Original paper

Activation of the P38-MSK1 axis in colorectal adenocarcinoma determines a good prognostic outcome

Mariana F. Gayyed[1], Medhat Monir Soliman[2], Magdy Fouad Ahmed[3], Taha Mohamed Hassanin[3], Rajai Munir Al Jehani[4*], Fatma El Zahraa Ammar Mohamed[1*]

*These authors contributed equally.

[1]Assistant professor of Pathology, Faculty of Medicine, Minia University, Minia, Egypt
[2]Consultant of Onco-surgery, Minia Oncology Centre, Minia, Egypt
[3]Assistant professor of Tropical Medicine, Faculty of Medicine, Minia University, Minia, Egypt
[4]Biomedical Scientist, UCL Institute for Liver and Digestive Health, Royal Free London NHS Foundation Trust, London, UK

Colorectal cancer (CRC) is the third most common cancer worldwide and is associated with a high level of mortality and morbidity. In this study we evaluate expression of p-p38 and p-MSK1 in CRC and determine whether there is an association between expression of these markers and any clinicopathologic parameters that could be of prognostic value.

Expression of p-p38, p-MSK1 and ki-67 were examined by immunohistochemistry in 135 archival CRC cases and the findings were correlated with the patient clinicopathological data.

P-p38 and p-MSK1 were expressed at high level in 58.5 % and 60.7% of CRC cases respectively. A statistically significant negative correlation was found between expression of p-p38 and Ki-67 ($p < 0.001$, $r = -0.63$) and between p-MSK1 and Ki-67 expression ($p < 0.001$, $r = -0.61$). The majority of CRC cases expressing high levels of p-p38 also expressed high levels of p-MSK1 and this correlation was highly significant ($p < 0.001$, $r = 0.863$). The high expression of p-p38 and p-MSK1 was also significantly associated with low Dukes and TNM stage. The elevated expression of p-38 and p-MSK1 in CRC was associated with a good prognosis and prolonged overall survival ($p < 0.001$, each).

Our finding showed that activation of the p38-MSK1 axis determines a good outcome in CRC.

Key words: colon adenocarcinoma, p-p38 and p-MSK1, Ki-67.

Introduction

Globally, colorectal carcinoma (CRC) is the third most common cancer in men, and second most common cancer in women [1]. According to the World Health Organization, around 1.8 million CRC cases were diagnosed in 2018 and 861,000 deaths were recorded [2, 3].

Mitogen activated protein kinases (MAPKs) are known to play a pivotal role in many physiological processes including cell growth, metabolism, differentiation, and tumour progression [4]. MAPK-activated protein kinase is activated by extracellular signal regulated kinase 1 and 2 (ERK1/2) and p38 MAPKs following mitogenic signalling or cellular stress. The (ERK1/2) and p38 MAPKs pathways also control vital cellular processes such as growth, proliferation, differentiation, migration and apoptosis.

Table I. Clinicopathological parameters in primary colorectal adenocarcinoma (n = 135)

Clinicopathological parameters	No (%)
Age	
≤ 51	69 (51.1)
> 51	66 (48.9)
Sex	
Male	77 (57)
Female	58 (43)
Site	
Caecum	7 (5.2)
Ascending	49 (36.3)
Transverse	11 (8.1)
Descending	18 (13.3)
Sigmoid	30 (14.8)
Rectum	20 (22.2)
Size	
≤ 7 cm	77 (57)
> 7 cm	58 (43)
Tumour Grade	
GI	9 (6.7)
GII	76 (56.3)
GIII	50 (37)
TNM Stage	
Stage I	15 (11.1)
Stage II	43 (31.9)
Stage III	52 (38.5)
Stage IV	25 (18.5)
Dukes stage	
A	1 (0.7)
B	44 (32.6)
C	66 (48.9)
D	24 (17.8)
LN Metastasis	
Yes	45 (33.3)
No	90 (66.7)
Peritoneal Dissemination	
Yes	30 (22.2)
No	105 (77.8)
Liver Metastasis	
Yes	25 (18.5)
No	110 (81.5)

The p38 MAPK pathway has been shown to have a dual function in cancer cells either supporting cell viability and survival or promoting cell death [4, 5, 6]. For example, treatment of colon cancer line HCT116 with the drug Oridonin has to been shown to induce antiproliferative and pro-apoptotic effects on the cancer cells through phosphorylation of P38 [5]. Other studies, however suggest the p38 MAPK pathway may play a fundamental role in the pathogenesis of colon cancer as well as other types of cancers such as those of the lung, liver, breast, prostate, and bladder [6, 7, 8, 9, 10, 11, 12, 13]. In addition, inhibition of p38 MAPK has been shown to increase the sensitivity of human colon cancer cells to 5-fluorouracil treatment [14]. Activation of p38 protein by phosphorylation can stimulate a variety transcription factors in addition to several kinases including Mitogen- and stress-activated protein kinase 1 (MSK1) [4]. MSK1 has been associated with cell proliferation and tumour transformation in many types of cancer such as skin, breast and nasopharyngeal carcinoma [15, 16, 17, 18]. Structurally MSK1 protein contains two kinase domains: an N-terminal kinase domain (NTKD) and C-terminal kinase domain (CTKD). The latter is the site where binding of Erk or p38MAPK takes place leading to phosphorylation of 3 sites on MSK1 [19].

The p38-MSK1 axis has previously been shown to be involved in many types of cellular response. For example, stress induced phosphorylation and activation of CREB protein is mediated by MSK1/2 signalling in a p38-dependent manner [20, 21]. In addition, phosphorylation of Histone 3S10 in gastric cancer cells has also been shown to be associated with MSK1 activation via the p38-MAPK pathway [22]. The p38- MSK1 pathway has also been reported to modulate the Wnt-β-catenin pathway in colon cancer which is essential for regulating cell proliferation and differentiation [23].

In light of the above, we decided to examine the p38-MSK1 pathway in CRC in relation to expression of other hallmarks of cancer, specifically the Ki-67 proliferation marker [24] which is associated with poor prognosis in CRC [25]. In this study, we evaluate expression of the phosphorylated, active form of p38 (p-p38), for the first time, also explore the expression of phosphorylated MSK1 in CRCs in order to determine whether there is any correlation between expression of these markers and patient outcome.

Material and methods

Patients and tissue samples

Archival tumour blocks obtained from 135 colorectal cancer patients which had been collected between January 2010 and December 2014 at Minia

University Hospital and Minia Oncology Centre were used in this study. The research was conducted following approval and written consent obtained from each patient. The clinicopathological data obtained from each patient including age, gender, tumour type, site, size, grade, lymph node status, stage, peritoneal deposits and liver metastasis were obtained from the patient medical records without access to any patient personal information (Table I). All patients had undergone endoscopic biopsy followed by surgical resection biopsy. Patients treated with neoadjuvant chemotherapy were excluded from this study.

The mean age of the patients was 48.9 ±1.37 years (range 24-76 years) with a median of 51 years. These included 77 (57%) males and 58 (43%) females. The mean primary tumour size was 7.25 ±2.29cm (4-14 cm) with a median of 7 cm. Fourteen (10.4%) cases were well-differentiated adenocarcinomas, 73 (54%) were moderately differentiated and 48 (35.6%) cases were poorly differentiated adenocarcinomas.

Immunohistochemical procedure

Immunohistochemical analysis was carried out on 4 μm sections from each formalin fixed paraffin embedded (FFPE) tissue block which were then mounted on positively charged glass slides. One section from each block was initially stained with hematoxylin-eosin to verify that adequate amounts of tumour were present.

Antibodies used were, anti- mouse p-p38 monoclonal antibody (Cell signaling) at 1 : 100 dilution, anti-rabbit phospho-MSK1 monoclonal antibody (ph-MSK1, Abcam) at 1 : 100 dilution, and an anti-rabbit Ki67 monoclonal antibody (Roche-30-9, ready to use) following the manufacturer's instructions. Immunohistochemistry was performed using an automated immune-stainer (Ventana Bench-Mark GX; Ventana Inc.). FFPE sections were deparaffinised followed by cell conditioning. Antigen retrieval was carried out using a Tris-based reaction buffer (pH 7.6) prior to application of primary antibody for 30 minutes. Visualisation was performed using Avidin Biotin detection. A bladder cancer sample was used as a positive control for p-p38, p-MSK1and Ki67. Omission of primary antibody during staining served as negative controls.

Interpretation of immunohistochemical staining

The stained slides were reviewed and scored independently by two pathologists. The p-p38 and p-MSK1 staining was scored according to intensity of staining (0 = no staining; 1 = weakly staining; 2 = strongly staining) and the percentage of positively stained cells (0 = < 5% positive cells; 1 = 5-25% positive; 2 = 26-50% positive; 3 = > 50% positive) [26]. The final expression score was obtained by multiplying the intensity of staining score with the percentage of positively stained cells. For statistical analysis, scores of more than 2 were designated as high expression, and scores below 2 were designated as low expression. The Ki-67 nuclear staining was categorized as according to the percentage of Ki-67-positive tumour cells as follows; expression was scored as high when the percentage of Ki-67 positive cells was > 14% and low when the percentage of Ki-67 positive cells was 14 or less. The Ki-67 scoring system used here is the same one currently used for grading breast cancer [27].

Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 20.0 (BM Corp, Armonk, NY, United States). The association of p-p38 and p-MSK1 expression with any of the patient clinicopathological parameters was determined using the Chi-squared test and Fisher's exact test. The correlation between the p-MSK1, p-p38 and Ki67 expression was determined using Spearman's correlation analysis. Cumulative patient survival was assessed using the Kaplan-Meier method. The effect of p-MSK1 and p-p38 expression on the prognosis of CRC patients were assessed via univariate and multivariate Cox regression. Hazard risk (HR) and relative 95% confidence interval (CI) were analyzed. A p-value less than 0.05 was considered significant for all statistical analyses.

Results

Phosphorylated p38 expression in primary colorectal carcinoma

Phosphorylated p38 was detected mainly in the nucleus of malignant cells whilst normal tissue adjacent to the tumours showed no staining of this marker. High p-p38 nuclear expression was detected in 79 cancer cases (58.5 %), whereas low expression was found in 56 cases (41.5%) (Fig. 1). No statistically significant correlation was found between p-p38 expression and tumour grade ($p = 0.299$). However, a significant inverse relationship was observed between p-p38 expression and Dukes stage and between p-p38 expression and TNM stage ($p < 0.001$, each). Additionally, a significant inverse correlation was found between high expression of p-p38 and tumour size ($p = 0.005$), lymph node involvement ($p = 0.007$), peritoneal deposits ($p = 0.001$) and liver metastasis ($p < 0.001$; Table II). No significant correlation was found between high expression of p-p38 and any of the other clinicopathological parameters,

Table II. The association between p-p38-p-MSK1 expression and different clinicopathological parameters in primary colorectal adenocarcinoma (n = 135)

Clinicopathological parameters	p-p38 expression		p-value	p-MSK1 expression		p-value
	Low (%)	High (%)		Low (%)	High (%)	
Age						
≤ 51	32 (46.4%)	37 (53.6%)	0.238	30 (43.5%)	39 (56.5%)	0.305
> 51	24 (36.4%)	42 (63.6%)		23 (34.8%)	43 (65.2%)	
Sex						
Male	32 (41.6%)	45 (58.4%)	0.983	30 (39%)	47 (61%)	0.935
Female	24 (41.4%)	34 (58.6%)		23 (39.7%)	35 (60.3%)	
Site						
Caecum	2 (28.6%)	5 (71.4%)	0.157	2 (28.6%)	5 (71.4%)	
Ascending	21 (42.9%)	28 (57.1%)		19 (38.8%)	30 (61.2%)	
Transverse	6 (54.5%)	5 (45.5%)		6 (54.5%)	5 (45.5%)	0.686
Descending	17 (56.7%)	13 (43.3%)		14 (46.7%)	16 (53.3%)	
Sigmoid	4 (22.2%)	14 (77.8%)		6 (33.3)	12 (66.7)	
Rectum	6 (30%)	14 (70%)		6 (30%)	14 (70%)	
Size						
≤ 7 cm	24 (31.2%)	53 (68.8%)	0.005*	24 (31.2%)	53 (68.8%)	0.027*
> 7 cm	32 (55.2%)	26 (44.8%)		29 (50%)	29 (50%)	
Tumour Grade						
GI	3 (33.3%)	6 (66.7%)	0.299	3 (33.3%)	6 (66.7%)	0.280
GII	28 (36.8%)	48 (63.2%)		26 (34.2%)	50 (65.8%)	
GIII	25 (50%)	25 (50%)		24 (48%)	26 (52%)	
Marked	29 (56.9%)	22 (43.1%)		27 (52.9%)	24 (47.1%)	
TNM Stage						
Stage I	2 (13.3%)	13 (86.7%)	0.000*	2 (13.3%)	13 (86.7%)	0.000*
Stage II	4 (9.3%)	39 (90.7%)		3 (7%)	40 (93%)	
Stage III	28 (53.8%)	24 (46.2%)		26 (50%)	26 (50%)	
Stage IV	22 (88%)	3 (12%)		22 (88%)	3 (12%)	
Dukes stage						
A	0 (0%)	1 (100%)	0.000*	0 (0%)	1 (100%)	0.000*
B	7 (15.9%)	37 (84.1%)		6 (13.6%)	38 (86.4%)	
C	27 (40.9%)	39 (59.1%)		25 (37.9%)	41 (62.1%)	
D	22 (91.7%)	2 (8.3%)		22 (91.7%)	2 (8.3%)	
LN Metastasis						
Yes	6 (13.3%)	19 (42.2%)	0.007*	28 (62.2%)	17 (37.8%)	0.001*
No	30 (33.3%)	60 (66.7%)		25 (27.8%)	65 (72.2%)	
Peritoneal Dissemination						
Yes	20 (66.7%)	10 (33.3%)	0.001*	19 (63.3%)	11 (36.7%)	0.002*
No	36 (34.3%)	69 (65.7%)		34 (32.4%)	71 (67.6%)	
Liver Metastasis						
Yes	23 (92%)	2 (8%)	0.000*	23 (92%)	2 (8%)	0.000*
No	33 (30%)	77 (70%)		30 (27.3%)	80 (72.7%)	

Test of significance: ^{2} *and Fisher exact tests*

p-values < 0.05 are considered significant

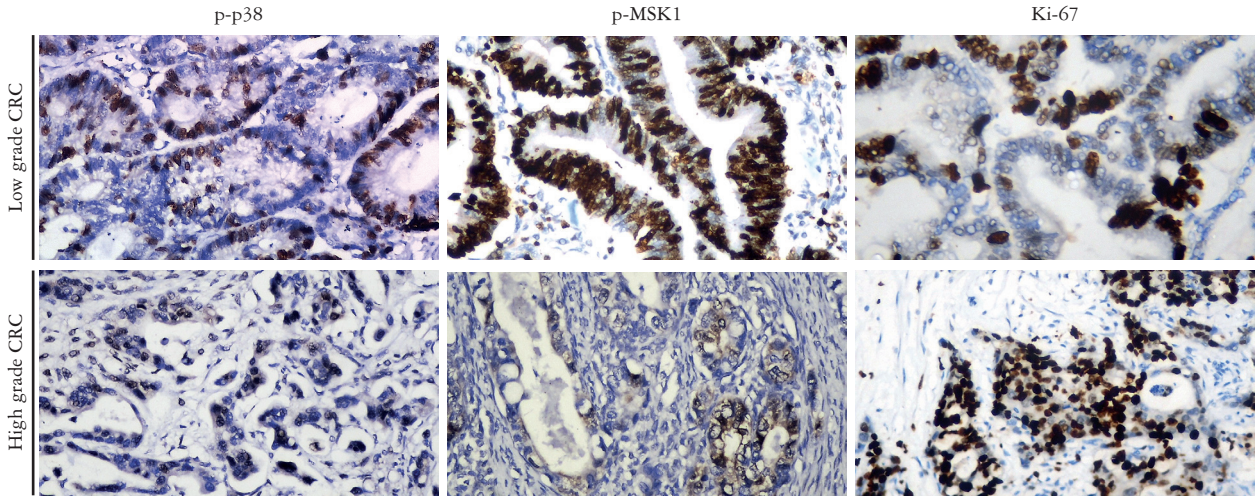

Fig. 1. Immunohistochemical staining of p-p38, p-MSK1 and Ki-67 in colorectal adenocarcinoma. The upper panel is of a low-grade CRC demonstrating high levels of p-p38 and p-MSK1 expression and a low level of Ki-67 expression. The lower panel is of a high-grade CRC demonstrating low levels of p-p38 and p-MSK1 expression and a high level of Ki-67 expression (magnification 200×)

Table III. Association between p-p38-p-MSK1 expression and Ki67 expression in primary colorectal adenocarcinoma (n = 135)

	Total (135)	p-p38 Expression		p-value	(r)	p-MSK1 Expression		p-value	(r)
		Low (%)	High (%)			Low (%)	High (%)		
Ki67 Expression	Low	39 (65)	21 (35)	**0.000***	**–0.632***	38 (63.3)	22 (36.7)	**0.000***	**–0.613***
	High	17 (22.7)	58 (77.3)			15 (20)	60 (89)		
p-MSK1 Expression	Low	50 (94.3%)	3 (5.7%)	**0.000***	**0.863***				
	High	6 (7.3%)	76 (92.7%)						

**Test of significance: Chi-square and Fisher exact tests*

** p values ≤ 05 are considered statistically significant*

** Immunoreactivitys scores for p-p38 and p-MSK1 were positively correlated to each other but inversely correlated to the Ki67 staining score using Spearman's correlation analysis*

r values were considered fair (0-0.24), weak (0.25-0.49), moderate (0.50-0.74) or strong (0.75-1).

such as age, sex and site. With Ki-67 a significant correlation was found between expression of this proliferation marker and expression of phosphorylated p-p38 ($p = 0.000$, $r = –0.637$; Table III).

Phosphorylated MSK1 expression in primary colorectal carcinoma

Phosphorylated MSK1 immunostaining was localized in the nucleus of normal and tumour cells. However, the expression of MSK1 in the normal tissue adjacent to the tumours was either weak or negative (Fig. 1). High expression of p-MSK1 was seen in 82 (60.7%) primary colorectal cancers and low expression was observed in 53 cases (39.3%). A Significant inverse correlation was found between p-MSK1 expression and Dukes stage ($p < 0.001$) as well as TNM stage ($p < 0.001$). High expression of p-MSK1 was also significantly inversely correlated with tumour size ($p = 0.027$), lymph node ($p = 0.001$) and peritoneal involvement ($p = 0.002$) as well as liver metastasis ($p < 0.001$; Table II). No significant correlation was found between high expression of p-p38 and the other clinicopathological parameters (age, sex, site and tumour grade). A statistically significant inverse correlation was found between expression of p-MSK1 and Ki-67 ($p < 0.001$; $r = –0.61$; Table III). Interestingly the majority of CRC cases expressing high levels of p-p38 also expressed high levels of p-MSK1 and this correlation was statistically highly significant ($p < 0.001$, $r = 0.863$).

Survival analysis

The median follow-up of patients was 40 months (range 6-58 month) with a mean and standard deviation of 37.65 ±1.3 months. Correlation between p-MSK1 or p-p38 expression in tumours and tumour metastasis was determined using the Wilcoxon test. With regard to patient outcome it was found that

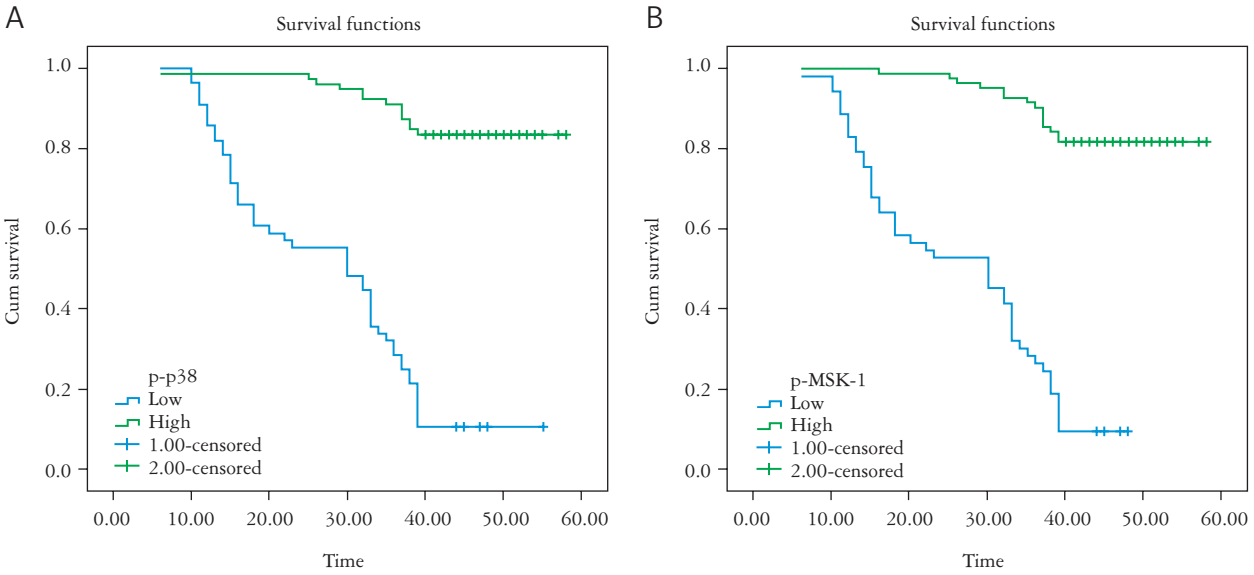

Fig. 2. Kaplan-Meier curves for overall survival stratified according to p-p38 and p-MSK1 expression. Longer overall survival is associated with high levels of p-p38 and p-MSK1 expressions

Table IV. Multivariate Cox regression analysis of the relationship between clinicopathological characteristics and prognosis in primary colorectal adenocarcinoma (n = 135)

						95.0% CI for Exp(B)	
	B	SE	Wald	Sig.	Exp(B)	Lower	Upper
p-MSK1	–1.047	0.512	4.179	0.041	0.351	0.129	0.958
p-p38	–1.216	0.543	5.007	0.025	0.297	0.102	0.860
KI67	0.400	0.288	1.938	0.164	1.492	0.849	2.623
Grade	0.129	0.227	0.324	0.569	1.138	0.729	1.776
Dukes stage	0.224	0.426	0.276	0.600	1.251	0.542	2.886
TNM	0.868	0.399	4.733	0.030	2.382	1.090	5.207

low expression of p-p38 and p-MSK1 was associated with a lower overall survival rate ($p < 0.001$; Fig. 2).

The relationship between expression of p-p38 and p-MSK1 in CRCs and patient prognosis was evaluated using univariate and multivariate Cox regression analysis. The multivariate regression analysis revealed low expression of p-p38 and p-MSK1 significantly increased the risk of adverse consequences and poor prognosis in CRC patients (HR = –1.216, 95% CI: 0.297, $p = 0.025$, for p-p38; and HR = –1.04, 95% CI: 0.351, $p = 0.041$ for p-MSK1; Table IV). Increased TNM staging, was found to be-associated with adverse consequences only (HR = 0.868, CI: 2.382, $p = 0.030$; Table IV).

Discussion

Despite recent improvements in the early diagnosis and treatment of CRC, the level of mortality and morbidity from this disease remains high [28] and identification of new diagnostic and prognostic markers that can improve the outcome in patients with CRC is a major priority.

Previous studies demonstrated higher levels of p-p38 had a growth inhibitory effect on cancer cells derived from breast, prostate and skin among others [29]. In colon cancer p38 activation has been shown to selectively induce cell death in K-ras-mutated HCT116 cells [30]. Moreover, p38 pathway has also been demonstrated to bring about cell cycle arrest and autophagic cell death in colon cancer cells [31].

One of the p38 downstream signalling targets is MSK1. Although the role of MSK1 in CRC is not fully understood, there is some evidence pointing to the possibility that MSK1 could be a biomarker of prognostic value [13, 23]. In this study we set out to determine whether the p38-MSK1 axis has a pathological value in colon cancer by examining expression of the phosphorylated active forms of these proteins in this malignancy.

We found phosphorylated p38 was expressed mainly in the nucleus of CRC cells. The level of p-p38

was high in 58.5% of CRCS cases whereas low expression was found in 41.5%. We next determined if there was any association between the level of p-p38 expression and any clinicopathological parameters that might suggest a possible role for p38 in CRC pathogenesis. We found high levels of p-p38 were mainly expressed in lower grade CRCs, although the association between tumour grade and p-p38 expression was not statistically significant ($p = 0.299$). In line with our findings, a previous study on breast cancer showed that high levels of p-p38 expression were associated with grade 1 and 2 cancers more often than grade 3 breast cancers [32]. In this case p38 was suggested as having a tumour suppressor role in breast cancer [33]. The concept of p38 as a tumour suppressor is further supported by murine models in which inactivation of p38 MAPK by direct gene targeting or targeting of the genes that regulate p-38 expression has been shown to be associated with increased tumorigenesis [34, 35]. An examination of the relationship between p38 activity and tumour proliferation, revealed there was a significant negative correlation between p-p38 and Ki-67 expression in CRC ($p < 0.001$) ($r = -0.632$). Contrary to our findings, however, a previous study on breast cancer demonstrated an association between the expression of p38 and highly proliferative tumours as determined by Ki-67 expression. P38 was considered to be marker for breast cancer prognosis in but the study was limited as only metastatic breast cancers within lymph nodes were evaluated in this case [36]. In agreement our findings the expression of p38 has been shown to play a major role in the suppression of cell proliferation and promotion of cell migration in mouse corneal epithelium organ cultures [37] In addition, the drug oxaliplatin, which is used in the treatment of colorectal cancer and other cancers, has been shown to exert its anti-tumour effects by activating p38 and/or JNK kinases [38]. Furthermore, p38 has been described as playing an important role in the metabolism of colon cancer cells and modulating tumour behaviour [39]. Interestingly, we found a significant inverse association between p-p38 expression and Dukes stage and TNM stage ($p < 0.001$, each) in our CRCs. In addition, a significant inverse correlation was found between high expression of p-p38 and lymph node metastasis, peritoneal dissemination and liver metastasis ($p = 0.007$, 0.001 and $p < 0.001$, respectively). This suggests that p38 may also play a role in the migration of colorectal cancer cells. In support of this, p38MAPK signalling has previously been shown to upregulate E Cadherin expression via activation of TAK1 [40], and thus impede the induction of epithelial mesenchymal transition which enables malignant cells to migrate from their primary site. In addition, inhibition of p38 has previously been associated with resistance to anoikic in metastasizing cancer cells which is critical for their survival and high levels of P38 expression have also been linked to tumour cell dormancy [35]. Contrary to our findings, however, the expression of p38 has previously been reported as being higher in advanced stage CRCs [41]. However, in this case expression of p-p38 alpha specifically was examined which may have a different pattern of expression in CRCs compared to the p-p38 kinase detected by the pan antibody used in our study.

We found low expression of p-p38 significantly correlates with a low survival rate in CRCs ($p < 0.001$). In contrast, increased p38 phosphorylation has previously been reported to be associated with poor survival rates in CRC [42]. However, the latter study was limited by the fact that samples from tissue microarrays only were examined which means that any heterogenous expression within tumours might have been missed. In addition, the investigators compared high and low levels of p-p38 expression on survival within each clinical stage separately rather than compare the levels of p-p38 expression on overall survival independently of stage.

Our data suggests there is a link between p38 activation and good prognosis. This is further supported by functional studies in which blockade of the p38 pathway in colorectal cancer cells, either by pharmacological inhibition or genetic ablation, leads to cell cycle arrest and autophagic cell death [23, 31]. One of the downstream signalling targets of p38 is MSK1. We found high expression of p-p38 significantly correlates with high expression of MSK1 in CRC ($p < 0.001$) which suggests the p38-MSK1 axis is activated in CRC. A few studies have also eluded to the possibility that MSK1 could be of prognostic value in CRCs [13, 23]. In this study we examined expression of the active, phosphorylated form of MSK1 and p38 in order to determine whether these markers could be of value in predicting disease outcome in CRC. MSK1 was found to be expressed at high levels in 60.7% of CRC cases and expressed at low levels in 39.3% of cases. A similar study by Fu et al reported high expression levels of MSK1 but in a smaller proportion, 45%, of CRCs cases. This discrepancy between the two studies could in part be due to the fact that we examined expression of the phosphorylated active form of MSK1 only whereas total MSK1 was targeted in the study of Fu *et al.* [13]. The high levels of pMSK1 expression in a large percentage of CRCs in our study suggest MSK1 may play an active role in CRC pathogenesis. This is supported by other studies in which the p38-MSK1 axis was shown to exert an influence on gene expression and phenotype in CRC [23]. According to Fu *et al*., high expression of MSK1 was more frequently associated with moderately differentiated than poorly differentiated CRCs. However, we found no statistically significant

association between p-MSK1 expression and CRC tumour grade ($p = 0.280$). The relationship between MSK1 expression and tumour grade appears to vary depending on the type of cancer. For instance, high-grade breast cancers have been associated with low levels of MSK1 expression [43], whereas increased levels of phosphorylated MSK1 expression have been associated with poorly differentiated nasopharyngeal cancers [16]. As is the case with p38, we found an inverse correlation between expression of p-MSK1 and Ki-67 expression in our CRCs ($p < 0.001$). This finding is in agreement with a previous study in which colon cancer cells expressing high levels of MSK1 were shown to exhibit a higher level of cell proliferation [13]. In this case a novel mechanism was described by which MSK1 overexpression suppressed oxidative stress by decreased NADP+/NADPH ratio. It should be stated, however, that total MSK1 was analysed in this study rather than just the active phosphorylated form investigated by us [13]. We also found a significant inverse correlation between pMSK1 expression and TNM stage ($p < 0.001$), Dukes stage ($p < 0.001$), tumour size ($p = 0.027$), lymph node metastasis ($p = 0.001$), peritoneal deposits ($p = 0.002$) and liver metastasis ($p < 0.001$). Similar findings, whereby low-levels of MSK1 expression were associated with higher tumour stage have been reported in breast cancer [43]. The only other published work on MSK1 in colon cancer reported expression of MSK1 was lower in stage IV compared to stage III CRCs [13]. In addition, the overall survival of patients with CRC expressing low levels of MSK1 was reported to be significantly shorter than that of patients with tumours expressing high levels of MSK1. These findings suggest MSK1 expression could be a useful independent prognostic indicator in CRC. However, this work was based on detection of total MSK1 expression so it is not possible to determine if the data could also be extrapolated to the phosphorylated active form of MSK1 [13]. Our multivariate analysis showed that increased TNM stage is an independent poor prognostic parameter whereas, high expression of p-p38 and p-MSK1 are independent good prognostic parameters.

In conclusion, although p38-MSK1 axis was investigated in previous studies, this is the first study looking at expression of p-p38 and p-MSK1 together in CRCs [44, 45, 46]. High expression of these markers in CRCs was associated with a good prognosis and longer overall survival of patients. Our data suggests p38 and MSK1 may have an important role in CRC pathogenesis and raises the possibility of exploiting the p38-MSK1 axis as a therapeutic target for treatment of CRC. Further studies with a larger number of cases which also take into account the K-ras mutation status of the tumours will enable us to throw more light on exact the role played by the p38-MSK1 pathway in development of CRC.

The authors declare no conflict of interest.

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Address for correspondence

Mariana F. Gayyed
Assistant professor of Pathology
Faculty of Medicine
Minia University, Minia, Egypt
tel. +201001143941
e-mail: mariana.gaid@mu.edu.eg