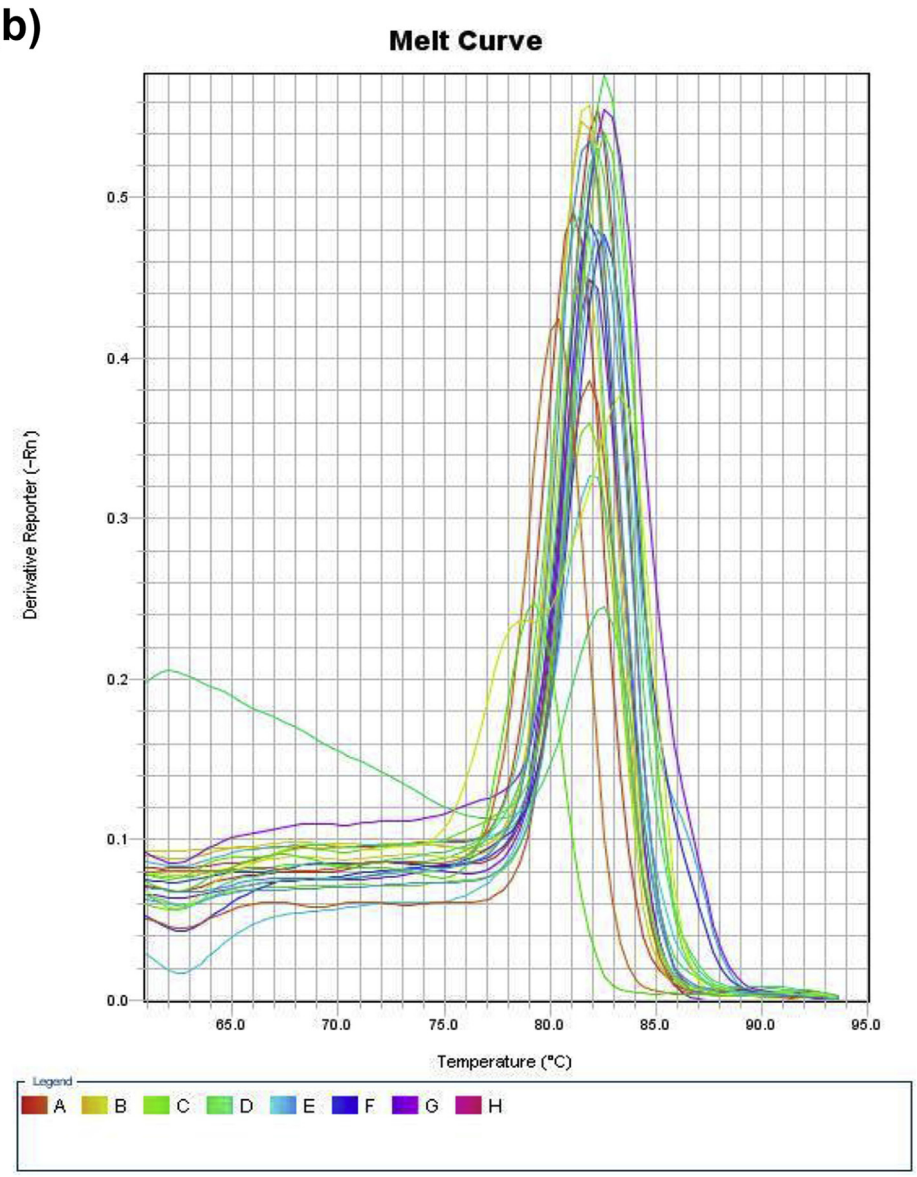

Fig. 1b. the melting curve of Livin gene expression.

RNA. Then the samples were incubated at 25 ˚C for 10 min and at 48 ˚C for 30 min. Heating to 95 ˚C for 5 min inactivated the reverse transcriptase on 2720 thermal cycler Singapore.

For cDNA amplification the sequence of primers of livin Forward primer TGAGGAGTTGCGTCTGG Reverse primer GCACGGCACAAAGA CGAT, BMF Forward primer CCCTTGGGGAGCAGCCCCCTG Reverse primer GCCGATGGAACTGGTCTGCAA, primers of P53, Forward primer AGTCTAGAGCCACCGTCCA Reverse primer TCTGACGCACACCTATTG CAAGC and primers of GAPDH as endogenous control, forward primer GTCTCCTCTGACTTCAACAGCG, reverse primer ACCACCCTGTTGCTG TAGCCAA were used with SensiFAST™ SYBR® Lo-ROX Kit, nuclease-free water, cDNA in a total reaction volume 25 μl and using GAPDH as endogenous control using the 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA).

Using the comparative CT method, endogenous controls were used to normalize the target genes expression levels by correcting differences in the amount of cDNA loaded into PCR reactions. Fig. 1a and b shows the amplification plot and melting curve of livin gene expression. While Fig. 2 a and 2b show the amplification plot and melting curve of BMF gene expression.

Regarding statistical analysis, Qualitative data were described using number and percent and was compared using Chi-square test or Fisher Exact test, while normally quantitative data were expressed in mean ± SD and was compared using F test (ANOVA) abnormally distributed data was expressed in median (Min. - Max.) And was compared using Kruskal Wallis test. Normally quantitative data were expressed in mean ± SD and was compared using F test (ANOVA) abnormally distributed data was expressed in median (Min. – Max.) and was compared using Mann Whitney test for two groups and Kruskal Wallis test for more than two groups. P-value ≤ 0.05 was considered statistically significant.

3. Results

Fifty pathologically proven cancer colon patients diagnosed and treated between June 2017 and August 2019; were included in this study. The study used 150 tissue specimens collected from 100 patients, underwent surgery or colonoscopic biopsy; for Livin and BMF gene expressions estimation. They were categorized according to histopathological diagnosis as follows: Group I: 50 tissue specimens for patients with colon adenocarcinoma, Group II: 50 tissue specimens for patients with colon adenoma, and Group III: 50 tissue specimens of

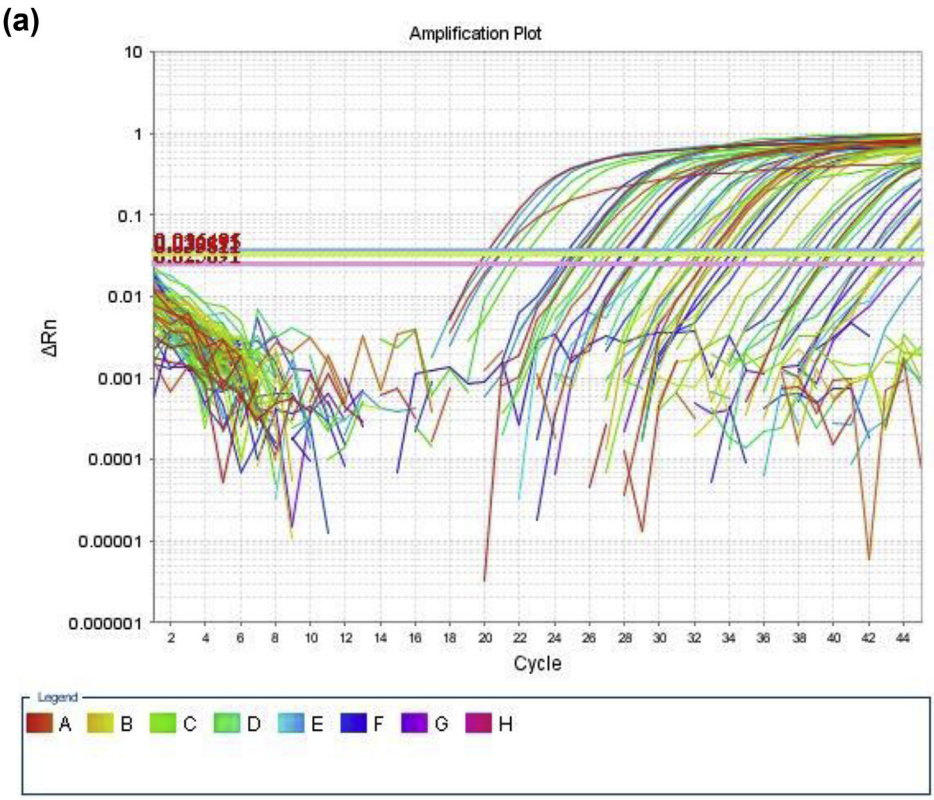

Fig. 2a. the amplification plot of BMF gene expression.

accompanying normal colon mucosa from same patients in group I for comparison.

The demographic features of the patients are summarized in table (1), where no significant difference was detected between patients with colorectal cancer (group I) and patients with benign colorectal polyp (group II), indicating they were well matched (Table 1).

The expression of livin gene was significantly higher in colorectal cancer tissue compared to benign and paired normal colorectal specimens ($P < 0.001$), however, the colorectal cancer group showed statistically significant lower BMF and p53 genes expression compared to the others two groups ($P < 0.001$).

Comparing the levels of livin, BMF and p53 mRNA among colon adenomas and normal mucosa did not show a significant difference (Table 2). A remarkable reciprocal relation between up regulation of livin and down regulation of BMF and p53 genes expressions in colon cancer tissue was observed.

Spearman's correlation analysis was conducted to display the impact of BMF gene expression on livin and p53 genes expression in colorectal specimens (cancer, benign and paired normal mucosa), and on cancer patients prognosis; in terms of overall survival (OS) and time to disease progression (TTP), (Table 3 and Fig. 3a,b,3c). There was a significant negative correlation between BMF, p53 genes and livin gene expressions, as lower BMF and p53 genes expression in cancer tissue was significantly associated with higher livin gene expression. But a positive correlation was detected with OS and TTP, only in cancer group.

Similarly, the impact of livin gene expression on the same parameters was summarized in Table (4) and fig (4a, 4b). There were significant inverse correlations between livin gene expression, BMF gene expression, TTP and OS in colorectal cancer patients; higher livin gene expression in cancer tissue was significantly associated with decreased BMF gene expression, ($P < 0.001$).

Spearman's correlation analysis, confirmed the reciprocal relationship between livin and BMF gene expressions in colon cancer tissue. Remarkably, only increased livin and decreased BMF genes expressions were significantly associated with decreased overall CRC patients survival ($r = 0.550$, $P < 0.001$ with BMF gene, $r = -0.505$, $P < 0.001$ with livin gene) and rapid disease progression (shorter TTP).

The expressions of BMF, p53 and livin genes in colon cancer tissue was significantly related to clinical tumor stage (TNM) and presence of metastasis ($P < 0.001$), Most of colon cancer patients were stage IV disease (46%) followed by stage III (40%) then stage II (14%). Notably, patients with higher BMF gene, p53 and lower livin gene tissue expressions were significantly presented in stage II cancer (7/50) and without metastasis (27/50; 47%), However, patients with higher livin gene and lower BMF, p53 gene expressions were totally presented in stage IV disease with tumor metastasis (23/50; 46%), (Table 5).

4. Discussion

Cancer colon is one of the leading causes of cancer associated death globally. This may be due to little understanding of the colon cancer molecular controls conferred by apoptotic and anti-apoptotic genes [9].

Apoptosis is considered the main protection against tumor, as the ability of tumor cells to evade apoptosis is the mainstay during ontogenesis. A complex network of pro-apoptotic and anti-apoptotic genes can govern the regulation of apoptosis, and their balance can determine the cellular fate and ensure human health [7].

Livin, is a novel member of the inhibitors of apoptosis protein family (IAP), suppresses apoptosis through inhibition of caspases, protein degradation, and also can affect mitosis. Livin has been implicated in the development and progression of CRC [10].

Proapoptotic BCL-2 modifying factor (BMF) gene is a member of BH3-only proteins, but, its role in apoptosis signaling, and oncogenesis, still unclear. In many cell lines, BMF serves a role in initiating cell apoptosis by binding to B-cell lymphoma (Bcl)2, Bcl-xL and Bcl-w proteins, and showed pro-apoptotic potential in many cell lines and cell death assays [7,11].

In this work, livin gene and BMF gene mRNA expression levels in

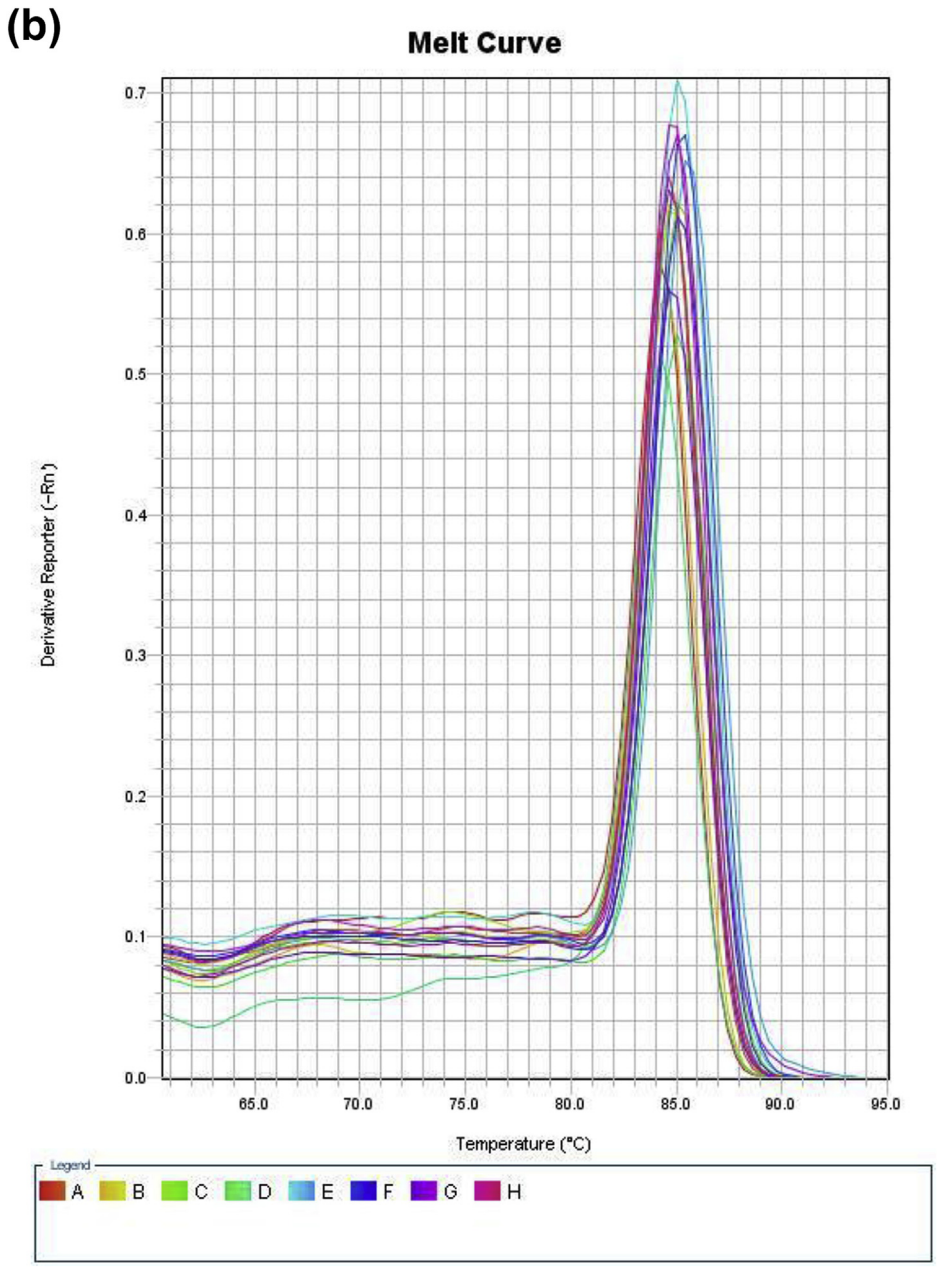

Fig. 2b. the melting curve of BMF gene expression.

Table 1
Demographic characteristics of the studied patients.

Variables	Cancer colon cases (n = 50)	Colon adenoma cases (n = 50)	Test of Sig.	P
Sex n (%)				
Male	**22(44%)**	**27 (54%)**	χ^2 =2.083	**0.353**
Female	**28 (56%)**	**23 (46%)**		
Age (years)				
Mean ± SD.	**49.90 ± 11.10**	**50.06 ± 10.96**	**F= 0.317**	**0.729**
BMI(kg/m^2)				
Mean ± SD.	**26.04 ± 5.1**	**25.70 ± 3.65**	**F=0.610**	**0.544**

There was a non significant difference of sex, age and BMI in different studied groups.

different colorectal tissue specimens (50 cancer, 50 benign and 50 paired normal mucosa samples) were assessed.

The hallmark finding in this study, is the reciprocal correlation between the up regulation of livin gene and down regulation of BMF gene expressions in colon cancer tissues (r = -0.550, P < 0.001). Livin mRNA was significantly higher, while BMF mRNA was significantly lower in colorectal cancer group compared to benign and normal colorectal tissue specimens (P < 0.001), however, such relation was absent between colon adenomas and normal mucosa.

This may indicate that, upregulation of livin gene in cancer tissues could suppress cell apoptosis in the phase of adenoma to adenocarcinoma sequence and possibly livin status had an influence on colorectal carcinogenesis by apoptosis inhibition [10]. Supportive evidence was added by Xi et al., as they suggested that livin had an oncogenic role in CRC and overexpression of this gene is a growth factor for development and proliferation of colon cancer cells [12]. Also, Myung et al. confirmed that livin expression in colon cancer tissues was up-regulated

Table 2
Livin, BMF and p53 genes expression levels among the three groups.

Gene	Cancer Colon specimens (n = 50)	Colon adenoma specimens (n = 50)	Normal mucosa specimens (n = 50)	P	Post hoc test (Dunn's)		
					Cancer vs. benign	Cancer vs. normal	Benign vs. normal
BMF gene expression Median (min.max.)	1.35 (0.45–5.8)	4.24 (3.09–29.92)	17.17 (7.12–29.9)	< 0.001*	< 0.001*	< 0.001*	0.060
Livin gene expression Median (min.–max.)	17.16 (4.23–26.5)	1.15 (0.06–16.04)	1.14 (0.06–15.84)	< 0.001*	< 0.001*	< 0.001*	0.090
P53 gene expression Median (min.–max.)	1.56 (0.93–6.1)	10.15 (8.6–12.4)	11.1 (8.3–15.8)	< 0.001*	< 0.001*	< 0.001*	0.43

P values for Kruskal Wallis test, sig. bet. groups was done using Post Hoc Test (Dunn's test).
*Statistically significant at $p \leq 0.05$.
There was a significant difference between the three groups regarding gene expression of livin, BMF and p53.

Table 3
BMF gene expression and clinical data in each group.

	BMF gene expression					
	Cancer colon cases (n = 50)		Colon adenoma cases (n = 50)		Normal mucosa specimens (n = 50)	
	r_s	P	r_s	P	r_s	p
BMI (kg/m^2)	−0.170	0.238	0.079	0.586	–	–
Age (years)	−0.008	0.953	0.040	0.784	–	–
Performance state	−0.082	0.570	–	–	–	–
Time to progress (TTP)	0.633*	< 0.001*	–	–	–	–
Overall survival	0.550*	< 0.001*	–	–	–	–
Livin gene expression	−0.565*	< 0.001*	−0.112	0.438	−0.009	0.952
P53 gene expression	0.436	0.035*	0.212	0.180	0.206	0.160

r_s: Spearman coefficient *Statistically significant at $p \leq 0.05$.
In cancer colon cases: There was a significant positive correlation between BMF gene expression and each of TTP, overall survival and p53 gene expression. While there was a significant negative correlation between BMF and livin gene expression.

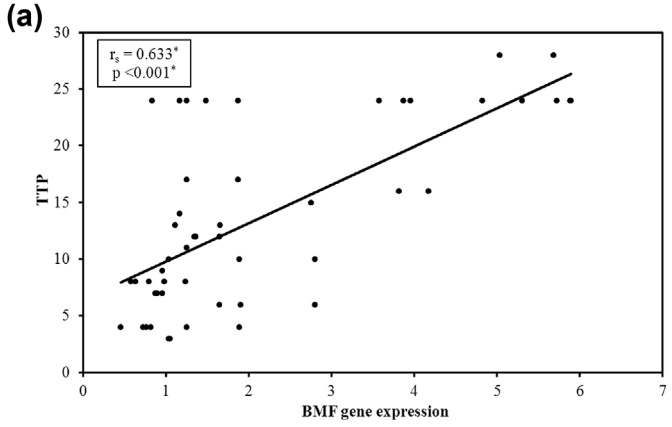

Fig. 3a. A positive correlation between BMF gene expressions in cancer colon patients and Time to progress (TTP).

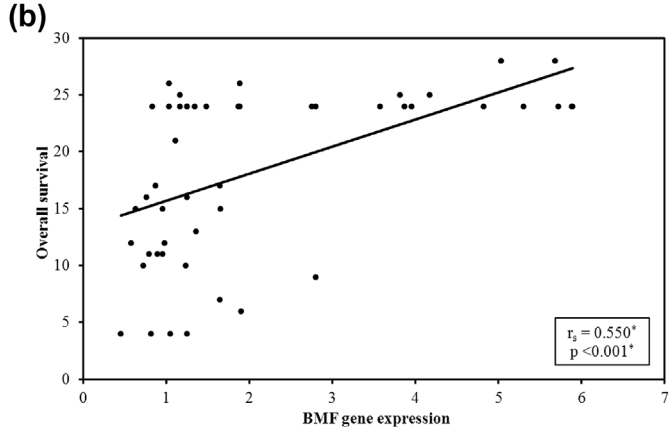

Fig. 3b. a positive correlation between BMF gene expression in cancer colon patients and Overall survival.

compared to that in paired normal mucosa at the mRNA and protein levels [13]. Notably, livin gene not only had a role in colorectal tumorigenesis, but also it blocks death receptors and chemotherapy-induced apoptosis during treatment [10].

The BCL-2 family proteins members comprise the sentinel network of apoptotic response. Knockdown of BMF gene and other members of the pro-apoptotic BH3-only proteins, facilitates tumor progression,and permits the survival of malignant clones [7].

Another pro-apoptotic factor is p53 protein that transactivates downstream targets that mediate apoptosis or cell cycle arrest. p53 is a transcriptional regulator that reduced AKT phosphorylation, induced BCL2-modifying factor (BMF) expression, sensitized BIM dissociation from BCL-XL and induced mitochondria-dependent apoptosis in cancer cells [14].

In this study, lower expression of BMF gene in colon cancer tissues compared to adenoma and normal tissues was detected. In accordance with this result, an array-based screen study on human colon cancer cells implicated BMF gene as a critical player and key regulator of apoptosis and tumor inhibition [15]. In a recent review, loss of BMF gene has been shown to accelerate tumor progressions in several tumors [11]. BMF gene serves a role of tumor suppressor gene, in a variety of tumor types including, lymphoma, colorectal cancer, and breast cancer,consequently, decreased its expression enhances tumorigenesis

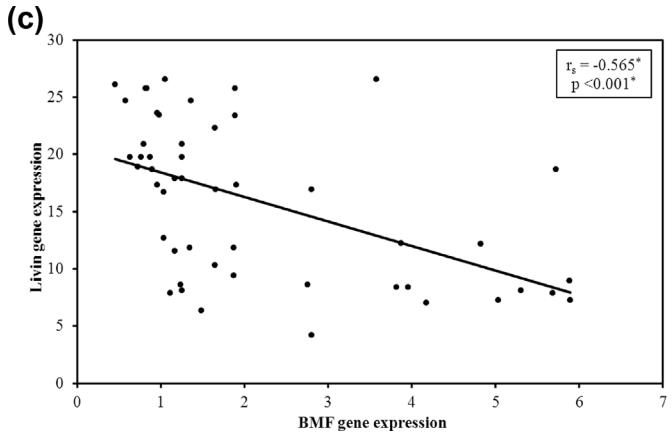

Fig. 3c. A negative correlation between BMF gene expression and Livin gene expression in cancer colon patients specimens.

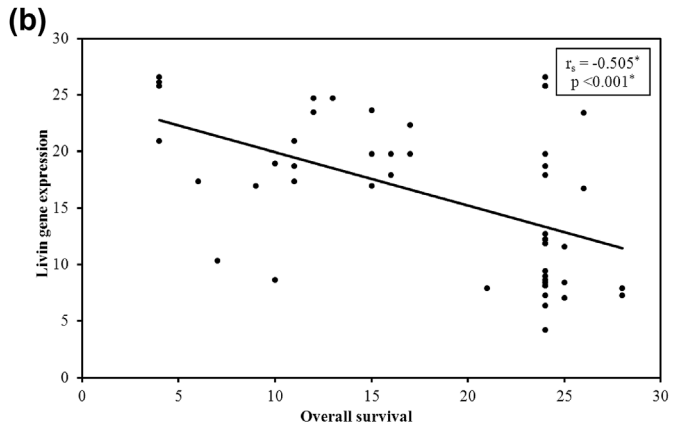

Fig. 4b. A negative correlation between livin gene expression in cancer colon patients and Overall survival.

Table 4
Livin gene expression and Clinical data in each group.

	Livin gene expression					
	Group I		Group II		Group III	
	r_s	P	r_s	p	r_s	p
BMI (kg/m^2)	0.081	0.577	−0.162	0.262		
Age (years)	0.077	0.594	−0.188	0.191		
Performance status	0.095	0.512	–	–		
Time to progress (TTP)	−0.486*	< 0.001*	–	–	–	–
Overall survival	−0.505*	< 0.001*	–	–	–	–
BMF gene expression	−0.565	< 0.001*	−0.112	0.438	−0.009	0.952
P53 gene expression	−0.693	< 0.001*	0.119-	0.403	−0.143	0.389

r_s: Spearman coefficient.
In cancer colon cases: There was a significant negative correlation between livin gene expression and each of time to progress (TTP), overall survival, BMF and p53 genes expression.

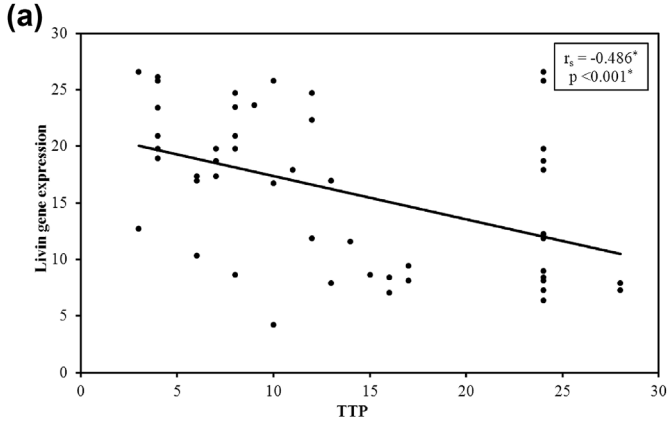

Fig. 4a. A negative correlation between livin gene expression in cancer colon patients and Time to progress (TTP).

[16].

Additionally, in a recent clinical trial to investigate the role of histone deacetylase (HDAC) inhibitor as an anticancer agent that regulate BMF gene activity and trigger BMF-mediated apoptosis, this drug showed improved therapeutic approach for treating colon cancer and other conditions, also the same study proved that HDAC can bind with BMF gene in colon cancer cells, and establish a pro-survival scenario in colon cancer cells [17].

In this study, for the first time we identified a reciprocal relationship between overexpression of livin gene and downregulation of BMF gene in colon cancer cells. Further studies are warranted to understand the key regulators of the apoptotic mechanisms linked to this reciprocal regulation of BMF and livin gene in human colorectal cancer. A similar inverse relationship between BMF gene and survivin gene (another IAP family member) in human colorectal cancer has been described [15]. Our study speculate that, regulation of apoptotic mechanism in colon cancer tissues centered on reciprocal overexpression of anti-apoptotic livin and decreased expression of proapoptotic BMF gene.

In this study, we investigated livin and BMF gene expressions correlation with clinicopathological features in patients with cancer colon; more aggressive (stage IV), undifferentiated tumors, with metastasis were linked to increased livin gene and downregulation of BMF gene expressions, the reciprocal relation of both genes is connected to colon cancer aggressiveness.

In line with this study finding, regarding livin gene, Myung et al., stated that livin overexpression was significantly associated with advanced tumor stage, and lymph node metastasis in patients with cancer colon [13]. Also, Liu et al. reported a positive correlation between livin gene expression and metastasis in gastric cancer [18]. In this study, livin overexpression was presented with undifferentiated colon cancer, however this relation was insignificant, also, Xi et al. didn't find any correlation between livin expression and the degrees of colon tumor differentiation [12].

In several tumor types upregulation of livin was predictive sign of more aggressive tumor [19], and was associated with tumor progression as it suppresses apoptosis and enhances metastasis by increasing tumor cell motility [20], those two results were reported and in agreement with our finding.

However, livin could not serve as a predictive sign in all kinds of cancers. Takeuchi et al. have demonstrated that livin expression in metastatic melanomas could not influence disease outcome and had no significant prognostic utility [21]. Kemphensteffen et al., also reported that high livin expression levels was present in well-differentiated renal cell and in early stage carcinomas and it did not correlate with pathological or clinical parameters and were not predictive of patient outcome [22].

Despite a lot of researches that suggested the role of livin as a predictive marker of tumor progression and patients prognosis in the majority of cancers. The tumor suppressive potential of BMF is just emerging [11].

In agreement with our finding, concerning downregulation of BMF expression in more aggressive (stage IV) undifferentiated colon tumor associated with metastasis, a recent study on human colon cancer cells reported that, lower expression of BMF gene promoted colon cancer cell

Table 5
Relation between BMF gene and livin gene expressions and Clinicopathologic data in group I (cancer colon cases) (n = 50).

	No(%)	BMF gene expression	p	P53 gene expression	p	Livin gene expression	p
		Median (min. – max.)		Median (min. – max.)		Median (min. – max.)	
Clinical tumor stage (TNM)							
I	**0**		< 0.001*		0.044*		0.003*
II	**7(14%)**	3.57(1.25–5.89)		2.99 (1.45–6.1)		8.12 6.38–26.57	
III	**20(40%)**	2.33(1.11–5.88)		1.45(1.04–5.89)		11.89 7.05–22.34	
IV	**23(46%)**	0.95(0.45–2.80)		0.98 (0.93–3.95)		19.80 4.23–26.57	
Presence of metastasis							
No		2.75(1.11–5.89	< 0.001*	2.34(0.99–6.1)	0.001*	11.89 (6.38–26.57)	0.001*
Yes		0.95(0.45–2.80)		0.95 (0.93–1.45)		19.80 (4.23–26.57)	
CEA mg/dl							
Normal	**43(86%)**	1.64(0.63–5.89)	0.008*	2.34(0.99–6.1)	0.435	17.34 (4.23–26.57)	0.386
Elevated	**7(14%)**	1.03(0.45–1.16)		1.98 (0.93–5.45)		16.71 (7.89–26.57)	
CA19–9 U/ml							
Normal	**40(80%)**	1.42(0.45–5.89)	0.280	2.55(0.93. - 6.1)	0.530	16.98 (6.38–26.57)	0.585
Elevated	**10(20%)**	1.04 (0.57–5.03)		2.38 (10.9–5.73)		18.02 (4.23–26.57)	

U, p: U and p values for **Mann Whitney test** *: Statistically significant at $p \leq 0.05$.
There was a significant difference in BMF, p53 and livin genes expression level a regard the tumor stage and presence of metastasis.
#: Excluded from the comparison due to small number of case (n = 1).

proliferation with enhanced metastasis, protected against apoptosis, and was negatively correlated with the transcription factor Eomes, as knocking down Eomes was associated with apoptosis induction and upregulation of BMF [15]. In another work done on breast cancer, restraining of BMF activation is a major signal that underpins tumor dissemination and growth, however, overexpression of BMF was sufficient to inhibit metastasis and was activated by the transcription factor FOXO3 [23].

Also, in a study of ovarian cancer tissues a nearer results to ours was detected, as BMF mRNA expression was significantly downregulated in ovarian cancer compared with normal tissues, with enhanced proliferation and reduced the apoptosis of ovarian cancer cells, which promoted progression and metastasis in ovarian cancer [16]. Additionally, He et al., indicated that miR-221 promoted the proliferation and migration of hepatocellular carcinoma cells and suppressed the apoptosis by targeting the inhibition of BMF expression, and he suggested that downregulation of BMF in liver cancer may be the factor that enhanced progression and metastasis [24]. Remarkable loss of BMF was detected in lymphoma, lung and breast cancer and was linked to tumor progress and metastasis [11].

In this study, 25/50 (50%) of patients died during the follow-up period, those patients had significantly higher livin gene and lower BMF gene expressions compared to alive patients. Moreover, according to Spearman's correlation analysis, an inverse relationship was detected between livin gene expression and overall survival in patients with colon cancer, as increased livin expression was associated with decreased OS and shorter TTP, hence rapid disease progression and poor prognosis of CRC patients.

Controversies still present on the prognostic value of livin gene. Our result was in agreement with, Xi et al. and Myung et al., results, as over expression of Livin gene was associated with increased tumor recurrence and decreased OS in patients with CRC [12,13]. Livin over expression may help predicting the poor outcome in colon cancer patients and considered to be an independent prognostic factor [25].

Although the negative impact of livin gene on survival was the main finding in literatures, Choi et al., suggested that livin over expression was a favorable prognostic factor with longer survival in childhood acute lymphoblastic leukemia [26].

Similarly, in accordance to our results, decreased overall survival in colorectal cancer patients was associated with lower BMF expression compared with individuals exhibiting high BMF levels in a study done to investigate the divergent role for Eomes in colon cancer etiology [14]. Bcl-2 down regulation was an indicator of decreased survival time in cancer colon patients [27].

Overall survival rate was considered to indicate prognosis, therefore, in our study up regulation of livin gene and down regulation BMF gene expressions were significant prognostic markers of poor outcome in CRC patients.

Regarding pathological subtypes, 11/50 (22%) of patients had mucinous adenocarcinomas (MACs) and those patients were associated with low livin gene expression, however these unconventional findings against worse prognosis and oncologic behavior of MAC as it was predictable that MAC will express high livin level. But, MACs represent a genetically distinct variant of colorectal adenocarcinoma that displayed a large number of up regulated genes involved in mucin metabolism and have distinct pathway from non-mucinous cancer colon [28].

5. Conclusion

Regulation of apoptotic mechanism in colon tissue centered on reciprocal relation between anti-apoptotic livin and proapoptotic BMF and p53 genes expressions. Up regulation of livin gene and down regulation of BMF and p53 genes expression were markers of poor prognosis in cancer colon patients. Gene expression of higher livin and lower BMF and p53 genes expression associated with more aggressive tumors with metastasis. A new staging system based on both genes expressions and new treatment strategies could be applied in the future.

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Ethical approval

Research Involving Human Participants. The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent, and the Ethics Committee of Faculty of Medicine, Menoufia University approved the study protocol.

Contributor-ship

I confirm that all the named authors have participated in the study

to a sufficient extent to be named as authors. Dr Eman, Azza and Mohamed Assar do the lab investigation, Dr. Enas treat and follow up cancer colon patients at Clinical Oncology Department, and all authors participate in written and revision of the paper and approved the final manuscript for submission.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2020.100747.

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