Evaluation of the significance of human class I homeobox A13 and STK4/MST1 expression in conventional colorectal adenoma and adenocarcinoma and correlation with clinicopathological parameters (immunohistochemical study)

Ebtehal M. Abdel-Aal, Amira El-Sayed Soliman

Department of Pathology, Faculty of Medicine, Benha University, Qalyubia, Egypt

Correspondence to Ebtehal M. Abdel-Aal, MD, Saad Zaghloul Square, Benha 13511, Central Delta, Qalyubia, Egypt
Tel: +20 109 009 5977;
e-mail: dr.ebtehal.maher@gmail.com

Received: 05 November 2022
Revised: 10 November 2022
Accepted: 16 November 2022
Published: 08 February 2023

Egyptian Journal of Pathology 2022, 42:102–111

Background
Colorectal carcinoma (CRC) is the third most common cancer in the world. Human class I homeobox A13 (HOXA13) was initially identified as a transcription factor and has an important role in embryonic development and malignant transformation. Mammalian STE20-like kinase 1 (STK4/MST1) encodes a serine/threonine kinase that is the mammalian homolog of the Hippo pathway and plays an important role in controlling cell growth, apoptosis, and organ size.
Aim
This work aimed to evaluate the role of HOXA13 and STK4/MST1 in conventional colorectal adenoma and adenocarcinoma.
Materials and methods
This retrospective study was carried out on 20 cases of conventional colorectal adenoma and 30 cases of conventional colorectal adenocarcinoma. HOXA13 and STK4/MST1 immunostaining was done and assessed for each case. Correlation with the clinicopathological findings and statistical analysis was studied.
Results
In the carcinoma cases, there was a highly significant direct statistical correlation between HOXA13 expression and tumor grade, lymph node metastasis, and TNM stage (P<0.01 for each). Inverse statistical correlation between STK4/MST1 expression and tumor grade, depth of tumor invasion (T), and TNM stage (P<0.01 for each) was found. HOXA13 and STK4/MST1 immunoexpression showed direct highly significant relation with the transition from adenoma to adenoma with dysplasia to adenocarcinoma (P<0.01). There was a highly significant inverse statistical correlation between HOXA13 and STK4/MST1 expression in the studied cases of CRC (P<0.01). According to receiver-operating characteristic curve, both markers were good in the prediction of metastatic potential in the carcinoma cases using the TNM stage as a parameter with a sensitivity of 85%.
Conclusion
HOXA13 was immunohistochemically overexpressed, while STK4/MST1 was downexpressed in progression from colorectal adenoma to adenoma with dysplasia to adenocarcinoma cases. HOXA13 and STK4/MST1 might have a potential role as independent prognostic factors in CRC and may have validity to predict metastatic potential of CRC.

Keywords:
colorectal adenocarcinoma, human class I homeobox A13, STK4/MST1

Egypt J Pathol 2022, 42:102–111

1687-4277

Introduction

Colorectal carcinoma (CRC) is one of the most common malignancies worldwide. It is associated with high mortality rate with increased incidence in females than males (Abou Gabal *et al*., 2022). In the world, it is the third most lethal and the fourth most common malignancy (Hafez *et al*., 2022). In Egypt, according to the National Cancer Institute Registry, Cairo University, CRC represents 35% from the total GIT tumors and 6.49% from the total malignancies (Mokhtar *et al*., 2016).

Many risk factors are expected to play a role in this high incidence such as higher age, change in dietary habits, cigarette smoking, alcohol intake, high red meat and processed meat consumption, high fat and protein diet intake, low physical exercise, and the increased prevalence of obesity. Clinicopathological parameters

 DOI: 10.4103/egjp.egjp_24_22

play a major role in determining the management of CRC, but are usually not reliable predictors of prognosis (Mostafa *et al*., 2021). Therefore, studying the novel biomarkers of CRCs and the molecular mechanisms underlying the occurrence and progression of CRC must be identified to provide novel markers for treatment options for CRC.

Several studies have shown that class I homeobox (*HOX*) genes belong to a family of highly conserved transcription factors known for their roles as master regulators of embryogenesis (Gu *et al*., 2020). Mammalians have 39 HOX genes, which are distributed into four gene clusters: human class I homeobox A (HOXA), HOXB, HOXC, and HOXD4, which play a relevant role in both tumor development and progression (Qiao *et al*., 2021; Di Mauro *et al*., 2021). Overexpression of HOXA13 promoted migration and invasion through the induction of epithelial–mesenchymal transition in cancer cell lines (Botti *et al*., 2019). It is overexpressed in gastric cancer (Qin *et al*., 2019), esophageal cancer (Nesteruk *et al*., 2020), and lung cancer (Wang *et al*., 2021). Although many studies have found that other members of the *HOX* gene family, such as *HOXA3*, *HOXA4*, *HOXA5*, and *HOXA9*, are overexpressed in colon cancer compared with normal tissues, the expression and biological function of *HOXA13* in colon cancer is not well studied (Yu *et al*., 2020).

Mammalian STE20-like protein kinase 1 (STK4/MST1) is a class II kinase that belong to the sterile (STE)-20 protein kinase family. It is widely expressed in most cells of the human body and is a core component of the Hippo signaling pathway (Hong *et al*., 2016). This pathway is associated with the control of organ size by cell proliferation and survival, and its deregulation results in cancer (Han, 2019). The STK4/MST1 is one of the core components of the Hippo pathway and plays as an important role in cell differentiation, adhesion, migration, apoptosis, and other physiological activities through phosphorylation, dimerization, and nuclear localization of target proteins (Ouyang *et al*., 2019). It was first reported as a proapoptotic cytoplasmic kinase and is critical for amplifying apoptotic signals (Graves *et al*., 1998). It was reported to promote the differentiation of tumor cells acting as a tumor suppressor in many cancers, including hepatic cell carcinoma (Zhou *et al*., 2009), malignant gliomas (Zhu *et al*., 2019), and lung cancer (Singh *et al*., 2020). However, the role of STK4/MST1 in colon cancer is still unclear.

The aim of this work is to evaluate the expression of HOXA13 and STK4/MST1 in conventional colorectal adenomas and adenocarcinoma and correlate their expression with different clinicopathological variables and with each other to clarify their actions hoping to find new prognostic and therapeutic options of colon cancer.

Materials and methods

Study groups

This is a retrospective study performed on formalin-fixed paraffin-embedded biopsy specimens of 50 cases of colorectal lesions categorized as 20 cases of conventional colorectal adenoma and 30 cases of conventional colorectal adenocarcinoma, collected from the Pathology Department, and Early Cancer Detection Unit, Faculty of Medicine, Benha University, during the period from January 2016 to December 2021. The study was approved by the Ethics Committee of Faculty of Medicine, Benha University (4 November 2022). Thirty cases were obtained by radical colectomy and 20 cases by colonoscopy. Six control cases were taken from viable margins in patients with intestinal infarction. Cases were selected on the basis of availability of clinicopathological data retrieved from the patients' files, which included age, sex, tumor size, histological grade, and clinical stage for carcinoma cases. The cases were categorized according to age into two groups: less than 60 years old and more than or equal to 60 years old (Gu *et al*., 2020).

Histopathological examination

Formalin-fixed paraffin-embedded blocks were cut into 5 μm thickness and stained using hematoxylin and eosin stain. Two observers reviewed the microscopic sections from all the cases, unaware of their diagnosis.

The conventional colorectal adenoma cases were categorized according to the presence or absence of dysplasia (Song *et al*., 2017). The conventional colorectal adenocarcinoma cases were graded into well-differentiated (G I), moderately differentiated (G II), and poorly differentiated (G III) tumors ((Awan *et al*., 2017). Pathological tumor-node metastasis (TNM) were retrieved from the patient archives, and staging was determined according to the criteria of the American Joint Committee on Cancer, 8th edition (Amin *et al*., 2017).

Immunohistochemical procedure

According to manufacturer's instructions, the sections were deparaffinized and then hydrated through a series of descending alcohols. Then they were put in 10 mm citrate buffer (pH=6) and were twice pretreated in a microwave oven for antigen retrieval. The endogenous peroxidase activity was inactivated by incubation in 3% hydrogen peroxide. One to two drops of the primary polyclonal antirabbit antibody, HOXA13 and STK4/MST1 at a dilution of 1: 200 for each (Ab106503;

Abcam, Cambridge, UK and ab212551; Abcam, respectively) was applied to each section. Slides were incubated in humid chamber overnight at 4°C. The sections were incubated with avidin-biotin-peroxidase system (DAKO, Glostrup, Denmark) for 30 min. Two or three drops of streptavidin enzyme label were put on each slide for 30 min at room temperature. Peroxidase reaction was detected by addition of diaminobenzidine tetrahydrochloride. Slides were rinsed well in tap water for 5 min and then slightly counterstained with Mayer's hematoxylin for 1–2 min and dehydrated in ascending alcohol. The slides were cleared in xylene for three changes and coverslides were applied.

Negative and positive controls

According to manufacturer's instructions, sections from human placenta were used as a positive control for HOXA13, and sections from human normal colon were used as a positive control for STK4/MST1.

For negative controls, samples were treated as described above, except that the primary antibody was replaced with a solution of BSA in phosphate-buffered saline.

Immunohistochemical assessment

Assessment of human class I homeobox A13 expression

For HOXA13, nuclear and cytoplasmic brown immunostaining was detected. The staining intensity was scored on a scale of 0–3: 0 (negative), 1 (weak), 2 (medium), and 3 (strong). The percentage of positive cells was evaluated on a scale of 0–4: 0 (negative), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The final immunoactivity scores were calculated by multiplying the above two scores, resulting in an overall score that ranges from 0 to 12. Each case was ultimately considered 'negative' if the final score ranges from 0 to 3, and 'positive' if the final score ranges from 4 to 12 (Qiao *et al*., 2021).

Assessment of STK4/MST1 expression

For STK4/MST1, cytoplasmic brown immunostaining was observed. It was scored according to the percentage of positive cells and staining intensity. The scoring parameters for staining intensity were as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The scoring parameters for the percentage of positive cells were as follows: negative, 0–5%; 1, 6–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100%. Sections with a total combined score of less than 4 were characterized as low STK4/MST1 expression, while sections with a score of more than or equal to 4 were characterized as high STK4/MST1 expression (Jin *et al*., 2021)

Statistical analysis

Results were analyzed using SPSS version 16 (SPSS Chicago, IL, USA) Statistical Package for Microsoft Windows as follows: *P* value more than 0.05 is nonsignificant, *P* value less than 0.05 is significant, and *P* value less than or equal to 0.001 is highly significant. Receiver-operating characteristic (ROC) analysis was carried out to evaluate the prognostic role of HOXA13 and STK4/MST1 expression in predicting the metastatic potential using TNM stages as a parameter.

Results

This study was carried out on 50 cases of colorectal tumors. In the 20 cases of conventional colorectal adenoma, 11 (55%) cases were males and nine (45%) cases were females. The age of the studied cases ranged from 19 to 55 years with a mean age of 36.5 ± 10.6 years. In the 30 cases of conventional colorectal adenocarcinoma, 18 (60%) cases were males and 12 (40%) cases were females. The age of the studied cases ranged from 30 to 72 years with a mean age of 55 ± 13.7 years. They were distributed into two age groups as follows: 14 (46.7%) cases were less than 60 years old and 16 (53.3%) cases were more than or equal to 60 years. The studied carcinoma cases ranged in size from 3.5 to 15 cm in the largest dimension, with a mean size of 5 cm, 17 (56.7%) cases were more than or equal to 5 cm, while 13 (43.3%) cases were less than 5 cm. According to the carcinoma site 17 (56.7%) cases were located in the right colon, while 13 (43.3%) cases were located in the left colon.

Histopathological results

There was a highly significant statistical correlation between tumor grade and depth of tumor invasion, lymph node metastasis, and TNM stage ($P<0.01$ for each). There was a significant statistical correlation between tumor grade and tumor size and distant metastasis ($P<0.05$ for both). On the other hand, there was insignificant statistical correlation between tumor grade and patient age, sex and the tumor site ($P>0.05$ for each) (Table 1).

Human class I homeobox A13 immunohistochemical expression in the studied cases

HOXA13 was negative in normal colonic mucosa of the control group. Its expression was detected as nuclear and cytoplasmic immunostaining. The results of HOXA13 immunohistochemical expression in the adenoma and adenocarcinoma cases are shown in Table 1. HOXA13 immunoexpression showed direct highly significant relation with the transition from adenoma to adenoma with dysplasia to adenocarcinoma ($P<0.01$) (Fig. 1a–d).

Correlation between human class I homeobox A13 immunohistochemical expression and clinicopathological features of the studied cases

For the adenoma cases, 76.9% of adenoma without dysplasia cases were negative for HOXA13 expression, while 71.4% of

Table 1 Correlation between human class I homeobox A13 immunohistochemical expression and clinicopathological features of the studied carcinoma cases

Clinicopathological features	HOXA13 immunohistochemical expression [*n* (%)]		Total	*P* value
	Negative (scores 0–3)	Positive (scores 4–12)		
Adenoma cases (20)				
Dysplasia				
No	10 (76.9)	2 (28.6)	12 (60)	<0.05*
Yes	3 (23.1)	5 (71.4)	8 (40)	
Adenocarcinoma cases (30)				
Tumor site				
Right colon	5 (50)	12 (60)	17 (56.7)	>0.05
Left colon	5 (50)	8 (40)	13 (43.3)	
Tumor size				
<5 cm	6 (60)	7 (35)	13 (43.3)	>0.05
≥5 cm	4 (40)	13 (65)	17 (56.7)	
Tumor grade				
Grade I	4 (40)	2 (10)	6 (20)	
Grade II	5 (50)	11 (55)	16 (53.3)	<0.05*
Grade III	1 (10)	7 (35)	8 (26.7)	
Depth of tumor invasion (T)				
T1	3 (30)	0	3 (10)	
T2	3 (30)	5 (25)	8 (26.7)	
T3	3 (30)	9 (45)	12 (40)	<0.05*
T4	1 (10)	6 (30)	6 (30)	
LN metastasis (N)				
N0	8 (80)	6 (30)	14 (46.7)	
N1	2 (20)	10 (50)	12 (40)	<0.01**
N2	0	4 (20)	4 (13.3)	
Distant metastasis (M)				
M0	9 (90)	14 (70)	23 (76.7)	>0.05
M1	1 (10)	6 (30)	7 (23.3)	
Tumor stage (TNM)				
Stage I	4 (40)	0	4 (13.3)	
Stage II	3 (30)	3 (15)	6 (20)	<0.01**
Stage III	2 (20)	11 (55)	13 (43.3)	
Stage IV	1 (10)	6 (30)	7 (23.3)	

HOXA13, human class I homeobox A13; LN, lymph node; TNM, tumor-node metastasis.
*Significant.
**Highly significant.

cases of adenoma with dysplasia were positive for HOXA13 expression. There was a significant direct statistical correlation between HOXA13 expression and presence of dysplasia (P<0.05).

Concerning the carcinoma cases, there was a highly significant direct statistical correlation between HOXA13 expression and lymph node metastasis and TNM stage (P<0.01 for all). There was a significant direct statistical correlation between HOXA13 expression and tumor grade and depth of tumor invasion (T) (P<0.05 for both). There was insignificant statistical correlation between HOXA13 expression and age, sex, tumor site, tumor size, and distant metastasis (M) of the studied cases (P>0.05) as illustrated in Table 1.

STK4/MST1 immunohistochemical expression in the studied cases

STK4/MST1 was highly expressed in normal colonic mucosa of the control group. Its expression was detected as cytoplasmic brown immunostaining.

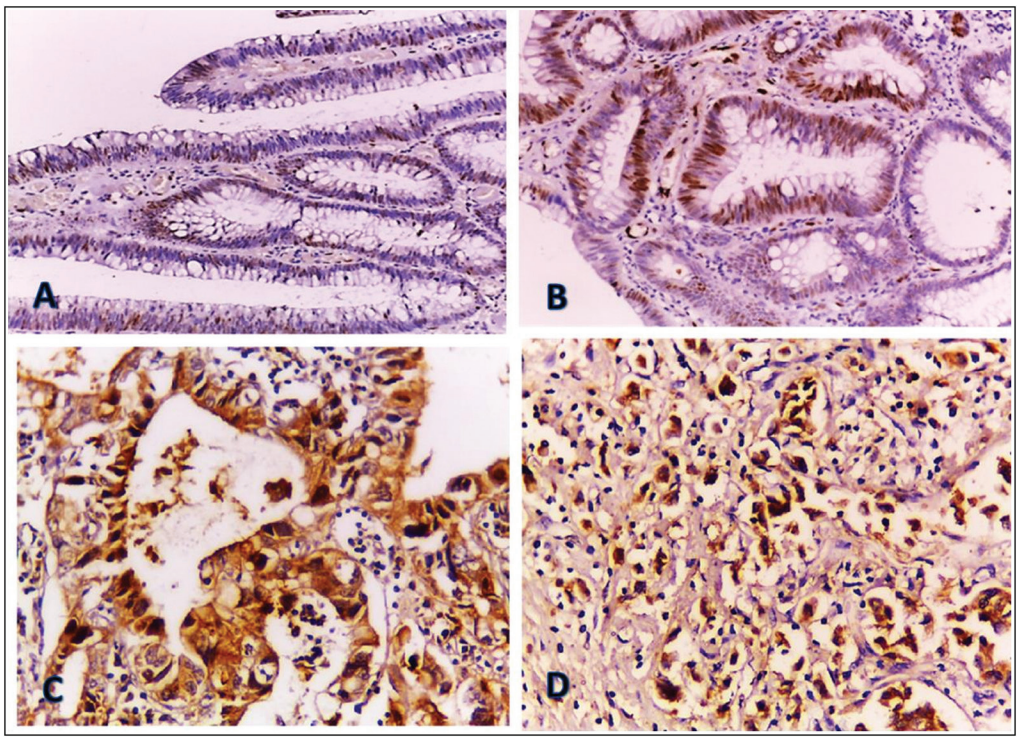
The results of STK4/MST1 immunohistochemical expression in the adenoma and adenocarcinoma cases are shown in Table 2. The STK4/MST1 immunoexpression showed inverse highly significant relation with the transition from adenoma to adenoma with dysplasia to adenocarcinoma (P<0.01) (Fig. 2a–c).

Correlation between STK4/MST1 immunohistochemical expression and clinicopathological features of the studied cases

In the adenoma cases, 100% of high expression cases were seen in adenoma without dysplasia cases, while 80% of low expression cases were seen in adenoma with dysplasia cases. There was a highly significant inverse statistical correlation between STK4/MST1 immunohistochemical expression and presence of dysplasia (P<0.01).

In the carcinoma cases, there was a highly significant inverse statistical correlation between STK4/MST1 immunohistochemical expression and tumor

Figure 1



(a) HOXA13 immunohistochemistry showing weak nuclear and cytoplasmic staining in less than 50% of cells of a tubulovillous adenoma without dysplasia, score 2 (negative) (ABC ×200). (b) HOXA13 immunohistochemistry showing strong nuclear and cytoplasmic staining in more than 50% of cells of a tubular adenoma with dysplasia, score 9 (positive) (ABC ×200). (c) HOXA13 immunohistochemistry showing strong nuclear and cytoplasmic staining in more than 75% of cells of a moderately differentiated colorectal adenocarcinoma, score 12 (positive) (ABC ×400). (d) HOXA13 immunohistochemistry showing strong nuclear and cytoplasmic staining in more than 75% of cells of a poorly differentiated colorectal adenocarcinoma, score 12 (positive) (ABC ×400). HOXA13, human class I homeobox A13.

grade, depth of tumor invasion (T), and TNM stage (P<0.01 for each). There was a significant inverse statistical correlation between STK4/MST1 immunohistochemical expression and both tumor size and distant metastasis (M) (P<0.05 for both). On the other hand, there was insignificant statistical correlation between STK4/MST1 immunohistochemical expression and age, sex, tumor site, and lymph node metastasis (P>0.05 for each) as illustrated in Table 2.

Correlation between human class I homeobox A13 expression and STK4/MST1 expression in the studied cases

There was a highly significant inverse statistical correlation between HOXA13 and STK4/MST1 expression in the studied cases of CRC (P<0.01) (Table 3).

Receiver-operating characteristic curve for the validity and predictivity of human class I homeobox A13 and STK4/MST1 expression in relation to TNM stage

ROC analysis was carried out to evaluate the prognostic role of HOXA13 and STK4/MST1 expression in predicting the metastatic potential using TNM stages as a parameter. For statistical purpose, stages I and II were grouped as low stage and stages III and VI were grouped as high stage.

According to the ROC curve, area under the curve of HOXA13 was 0.77 (good). The sensitivity, specificity, and accuracy of HOXA13 expression in the prediction of metastatic potential using TNM stage in the carcinoma cases were 85, 70, and 80%, respectively (Fig. 3a). For STK4/MST1, area under the curve was 0.74 (good). The sensitivity, specificity, and accuracy of STK4/MST1 expression in the prediction of metastatic potential using TNM stage in the carcinoma cases were 85%, 60%, and 77%, respectively (Fig. 3b).

Discussion

CRC is the world's third most lethal and fourth most common malignancy. In Egypt, CRC represented the seventh most common cancer and the third most common male neoplasm and fifth most common female neoplasm (Elhadidy and Haydara, 2022)

The present study aims to detect immunohistochemical expression of HOXA13 and STK4/MST1 in conventional colorectal adenoma and adenocarcinoma and its relevance to the various clinicopathological features. Besides, the study revealed the correlation between HOXA13 and STK4/MST1.

Table 2 Correlation between STK4/MST1 immunohistochemical expression and clinicopathological features of the studied carcinoma cases

Clinicopathological features	STK4/MST1 immunohistochemical expression [*n* (%)]		Total	*P* value
	Low (scores <4)	High (Scores ≥4)		
Adenoma cases (20)				
Dysplasia				
No	2 (20)	10 (100)	12 (60)	<0.01**
Yes	8 (80)	0	8 (40)	
Adenocarcinoma cases (30)				
Tumor site				
Right colon	12 (57.1)	5 (55.6)	17 (56.7)	>0.05
Left colon	9 (42.9)	4 (44.4)	13 (43.3)	
Tumor size				
<5 cm	6 (28.6)	7 (77.8)	13 (43.3)	<0.05*
≥5 cm	15 (71.4)	2 (22.2)	17 (56.7)	
Tumor grade				
Grade I	2 (9.5)	4 (44.4)	6 (20)	
Grade II	11 (52.4)	5 (55.6)	16 (53.3)	<0.01**
Grade III	8 (38.1)	0	8 (26.7)	
Depth of tumor invasion (T)				
T1	0	3 (33.3)	3 (10)	
T2	4 (19)	4 (44.4)	8 (26.7)	
T3	10 (47.6)	2 (22.2)	12 (40)	<0.01**
T4	7 (33.3)	0	6 (30)	
LN metastasis (N)				
N0	8 (38.1)	6 (66.7)	14 (46.7)	
N1	9 (42.9)	3 (33.3)	12 (40)	>0.05
N2	4 (19)	0	4 (13.3)	
Distant metastasis (M)				
M0	14 (66.7)	9 (100)	23 (67.7)	<0.05*
M1	7 (33.3)	0	7 (23.3)	
Tumor stage (TNM)				
Stage I	0	4 (44.4)	4 (13.3)	
Stage II	4 (19)	2 (22.2)	6 (20)	<0.01**
Stage III	10 (47.6)	3 (33.3)	13 (43.3)	
Stage IV	7 (33.3)	0	7 (23.3)	

LN, lymph node; TNM, tumor-node metastasis.
*Significant.
**Highly significant.

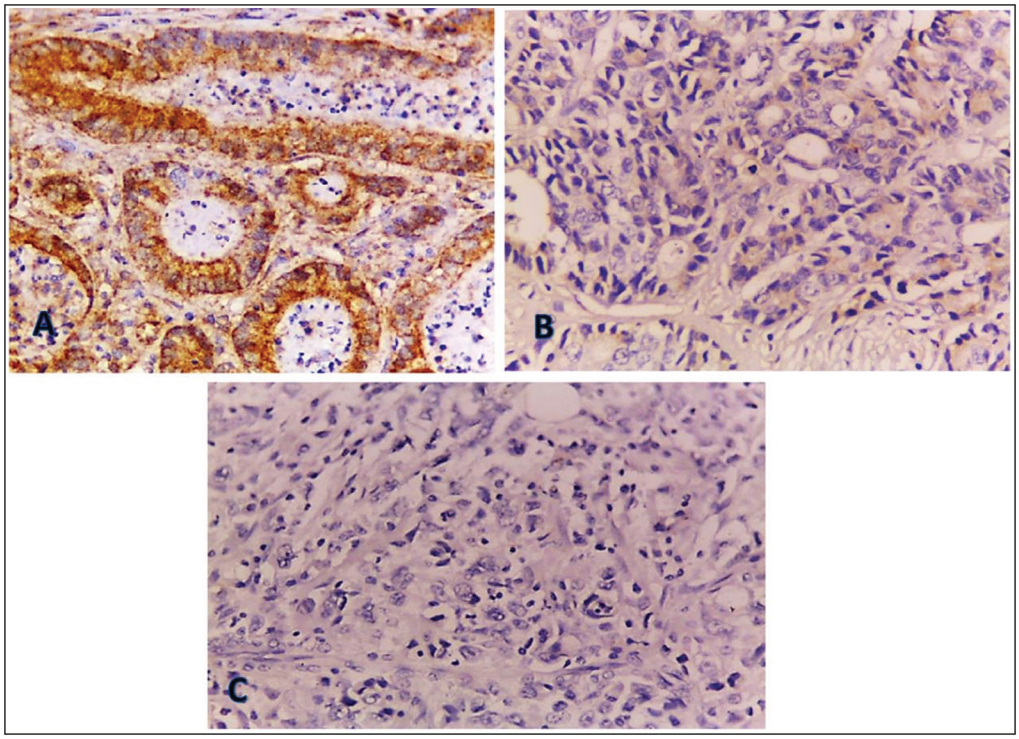
Statistical analysis was performed on the relation between tumor grade, and clinicopathological variables. It revealed a positive statistical highly significant correlation between tumor grade and depth of tumor invasion, lymph node metastasis, and TNM stage (P<0.01 for each). There was a significant statistical correlation between tumor grade and tumor size and distant metastasis (P<0.05 for both). On the other hand, there was insignificant statistical correlation between tumor grade and patient age, sex and tumor site (P>0.05 for each). This was in contrast with Said *et al.* (2021), who found that there was no statistical relation between tumor grade and TNM stage (P=0.095). This may be explained by the difference in histopathological subtypes of the selected cases.

Homeobox (*HOX*) genes regulate normal cell proliferation and differentiation in embryogenesis and were involved in tumorigenesis when dysregulated (Shah and Sukumar, 2010). Among which, HOXA13, a highly conserved transcription factor in the *HOX* gene family, is associated with a variety of human tumors (Qin *et al.*, 2019).

In this study, HOXA13 was highly expressed in 66.7% of CRC cases while in adenoma cases, 76.9% of adenoma without dysplasia cases were negative for HOXA13 expression while 71.4% of cases of adenoma with dysplasia were positive for HOXA13 expression. It showed a negative expression in normal colonic mucosa of the control group of viable margins of intestinal infarction cases and in 65% of the colonic adenoma cases with presence or absence of dysplasia.

This is in agreement with the study by Ma *et al.* (2014), who found that *HOXA13* was overexpressed

Figure 2



(a) STK4/MST1 immunohistochemistry showing strong cytoplasmic staining in more than 50% of cells of a tubulovillous adenoma without dysplasia, score 6 (high) (ABC ×400). (b) STK4/MST1 immunohistochemistry showing weak cytoplasmic staining in 50% of cells of a moderately differentiated colorectal adenocarcinoma, score 3 (low) (ABC ×400). (c) STK4/MST1 immunohistochemistry showing negative cytoplasmic staining in the cells of a poorly differentiated colorectal adenocarcinoma, score 0 (low) (ABC ×400).

Table 3 Correlation between human class I homeobox A13 and STK4/MST1 immunoexpression in studied cases

HOXA13 expression	STK4/MST1 expression [*n* (%)]		Total [*n* (%)]	*P* value
	Low	High		
Negative	4 (19)	6 (66.7)	10	
Positive	17 (81)	3 (33.3)	20	<0.01**
Total	21 (70)	9 (30)	30	

HOXA13, human class I homeobox A13.
**Highly significant.

in esophageal squamous cell carcinoma tissues when compared with that in normal tissues.

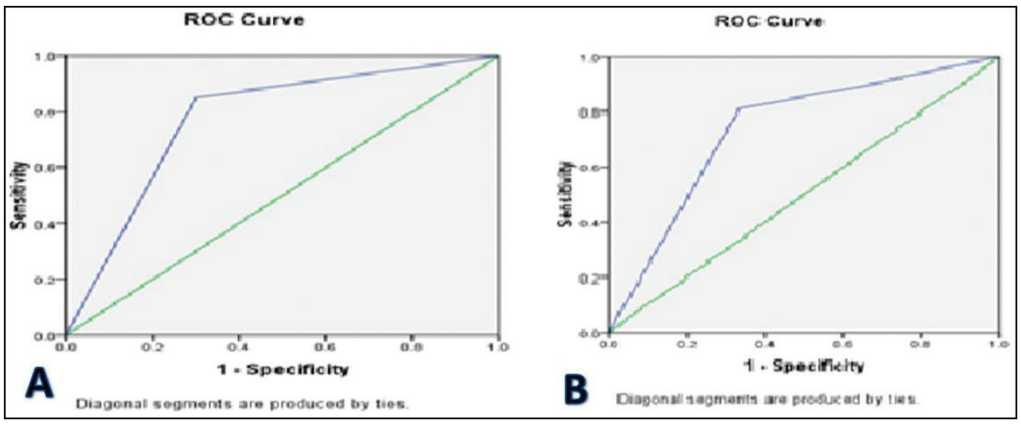
Statistical analysis was performed on the relation between high HOXA13 expression and lymph node metastasis and TNM stage. It revealed a highly significant direct statistical relation (P<0.01 for each) and a significant direct statistical relation with tumor grade and depth of tumor invasion (T) (P<0.05 for both).

These findings are consistent with the study by Gu *et al*. (2020), who found that HOXA13 was highly expressed in colon cancer tissues than adjacent normal tissues, and its expression was associated with histological grade (P=0.030), T stage (P=0.027), and N stage (P=0.045). This was explained by that *HOXA13* promoted tumor formation through the activation of Wnt/β-Catenin pathway. *HOXA13* promotes the nuclear translocation of β-catenin which is a critical step in colorectal tumorigenesis. Our study confirmed that HOXA13 may potentially serve as an oncogenic factor in colon cancer.

Also Qiao *et al*. (2021) stated that HOXA13-elevated expression was positively correlated with tumor grade (P<0.001), tumor invasion (P=0,002), lymph node metastasis (P<0.001), distant metastasis (P<0.001), and higher TNM stage (P<0.001). HOXA13 immunoexpression seems to gradually increase in the transition from normal colonic tissue, adenomatous dysplastic area, and adenocarcinoma with a prevalent nuclear staining in CRC samples. Our study suggested that this may provide a potential therapeutic strategy for the treatment of HOXA13-driven CRC metastasis. In the same context, Qin and Zhou (2022) declared that the expression of HOXA13 was elevated in gastric carcinoma tissues compared with adjacent normal tissues.

In contrast, Bhatlekar *et al*. (2014) stated that HOXA13 was expressed more in the normal tissue than in malignant tissues. As HOXA family genes were found to be downregulated in primary nonsmall cell lung cancers compared with normal lung tissues. Relative to

Figure 3



(a) Validity of HOXA13 and (b) STK4/MST1 expression in the prediction of metastatic potential using TNM stage in carcinoma cases. HOXA13, human class I homeobox A13.

genes of the HOXA family while genes of the HOXC and HOXD families were strongly upregulated in primary lung tumors through the activation of integrin vß3 and TGF-ß pathways. This may be explained by the heterogeneity of expression of HOX gene members in the same tumor.

STK4/MST1 is a component of the Hippo pathway and is shown to have an important role to control the organ size and cell to cell contact inhibition ability and is considered a potential tumor suppressor (Han, 2019).

In this study, STK4/MST1 was highly expressed in 100% of normal colonic mucosa of the control group. In adenoma cases, 100% of high expression cases were adenomas without dysplasia cases, while 80% of low expression cases were seen in adenomas with dysplasia cases. According to carcinoma cases, it showed low expression in 70% of CRC cases. So STK4/MST1 was significantly downregulated in tumor tissues compared with their corresponding nontumor part. The current study supported that STK4/MST1 might act as a tumor suppressor kinase and may have a role in the carcinogenesis of CRC. This was in agreement with Hsu *et al*. (2020), who stated that STK4/MST1 was highly expressed in the benign section but low or no expression of STK4 in colorectal tumors and metastatic tissues (P<0.01).

In this study, there was a highly significant inverse statistical relation between STK4/MST1 immunohistochemical expression and tumor grade, depth of tumor invasion (T) and TNM stage (P<0.01 for each), and significant inverse statistical relation with both tumor size and distant metastasis (M) (P<0.05 for both).

This is matched with the Lin *et al*. (2020) study who found significant inverse relation between STK4/MST1 and higher grade (P=0.001), depth of invasion of the primary tumor (P=0.003), and distant metastasis (P=0.003). This may be explained by that STK4 defect results in the failure of β-catenin phosphorylation and ubiquitination that may subsequently lead to β-catenin accumulation and consequently result in colon cancer metastasis. Consequently, our results might provide further evidence of being STK4 as a biomarker of aggressiveness and poor prognosis in CRC.

Previous studies have found a correlation between STK4/MST1 expression and poor prognostic factors of other malignant tumors such as pancreatic carcinoma (Cui *et al*., 2019), breast cancer (Jin *et al*., 2021), and hepatocellular carcinoma (Qiu *et al*., 2021).

In contrast with this study, Drexler *et al*. (2021) found that there was no statistical relation between STK4/MST1 and tumor grade (P=0.13) and TNM stage (P=0.55) in pancreatic carcinoma but significant correlation with distant metastasis (P>0.001). As they found significant upregulation of nearly all Hippo pathway components including STK4/MIST1 in pancreatic duct adenocarcinoma compared with healthy pancreatic tissue in nonmetastatic carcinoma with a highly significant inactive shift in patients with metastases. This is explained by that the Hippo pathway is inactive in metastasized patients resulting in nuclear translocation of YAP and an enhanced target gene expression through transcriptional factors TEAD2 and TEAD3 with prometastatic and proliferative effects. So, we revealed that the Hippo pathway has a huge impact on disease progression with metastatic spread and is clinically highly relevant as a shift in the balance toward the inactive pathway which predicts an unfavorable prognosis.

Concerning the relation between HOXA13 and STK4/MST1 expression, HOXA13 expression showed a highly significant inverse statistical correlation with STK4/MST1 expression in the studied cases of

CRC (P<0.01).This may explained by the fact as we noted before that β-catenin overexpression or downregulation regulates anchorage-independent cell growth, β-catenin expression is negatively associated with STK4/MST1 levels, and it can be directly phosphorylated by STK4/MST1 that subsequently leads to β-catenin ubiquitination. On the other side HOXA13 overexpression leads to nuclear translocation of β-catenin, thereby maintaining the proliferation and metastasis of colon cancer. So STK4/MST1 downregulation or HOXA13 overexpression leads to β-catenin activation and promotes cell growth and CRC progression.

Conclusions

In this study, HOXA13 was found to be overexpressed, while STK4/MST1 was downexpressed in progression from colorectal adenoma to adenoma with dysplasia to adenocarcinoma cases in comparison to normal colonic mucosa. So, these results support that dysregulated expressions of HOXA13 and STK4/MST1 genes play an important role in carcinogenesis and malignant progression of CRCs.

HOXA13 and STK4/MST1 might have a potential role as independent prognostic factors in CRC and may have validity to predict metastatic potential of CRC.

Recommendations

Further studies using different molecular methods on HOXA13 and STK4/MST1 are recommended to explore more about the mechanisms by which HOXA13 and STK4/MST1 may contribute to the progression of CRC. Using STK4 to target the Hippo pathway could improve the outcome of patients and help develop new strategies in CRC therapy.

Conflicts of interest

There are no conflicts of interest.

References

Abou Gabal H, Ahmed N, Meckawy G, Yassin R, Hakim S (2022). Evaluation of EZH2 and ERRα in colorectal carcinoma: an immunohistochemical study. Pol J Pathol 72:200–210.

Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Winchester DP (2017). The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more 'personalized' approach to cancer staging. CA Cancer J Clin 67:93–99.

Awan R, Sirinukunwattana K, Epstein D, Jefferyes S, Qidwai U, Aftab Z, Rajpoot N (2017). Glandular morphometrics for objective grading of colorectal adenocarcinoma histology images. Sci Rep 7:1–12.

Bhatlekar S, Fields JZ, Boman BM (2014). HOX genes and their role in the development of human cancers. J Mol Med 92:811–823.

Botti G, Cillo C, De Cecio R, Malzone MG, Cantile M (2019). Paralogous HOX13 genes in human cancers. Cancers 11:699.

Cui J, Zhou Z, Yang H, Jiao F, Li N, Gao Y, Quan M (2019). MST1 suppresses pancreatic cancer progression via ROS-induced pyroptosis. Mol Cancer Res 17:1316–1325.

Di Mauro A, Scognamiglio G, Aquino G, Cerrone M, Liguori G, Clemente O, Tatangelo F (2021). Aberrant expression of long non coding RNA HOTAIR and de-regulation of the paralogous 13 HOX genes are strongly associated with aggressive behavior of gastro-entero-pancreatic neuroendocrine tumors. Int J Mol Sci 22:7049.

Drexler R, Küchler M, Wagner KC, Reese T, Feyerabend B, Kleine M, Oldhafer KJ (2021). The clinical relevance of the Hippo pathway in pancreatic ductal adenocarcinoma. J Cancer Res Clin Oncol 147:373–391.

Elhadidy AA, Haydara T (2022). Increase young age incidence of colorectal carcinoma among cohort of Egyptian population. Al-Azhar Int Med J 3:71–75.

Graves JD, Gotoh Y, Draves KE, Ambrose D, Han DK, Wright M, Krebs EG (1998). Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1. EMBO J 17:2224–2234.

Gu Y, Gu J, Shen K, Zhou H, Hao J, Li F, Dong Y (2020). HOXA13 promotes colon cancer progression through β-catenin-dependent WNT pathway. Exp Cell Res 395:112238.

Hafez FS, Meckawy GR, Alorabi M, Shakweer MM (2022). Interpretation of P16 expression as a marker of HPV in colorectal cancer. Histol Histopathol 37:18439–18439.

Han Y (2019). Analysis of the role of the Hippo pathway in cancer. J Transl Med 17:1–17.

Hong AW, Meng Z, Guan KL (2016). The Hippo pathway in intestinal regeneration and disease. Nat Rev Gastroenterol Hepatol 13:324–337.

Hsu TI, Lu PJ, Hsiao M (2020). Serine/theonine kinase 4 is a potential biomarker for assessment of β-catenin-mediated colon cancer prognosis. FASEB J 34(**S1**):1–1.

Jin X, Zhu L, Xiao S, Cui Z, Tang J, Yu J, Xie M (2021). MST1 inhibits the progression of breast cancer by regulating the hippo signaling pathway and may serve as a prognostic biomarker. Mol Med Rep 23:1–12.

Lin CH, Hsu TI, Chiou PY, Hsiao M, Wang WC, Chen YC, Lu PJ (2020). Downregulation of STK4 promotes colon cancer invasion/migration through blocking β-catenin degradation. Mol Oncol 14:2574–2588.

Ma RL, Shen LY, Chen KN (2014). Coexpression of ANXA2, SOD2 and HOXA13 predicts poor prognosis of esophageal squamous cell carcinoma. Oncol Rep 31:2157–2164.

Mokhtar N, Salama A, Badawy O, Khorshed E, Mohamed G, Ibrahim M, Abdelazim H (2016). Cancer pathology registry 2000–2011. Cairo, Egypt: National Cancer Institute Cairo University.

Mostafa MAE, Kayser HH, Allam AS, Saeed Hakim AR (2021). Serum osteopontin and its relation to colorectal carcinoma in egyptian patient. QJM 114(Supplement_1):hcab100.

Nesteruk K, Janmaat VT, Liu H, Ten Hagen TL, Peppelenbosch MP, Fuhler GM (2020). Forced expression of HOXA13 confers oncogenic hallmarks to esophageal keratinocytes. Biochim Biophys Acta 1866:165776.

Ouyang H, Zhou E, Wang H (2019). Mst1-hippo pathway triggers breast cancer apoptosis via inducing mitochondrial fragmentation in a manner dependent on JNK–Drp1 axis. Onco Targets Ther 12:1147.

Qiao C, Huang W, Chen J, Feng W, Zhang T, Wang Y, Xia L (2021). IGF1-mediated HOXA13 overexpression promotes colorectal cancer metastasis through upregulating ACLY and IGF1R. Cell Death Dis 12:1–18.

Qin Z, Zhou C (2022). HOXA13 promotes gastric cancer progression partially via the FN1-mediated FAK/Src axis. Exp Hematol Oncol 11:1–16.

Qin Z, Chen Z, Weng J, Li S, Rong Z, Zhou C (2019). Elevated HOXA13 expression promotes the proliferation and metastasis of gastric cancer partly via activating Erk1/2. Onco Targets Ther 12:1803.

Qiu Y, Huang D, Sheng Y, Huang J, Li N, Zhang S, Yan J (2021). Deubiquitinating enzyme USP46 suppresses the progression of hepatocellular carcinoma by stabilizing MST1. Exp Cell Res 405:112646.

Said EM, Abostate HM, Emara NM, Agina HA (2021). The significance of aldehyde dehydrogenase 1A1 expression in colorectal carcinoma. Benha Med J 39:2357-0016.

Shah N, Sukumar S (2010). The Hox genes and their roles in oncogenesis. Nat Rev Cancer 10:361–371.

Singh K, Pruski MA, Polireddy K, Jones NC, Chen Q, Yao J, Bailey JM (2020). Mst1/2 kinases restrain transformation in a novel transgenic model of Ras driven non-small cell lung cancer. Oncogene 39:1152–1164.

Song L, Peng X, Li Y, Xiao W, Jia J, Dong C, Han X (2017). The SEPT9 gene methylation assay is capable of detecting colorectal adenoma in opportunistic screening. Epigenomics 9:599–610.

Wang Y, He B, Dong Y, He GJ, Qi XW, Li Y, Li JJ (2021). Homeobox-A13 acts as a functional prognostic and diagnostic biomarker via regulating P53 and Wnt signaling pathways in lung cancer. Cancer Biomark 31:1–16.

Yu M, Zhan J, Zhang H (2020). HOX family transcription factors: related signaling pathways and post-translational modifications in cancer. Cell Signal 66:109469.

Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, Bardeesy N (2009). Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell 16:425–438.

Zhu D, Sun C, Qian X (2019). MST1 suppresses viability and promotes apoptosis of glioma cells via upregulating SIRT6 expression. J Integr Neurosci 18:117–126.