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ORIGINAL ARTICLE

NCK-Associated Protein 1 (NCKAP1) And Angiopoietin-Like Protein 1 (ANGPTL1): A novel Prognostic Markers and Promising Therapeutic Target for Colorectal Carcinoma Patients

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ABSTRACT

Background: NCK-associated protein 1 (NCKAP1) may have tumor-promoting or suppressive impacts according to the type of tissue involved. Transforming growth factor- β1 (TGFβ1) promotes tumor invasion and metastasis by inducing epithelial-mesenchymal transition (EMT). NCKAP1 may enhance EMT by promoting the activities of TGFβ1. Angiopoietin-like protein 1 (ANGPTL1) was suggested to be a tumor suppressor by lessening the expression of stem cell transcription factor SOX2, which reduces the invasive and metastatic abilities of tumor cells. Hence, the aim of this current study explore the effect of each of the following biomarkers (NCKAP1, ANGPTL1, TGFβ1, and SOX2) on colorectal cancer (CRC) prognosis and its clinical outcome, which could help in novel treatments' development against CRC.

Methods: Seventy-two CRC tissue blocks were obtained retrospectively, spanning January 2019 and December 2023. CRC tissue sections are then exposed to all steps of the immunohistochemical (IHC) technique for staining NCKAP1, ANGPTL1, TGFβ1, and SOX2.

Results: Positive ANGPTL1 expression was significantly correlated with good clinical parameters, lower mortality rates, and better prognosis in CRC patients. On the other hand, SOX2, NCKAP1, and TGFβ1 were significantly correlated with poor prognosis and negatively associated with ANGPTL1 in CRC patients.

Conclusion: ANGPTL1 can be considered a good prognostic indicator in CRC patients. On the contrary, the other three biomarkers SOX2, NCKAP1, and TGFβ1 could act as worse outcome indicators and blockage of these markers could be beneficial in preventing CRC progression.

Keywords: ANGPTL1; NCKAP1; CRC; IHC

INTRODUCTION

Colorectal cancer (CRC) may be considered the third most common fatal tumor and the third driving cause of death from cancer around the world **(1).** Despite movement in medications, CRC is still a lethal disease with a high rate of death, about half of CRC patients have metastatic disease with low survival rates **(1).** Both relapse and metastasis are two critical factors for the assessment of prognosis and survival of colon cancer patients. The process of tumor metastasis is complicated and includes hereditary changes within the malignant cells and their microenvironment **(2).** Much is still obscure about the CRC metastatic mechanisms. In this manner, it is imperative and pressing to investigate and clarify novel molecules related to CRC metastasis that could give new biomarkers for CRC diagnosis.

NCK-associated protein 1 (NCKAP1) is one of the proteins involved in the pathogenesis of Alzheimer's disease as a portion of the WASF complex (Wiskott-Aldrich syndrome protein family) in conjunction with CYFIP1, ABI2, WASF2, and BRK1. NCKAP1 plays a crucial role in cancer pathogenesis and directs different intracellular activities such as apoptosis, invasion, and migration of tumor cells **(3).** NCKAP1 overexpression is related to tumor advancement and poor outcomes in many cancer types as breast

(4), lung **(5)**, and colon cancers **(6)**. On the other hand, **Zhong et al., 2019** and **Chen et al., 2022** already declared that low expression of NCKAP1 in renal and liver cell cancers is related to diverse prognoses **(7-8).** This information proposes that NCKAP1 may have cancer-suppressive or promoting impacts on different cancer types.

Previous research has investigated the possible mechanisms underlying NCKAP1 tumor-promoting or suppressive activity. **Cowell et al., 2017** proposed that NCKAP1 has a basic role in tumor invasion and migration by directing the stability and function of WASF3 **(9)**, and **Swaminathan et al., 2021** reported that focused suppression of NCKAP1 repressed melanoma progression through using an experimental model deficient in BRAF/PTEN **(10). Zhu et al., 2019** suggested that NCKAP1 is an autophagy-related gene and is essentially related to disease-free survival in melanoma patients **(11)**. These findings demonstrate that NCKAP1 may have oncogenic effects in certain cancers. On the contrary, NCKAP1 has tumor-suppressing effects on hepatocellular carcinoma cells through regulating Rb1/p53 **(7)**. **Chen et al., 2022** identified that NCKAP1 may have a crucial tumor-suppressive function in CRC through Epithelial-mesenchymal transition (EMT), ribosomal signaling, and oxidative phosphorylation-related signaling pathways **(8).**

Epithelial-mesenchymal transition (EMT) is the method enabling malignant epithelial cells to acquire mesenchymal properties such as resisting apoptosis, invasion, and spread **(12).** Transforming growth factor- β1 (TGFβ1) advances tumor invasion and metastasis by activating EMT **(13)**. **Kwon et al., 2023** detailed that the knockdown of the NCKAP1 gene plays a critical part in suppressing TGFβ1-mediated EMT in CRC **(6)**. Consequently, NCKAP1 may be used as a reliable biomarker in CRC prognosis, and to the leading of our information, there are no adequate investigations about the expression and clinical significance of NCKAP1 at CRC has been recognized.

Angiopoietin-like protein 1 (ANGPTL1) helps in different pathophysiological processes, including angiogenesis **(14)**, tissue inflammation **(15)**, hematopoietic stem cell behavior **(16)**, metabolism of lipids **(17)**, and progression of malignancies **(18)**. ANGPTL1 is downregulated in different cancers such as lung **(19)**, thyroid **(20)**, hepatocellular carcinoma **(21)** and colorectal cancer **(22).** ANGPTL1 low expression is related to poor prognostic factors in breast and lung cancers, including progressive cancer stage, grade as well as lymphatic spread **(23)**. In truth, ANGPTL1 was suggested to have a cancer suppressor role in many cancers.

The tumor suppressive action of ANGPTL1 may be explained by different mechanisms. ANGPTL1 restrains the angiogenic activity of VEGF and initiates extracellular signal-regulated kinase 1/2 (ERK1/2)-related antiapoptotic action **(24)**. ANGPTL1 may modify cellular morphology through restraint of actin stretch fiber arrangement. Moreover, ANGPTL1 might initiate the process of mesenchymal-to-epithelial transition (MET) via many pathways such as miR-630, SLUG (SNAIL-related zinc-finger transcription factor) and integrin α1β1, hence permitting malignant cells to regain the properties of epithelial tissue **(25).**

Cancer stem cells (CSCs) constitute a small group of tumor cells, that have self-renewal and uncontrolled proliferative capacity, they are significant in carcinogenesis and cancer progression via enhancing tumor invasion and migration through the EMT **(26).** ANGPTL1 downregulated the markers of CSCs by upgrading the expression of FOXO3a, which helped in the downregulation of SOX2 expression (a stem cell transcription factor) which diminished the migration/invasion capacities of tumor cells in CRC. ANGPTL1 expression was indirectly related to CSC markers expression and significantly associated with favorable outcomes in colorectal cancer cases **(22).** However, the detailed explanations of ANGPTL1's effects on CSC in CRC cases are not completely understood.

In this way, the current study aimed to examine the immunohistochemical (IHC) expression of NCKAP1, ANGPTL1, SOX2, and TGFβ1 within the CRC patients and identify their correlations with clinic-pathological results and patients' outcomes. Besides, to investigate whether NCKAP1 and ANGPTL1 may be utilized as potential targets for treatment and prognosis assessment in CRC patients.

METHODS

This work is a cross-sectional study that used Seventy-two sections from formalin settled paraffin inserted tissue blocks diagnosed as primary colorectal carcinoma. The cases were provided by Pathology Department archives, Faculty of Medicine, and Surgical Oncology Department from January 2019 to December 2023. The clinicopathologic information, and histopathology reports, together with hematoxylin and eosin (H&E) stained slides from CRC cases were surveyed to affirm the diagnosis. This work

included cases with sporadic CRC during the study period with accessible clinical & statistical information, tissue blocks, and reports. Exclusion criteria incorporate cases with; broadly necrotic tumors, missing agreement on the diagnosis histologically, grading, or IHC markers scoring. This work was conducted in agreement with the Helsinki Declaration and written consent was obtained from each member. Moreover, the current study was permitted by the Ethical Committee of our university according to the Egyptian Ethical Guidelines (ZU-IRB#11223-17/10-2023).

Immunohistochemistry

The immunostaining method was a streptavidin-biotin amplified system. The slides were subjected to consequent steps of deparaffinization, rehydration, and endogenous peroxidase activity blocking. Antigen recovery was done by boiling in citrate buffer saline (pH= 6) taken after by cooling at room temperature. Primary antibodies against, NCKAP1 (rabbit polyclonal antibody, Catalog #ab198924, 1:50 dilution, Abcam, Cambridge, UK), ANGPTL1 (rabbit polyclonal antibody, Catalog # sc-271841, 1:100 dilution Santa Cruz Biotechnology, CA, USA), TGFβ1 (rabbit Monoclonal antibody, Catalog # ab170874, 1:100 dilution, Abcam, Cambridge, UK), SOX2 (rabbit Monoclonal antibody, Catalog # ab92494, 1:100 dilution, Abcam, Cambridge, UK) were applied to the slides. The primary antibody was brooded overnight at room temperature and after that applied secondary antibody with diaminobenzidine (DAB) as a chromogen substrate and counter staining by Mayer's hematoxylin.

Immunostaining interpretation

NCKAP1: Patients were divided into two groups, the NCKAP1−ve group and the + group, depending on aberrant NCKAP1 cytoplasmic expression in tumor cells (7).

ANGPTL1: Cytoplasm and nuclear expression of ANGPTL1 in CRC tissue is considered positive. Intensity of IHC staining was scored as 0 (negative), 1 (weak), 2 (medium), and 3 (strong). Regarding the percentages of the positively stained cells in the whole cancer area, the extent of staining was scored as 0 (5%), 1(5–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The final staining scores (0–7) were assessed by the sum of the intensity and extent scores. The final staining score ≥ 2 was considered positive ANGPTL1 expression (27).

SOX2: was evaluated as a nuclear expression, any expression in cancer cells above 5% was considered positive (28).

TGF-β1: Cytoplasmic expression of TGF-β1 in more than 10% of the cancer cells was considered positive and negative if staining was present in less than 10% of the tumor cells (29).

STATISTICAL ANALYSIS

Categorical variables are presented as a number (percentage). Continuous variables presented as the (mean±SD) and median (range) with the Shapiro-Wilk test were used for checking the normality. Mann Whitney U test was used to comparison between 2 groups of non-normally distributed variables, while the Kruskal Wallis H test was used to comparison between greater than 2 groups of not-normally distributed variables. Fisher's exact test or Pearson's Chi-square test when appropriate compared the percent of categorical variables. In survival analysis, the Kaplan-Meier method was used to calculate survival curves and analyzed using the log-rank test. P value ≤0.05 was considered statistically significant. SPSS 22.0 for Windows and MedCalc Windows performed all statistics.

RESULTS

Patients' characteristics

The present study included 72 patients with CRC. The patients' age ranged from 22 to 70 years. The mean age was equal to 52.3+10.38. 14 of 72 patients (19.4%) had an early-stage disease and were exposed to radical surgery followed by adjuvant chemotherapy. On the other hand, 26 of CRC patients (36.1%) were presented with advanced disease. These patients had received adjuvant chemotherapy and were evaluated for response according to RECIST criteria. The follow-up time had a median of 32.71 months (range: 18-36 months). During this period, 50% of the patients were free from disease relapses. Recurrence took place in 50% of the following patients, while 55.6% of the patients died during the follow-up period. The clinicopathological characteristics of the seventy-two cases with CRC are summarized in Table (1).

Association of NCKAP1 and TGFβ1, ANGPTL1 and SOX2 expression with clinicopathological parameters

Regarding CRC patients, NCKAP1 was positive in 37 (51.4%) of the cases, TGFβ1 in 42 (58.3%), ANGPTL1 in 26 (36.1%) and SOX2 in 40 (55.6%) (Table 1). Both NCKAP1 and TGFβ1 were stained in cytoplasm**,** while ANGPTL1 was stained in both cytoplasm and nucleus and SOX 2 was stained in the nucleus of cancer cells (Figure 1).

Positive ANGPTL1 expression was significantly correlated with lower tumor grade, stage, and absent lymphatic spread. Moreover, ANGPTL1

was found to be associated with lower patient mortality rates. These findings propose that ANGPTL1 significantly correlates with good prognosis in CRC patients. In addition, there was a significant indirect association between ANGPTL1 and other markers SOX2, NCKAP1, and TGFβ1 immune expressions (P= 0.000, 0.003, 0.049 respectively).

SOX2 expression showed a significant correlation with larger tumor size, higher stages, and grades of tumor. A significant correlation has also been found between SOX2 positive expression and lymph nodal spread (P= 0.047). There was a significant correlation between SOX2 and both NCKAP1 and TGFβ1 expressions. On the other hand, SOX2 expression was in a significant indirect correlation with ANGPTL1 expression (P=0.000).

Positive NCKAP1 expression revealed a significant association with high tumor stage (P= 0.02). NCKAP1 was positively correlated with SOX2 and TGFβ1 staining (P=0.000) for both markers. On the contrary, NCKAP1 showed a negative correlation with ANGPTL1 (P= 0.003). TGFβ1 expression is correlated with older age, high tumor stage, and presence of distant metastases. TGFβ1 is significantly correlated with NCKAP1 and SOX2 (P=0.000) for both markers. On the opposite side, TGFβ1 showed a negative association with ANGPTL1 (P= 0.049).

Association between NCKAP1 and TGFβ1, ANGPTL1 and SOX2 expression and survival

Analysis of overall survival (OS) and disease-free survival (DFS) of CRC patients using the Kaplan-Meier method (Figure 2) clearly revealed that shorter OS & DFS are associated with positive expressions of SOX2, NCKAP1, and TGFβ1. On the contrary, cases positive for ANGPTL1 had longer OS & DFS (Table 3).

Association between NCKAP1 and TGFβ1, ANGPTL1 and SOX2 expression with both tumor relapse and mortality.

Concerning recurrence and mortality rates, a significant association between lower rates of disease recurrence and positive ANGPTL1 IHC staining was observed, which suggests the role of ANGPTL1 in protection against cancer progression. On the other hand, a significant correlation between SOX2, NCKAP1, and TGFβ1 positive IHC staining with both relapse and mortality was observed, which denotes that SOX2, NCKAP1, and TGFβ1 can be considered poor prognostic indicators Tables (2).

Table 1: Clinicopathological parameters of 72 CRC patients

Variable	No (%)
Gender	
Male	43(59.7)
Female	29(40.3)
Age	
Mean±SD	52.3+10.38
Median	52.5
Range	48
Age	
<50	30(41.7)
≥50	42(58.3)
Tumor size (cm)	
Mean±SD	1.777+0.418
Median	2
Range	1
Tumor size	
<3	16(22.2)
≥3	56(77.8)
Tumor grade	
low	34(47.2)
High	38(52.8)
Tumor stage	
T1	14(19.4)
T2	12(16.7)
T3	20(27.8)
T4	26(36.1)

Variable	No (%)
Nodal stage	
N0	13(18.1)
N1	59(81.9)
Distal metastasis	
M0	37(51.4)
M1	35(48.6)
Relapse	
Absent	36(50)
present	36(50)
Death	
Alive	32(44.4)
Dead	40(55.6)
ANGPTL1	
Negative	46(63.9)
Positive	26(36.1)
SOX2	
Negative	32(44.4)
Positive	40(55.6)
NCKAP1	
Negative	35(48.6)
Positive	37(51.4)
TGFB1	
Negative	30(41.7)
Positive	42(58.3)

Table 2: Correlation between NCKAP1, ANGPTL1, SOX2 and TGFB1 immunostaining with clinicopathologic parameters, mortality and relapse

Variable	ANGPTL1 expression		P value	SOX2 expression		P value	NCKAP 1 ssion		P Value	TGFB1 expression		P value
	Negative No (%)	Positive No (%)		Negative No (%)	Positive No (%)		Negative No (%)	Positive No (%)		Negative No (%)	Positive No (%)	
Gender												
Male	26(60.5)	17(39.5)	0.618	18(41.9)	25(58.1)	0.635	25(58.1)	18(41.9)	0.058	20(46.5)	23(53.5)	0.340
Female	20(69)	9(31)		14(48.3)	15(51.7)		10(34.5)	19(65.5)		10(34.5)	19(65.5)	
Age												
<50	19(63.3)	11(36.7)	1.000	16(53.3)	14(46.7)	0.235	18(60)	12(40)	0.151	19(63.3)	11(36.7)	0.003
≥50	27(64.3)	15(35.7)		16(38.1)	26(61.9)		17(40.5)	25(59.5)		11(26.2)	31(73.8)	
Tumor size	7(43.8)	9(56.3)	0.078	11(68.8)	5(31.3)	0.044	11(68.8)	5(31.3)	0.09	8(50)	8(50)	0.567
<3	39(69.6)	17(30.4)		21(37.5)	35(62.5)		24(42.9)	32(57.1)		22(39.3)	34(60.7)	
≥3												
CRC grade	17(50)	17(50)	0.028	21(61.8)	13(38.2)	0.009	18(52.9)	16(47.1)	0.637	14(41.2)	20(58.8)	1.000
Low	29(76.3)	9(23.7)		11(28.9)	27(71.1)		17(44.7)	21(55.3)		16(42.1)	22(57.9)	
High												
Tumor stage												
T1	4(28.6)	10(71.4)		9(64.3)	5(35.7)		10(71.4)	4(28.6)		13(92.9)	1(7.1)	
T2	2(16.7)	10(83.3)	0.000	10(83.3)	2(16.7)	0.002	9(75)	3(25)	0.02	7(58.3)	5(41.7)	0.000
T3	17(85)	3(15)		5(25)	15(75)		7(35)	13(65)		4(20)	16(80)	
T4	23(88.5)	3(11.5)		8(30.8)	18(69.2)		9(34.6)	17(65.4)		6(23.1)	20(76.9)	
Nodal stage	4(30.8)	9(69.2)		9(69.2)	4(30.8)	0.04	8(61.5)	5(38.5)	0.367	5(38.5)	8(61.5)	
N0	42(71.2)	17(28.8)	0.01	23(39)	36(61)		27(45.8)	32(54.2)		25(42.4)	34(57.6)	1.000
N1												

Distal metastasis M0 M1	23(62.2) 23(65.7)	14(37.8) 12(34.3)	0.809	18(48.6) 14(40)	19(51.4) 21(60)	0.48	21(56.8) 14(40)	16(43.2) 21(60)	0.167	22(59.5) 8(22.9)	15(40.5) 27(77.1)	0.002
Relapse Absent present	17(47.2) 29(80.6)	19(52.8) 7(19.4)	0.006	23(63.9) 9(25)	13(36.1) 27(75)	0.002	28(77.8) 7(19.4)	8(22.2) 29(80.6)	0.000	29(80.6) 1(2.8)	7(19.4) 35(97.2)	0.000
Mortality Alive dead	16(50) 30(75)	16(50) 10(25)	0.047	20(62.5) 12(30)	12(37.5) 28(70)	0.009	26(81.3) 9(22.5)	6(18.8) 31(77.5)	0.000	24(75) 6(15)	8(25) 34(85)	0.000
ANGPTL1 Negative Positive				10(21.7) 22(84.6)	36(78.3) 4(15.4)	0.000	16(34.8) 19(73.1)	30(65.5) 7(26.9)	0.003	15(32.6) 15(57.7)	31(67.4) 11(42.3)	0.049
SOX2 Negative Positive	10(31.3) 36(90)	22(68.8) 4(10)	0.000				26(81.3) 9(22.5)	6(18.8) 31(77.5)	0.000	21(65.6) 9(22.5)	11(34.4) 31(77.5)	0.000
NCKAP1 Negative Positive	16(45.7) 30(81.1)	19(54.3) 7(18.9)	0.003	26(74.3) 6(16.2)	9(25.7) 31(83.8)	0.000				24(68.6) 6(16.2)	11(31.4) 31(83.8)	0.000
TGFB1 Negative Positive	15(50) 31(73.8)	15(50) 11(26.2)	0.049	21(70) 11(26.2)	9(30) 31(73.8)	0.000	24(80) 11(26.2)	6(20) 31(73.8)	0.000			

Table 3: Multivariate COX-regression analysis for detection of the most independent prognostic factor affecting patient overall & Disease-free survival

variable	OS		DFS	
	HR (CI 95%)	P value	HR (CI 95%)	P value
ANGPTL1 Negative Positive	26.162 32.571	0.013	20.436 29.114	0.015
SOX 2 Negative Positive	32.22 25.442	0.002	28.989 18.989	0.004
NCKAP 1 Negative Positive	32.735 24.578	0.000	30.769 17.081	0.000
TGFB1 Negative Positive	35.448 23.376	0.000	34.367 14.434	0.000

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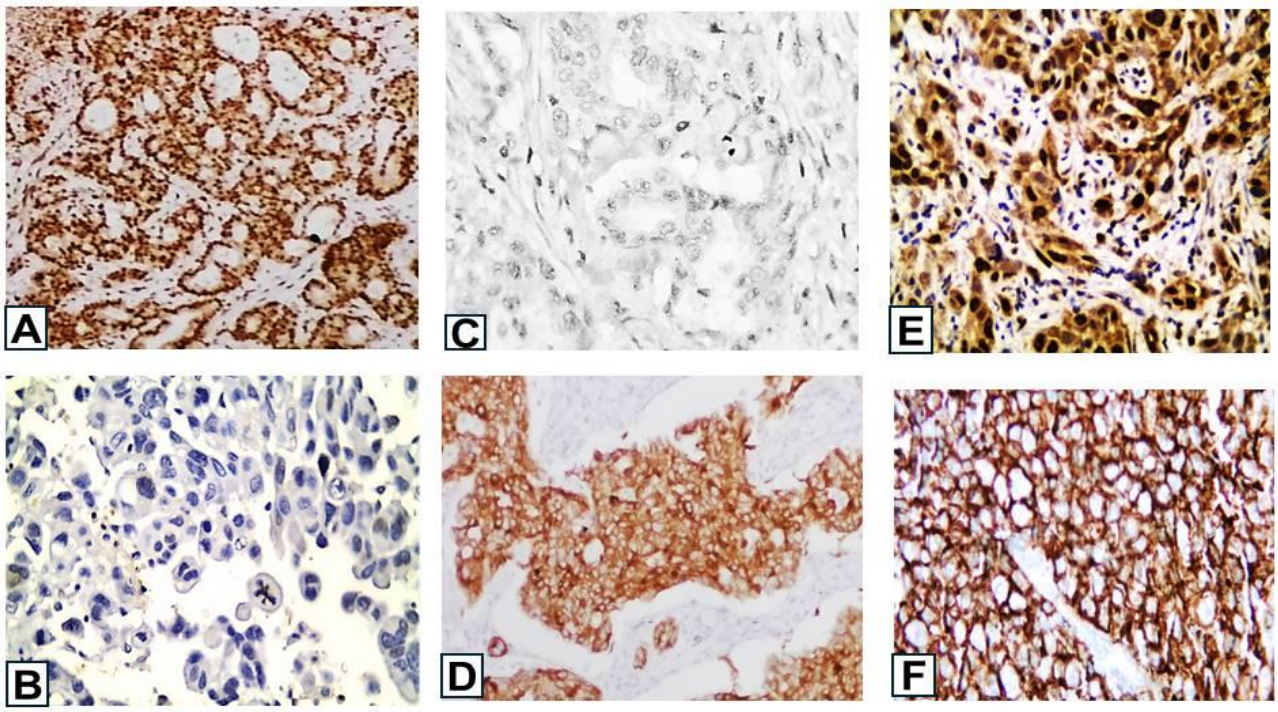

Figure 1: IHC expression of ANGPT1 (A: low grade CRC, B: high grade CRC), NCKAP1 (C: low grade CRC, D: high grade CRC), SOX2 (E: high grade CRC) and TGFB1 (F: high grade CRC). (A: magnified x 200) ,(B,C,D ,E and F : magnified x 400)

DISCUSSION

Colorectal cancer (CRC) is considered one of the most common and most aggressive GIT cancers around the world. The patient's prognosis varies widely according to certain determinants; one of these is the disease stage, as cases with localized disease have a 5-year survival of about 90%, which declines to 14% if there is a distant spread **(22)**. Most CRCs do not have early alarming symptoms and require regular investigations and novel markers for early detection.

NCKAP1 is considered one of the members of the WASF. Overexpression of WASF1 is suggested to down-regulate E-cadherin. NCKAP1 is responsible for the remodeling of the cytoskeleton, which is essential for metastasis. Therefore, the downregulation of NCKAP1 expression may restrict metastatic spread **(30).**

The current study results revealed NCKAP1 over-expression in the tumor cells. Positive NCKAP1 IHC expression was detected in 51.4% of CRC cases, which showed a significant correlation with high tumor stage, shorter both overall and disease-free survival as well as more relapse and mortality rates. This result supports the tumor-promoting action of NCKAP1 and its role as a novel predictive biomarker in CRC patients.

The present study results are in accordance with former studies, which declared that high NCKAP1 expression is correlated with adverse outcomes and progression of tumors in many cancers as breast, lung, and colon cancers **(4-5-6).** In addition, **Zhu et al., 2021** found that high NCKAP1 levels are significantly associated with invasion and spread in lung cancer non-small cell type **(5).** However, this study finding was contradicting to **Zhong et al., 2019** and **Chen et al., 2022** who priory stated that lower expression of NCKAP1 in hepatic and renal cell cancers is related to adverse outcomes **(7-8).** These differences can be explained by that target genes of NCKAP1-related pathways are selectively activated among different tissues **(7).**

Kwon et al., 2023 examined the relation between NCKAP1 and CRC metastasis and declared that invasion and migration of cancer cells were suppressed by inhibiting the activity of NCKAP1 in CRC cell lines. When NCKAP1 expression was inhibited, the levels of epithelial markers such as CDH1 and CTNNB1 were increased, and actin organization was destroyed. Therefore, it can be suggested that low NCKAP1 inhibits EMT. TGF-β1 facilitates malignant cell invasion and metastasis through enhancing EMT. NCKAP1 plays a vital role in promoting the TGFβ1-mediated EMT in CRC. EMT activity of TGFβ1 could be blocked by blocking NCKAP1, concluding that NCKAP1 is vital for completing the metastatic activity of colon cancer cells **(6).**

In line with a previous study, the current study investigated TGFβ1 immune expression and found

that it was expressed in 58.3 % of cases. Its expression was correlated to poorer clinicopathological parameters like stage IV disease, presence of distant metastatic lesions, shorter overall survival time, and higher relapse and mortality rates, which denotes that TGFβ1 can be considered a poor prognostic indicator. Regarding the association between TGFβ1 and NCKAP1 expressions, there was a strong positive correlation between the two biomarkers, which reveals that NCKAP1 may enhance EMT by promoting the activities of TGFβ1.

From the above findings, NCKAP1 may provide a piece of the puzzle for overcoming cancer and help in providing a diagnostic marker for CRC metastasis.

Angiopoietin-like proteins (ANGPTLs) are genes that have been found to regulate the EMT process. Down-regulation of ANGPTL1 may induce invasion and metastasis in certain cancers such as lung, breast, and thyroid in addition to hepatic carcinoma **(17-20)**, which makes ANGPTL1 an attractive tumor suppressor **(22).** However, inadequate information is widely known about the function of ANGPTL1 in colorectal carcinogenesis and tumor recurrence.

The finding of the present study found that ANGPTL1 expression was lower in CRC tissues. This study's results resemble the results of a previous study that revealed that ANGPTL1 mRNA is low expressed significantly in many cancers such as lung, prostate, bladder, and kidney **(20).** Furthermore, **Zhang et al., 2022** stated that ANGPTL transcriptions are downregulated in CRC tissues than those in surrounding normal tissues **(31).**

Moreover, the current study declared that the ANGPTL1 expression is noticed in about 36% of the studied CRC patients. It was significantly correlated with many favorable prognostic determinants such as lower tumor grade, stage, absent lymphatic spread, and lower mortality rates. These findings proposed that ANGPTL1 might help in suppressing the CRC progression.

Tumor-suppressing activities of ANGPTL1 can be explained by its ability to control both EMT and stemness of CRC cancer cells. ANGPTL1 down-regulates the cancer cell's ability to invade and spread by EMT pathway blocking through controlling Twist or Slug. Moreover, ANGPTL1 may enhance MET via integrin α1β1, which helps cancer cells to recover their epithelial properties **(27).**

In addition, ANGPTL1 may suppress the metastasis of CRC through CSCs regulation via SOX2 down-regulation helped by FOXO3a stimulation. Moreover, it may have antiangiogenic properties through inhibition of VEGF-induced endothelial cell proliferation, migration, and adhesion to extracellular matrix proteins **(22).**

In the present study, SOX2 IHC staining was observed in (55.6%) of CRC patients. Its immune expression was significantly associated with larger tumor size, higher grades and stages of tumor, and shorter survival time. Regarding the association between SOX2 and ANGPTL1 expression, there was a strong indirect association between the two biomarkers, which reveals that ANGPTL1 may work by suppressing the activities of SOX2.

Chang et al., 2022 presented near results to our study by explaining the mechanism of ANGPTL1/SOX2 axis that suppresses tumor spread and stemness in CRC. Moreover, they uncovered an indirect association between tumor ANGPTL1 and stem cell markers in CRC cases **(19).** From the previous results, it can be demonstrated that ANGPTL1 may provide a new therapeutic target for CRC.

CONCLUSION

In conclusion, ANGPTL1 could be used as an indicator for good prognosis and prolonged survival in CRC patients. The other three IHC biomarkers NCKAP1, TGFβ1, and SOX2 are related to the worse outcome of CRC cases and can be considered poor prognostic biomarkers, in addition, blockage of these markers could be beneficial in preventing tumor progression and may play a role in treatment of CRC patients.

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