Research Article

Immunohistochemical Expression of TWIST1 in Colorectal Adenocarcinoma

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Abstract
Background: Colorectal cancer is one of the most common cancers worldwide. It is the result of a complex genetic mutations and environmental factors. TWIST1 is expressed in a wide range of normal and neoplastic tissues and has emerged as an important regulator of diverse functions critical in cancer biology including cellular transition, proliferation, metastasis and gene expression. **Methods:** Immunohistochemical staining of TWIST1 was conducted on 50 tissue specimens of colorectal adenocarcinoma by using the avidin biotin-peroxidase complex method with diaminobenizidine chromagen detection system. **Results:** High TWIST1 expression was detected in 62% of cases. TWIST1 expression showed statistically significant correlation with lymph node metastasis (p= 0.007) and advanced tumor stage assessed by modified Dukes staging (p = 0.043). **Conclusions:** TWIST1 expression is significantly associated with poor prognostic factors and thus can be used as a prognostic indicator for colorectal carcinoma patients.
Key words: TWIST1, Colorectal Carcinoma, Immunohistochemistry. All authors have no conflict of interest.

Introduction
Colorectal cancer (CRC) is the third most common cancer worldwide and a leading cause of mortality and morbidity, representing a major public health problem (Torre et al., 2015). In Egypt, the cancer incidence in Egyptian males is 2.63% while in females is 2.28% among cancers. Rectal cancer incidence in Egyptian males is 0.84% while in females is 0.72% among cancers (Ibrahim et al., 2014).

TWIST proteins are mainly present in precursor cells including the myogenic, osteoblastic, chondroblastic, odontoblastic and myelomonocytic lineages (Norozi et al., 2016). TWIST1 is therefore involved in many processes such as myogenesis, apoptosis, cell–cell interaction, cell migration and cell signaling. (Zhao et al., 2017). In colorectal carcinoma, studies have demonstrated an increased expression of TWIST1 (Raouf et al., 2019).

Material and Methods
1. Tissue specimens:
The present study comprised 50 randomly selected cases of primary colorectal adenocarcinomas. Tumor type and grade were evaluated according to WHO criteria (Hamilton et al., 2010). Tumor stage was estimated by TNM staging (Edge and Compton, 2010) and modified Dukes staging (Li et al., 2013).

2. Immunohistochemistry:
Primary antibody against TWIST1: monoclonal mouse antibody (100µl, concentrated, clinilab Laboratories), diluted at (1:300). For negative control the primary antibody was replaced with PBS. Positive control was cervical carcinoma.

3. Scoring of Immunostaining:
Regarding nuclear TWIST1 expression, results are evaluated according to a score that combines the intensity with the scope of the positive staining area. The intensity (I) of staining is scored as 0(no staining), 1(weak), 2(moderate), 3(strong). The density (D) of staining is scored as 1 (<10%), 2 (10-50%), 3 (50-80%) or 4 (80-100%) according to the percentage of positively stained regions in relation to the total tumor area. The final staining scores (0-12) are the product of IxD. Samples which are >4 are negative/low expression, those with ≥4 are considered high expression (Yusup et al., 2017).

Statistical analysis
Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS software version 16).

Results

1. Clinicopathological Features: Data regarding different clinical and histopathological features for colorectal adenocarcinoma patients are summarized in Table (1).

Clinicopathological features	No. (%)
Age	
<50	17 (34%)
≥50	33 (66%)
Gender	
Male	28 (56%)
Female	22 (44%)
Location	
Colon	32 (64%)
Rectum	18 (36%)
Tumor grade	
Grade II	40 (80%)
Grade III	10 (20%)
Nodal status	
Negative	22 (44%)
Positive	28 (56%)
Modified Dukes stage	
B1	7 (12%)
B2	16 (32%)
C1	8 (18%)
C2	19 (38%)

2. Immunohistochemical expression of nuclear TWIST1 and its association with patients' clinicopathological features.

Table (2): Association between nuclear TWIST1 expression and clinicopathological features for patients with CRC (n=50).

Clinicopathological features	*Total* 50 (100%)	Nuclear TWIST1 expression		*P* value
		Negative/low expression 19 (38%)	High expression 31 (62%)	
Age				
<50	17 (34)	8 (47.1)	9 (52.9)	0.260
≥50	33 (66)	11 (33.3)	22 (66.7)	
Gender				
Male	28 (56)	11 (39.3)	17 (60.7)	0.534
Female	22 (44)	8 (36.4)	14 (63.6)	
Location				
Colon	32 (64)	13 (40.6)	19 (59.4)	0.421
Rectum	18 (36)	6 (33.3)	12 (66.7)	
Tumor grade				
Grade II	40 (80)	18 (45)	22 (55)	0.042*
Grade III	10 (20)	1 (10)	9 (90)	
Nodal status				
Negative	22(44)	13 (59.1)	9 (40.9)	0.007*
Positive	28 (56)	6 (21.4)	22 (78.6)	
Modified Dukes stage				
B1	7 (14)	6 (85.7)	1 (14.3)	0.043*
B2	14 (32)	5 (35.7)	9 (64.3)	
C1	10 (16)	3 (30)	7 (70)	
C2	19 (38)	5 (26.3)	14 (73.7)	

*P - value < 0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.

In the current study, a statistically significant association was detected between nuclear TWIST1 expression and tumor grade (p=0.042). Also, a statistically significant asso-ciation was detected between TWIST1 expression and lymph node metastasis (p=0.007) and advanced tumor stage assessed by modified Dukes staging (p =0.043).

Discussion

In the present study, 62% of cases showed high expression of TWIST1, while 38% showed negative/low expression. Raouf et al., (2019) reported similar results, while Yusup et al., (2017) reported lower expression (54.7%).

In the current study, a statistically significant positive association between nuclear TWIST1 expression and regional lymph node involvement was detected where 78.6% of cases with positive lymph node metastases showed high expression. This was in accordance with Zhu et al., (2018) and Raouf et al., (2019) who detected higher expressions in cases with positive nodal metastasis.

However, this was on the contrary to what was reported by Gomez et al., (2011);Kim et al., (2014) who found non-significant association between TWIST1 expression and nodal metastasis.

Concerning modified Dukes classification, we also detected a statistically significant positive association where (14.3%) of stage B1 cases and (64.3%) of stage B2 cases showed high expression while (70%) of stage C1 cases and (73.7%) of stage C2 cases showed high expression. This finding was consistent with other studies who reported significant higher expressions in Dukes B and C cases (Abd El Naby et al., 2015).

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