MINAR International Journal of Applied Sciences and Technology
ISSN: 2717-8234
Article type: Research Article

Received: 05/09/2022 **Accepted:** 22/09/2022 **Published:** 01/12/2022

NAIF1 EXPRESSION IN COLORECTAL CANCER PATIENTS: CLINICAL AND PATHOLOGICAL IMPLICATIONS

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Abstract:

Background: In advanced human malignancies, the nuclear apoptosis-inducing factor 1 (NAIF1) is commonly silenced or not expressed. The purpose of this study was to look into potential relationships between clinical, pathological characteristics and NAIF1 expression.

Methods: This cross-sectional study used western blot and immunohistochemistry staining to assess the level of NAIF1 expression in 100 colorectal cancer samples. The outcomes of the immunohistochemistry staining were then contrasted with those of the clinicopathological characteristics.

Results: 68 out of 100 colorectal cancer samples were negative for NAIF1 expression.

In contrast to other clinicopathological parameters, loss of NAIF1 expression was significantly linked with lymphovascular invasion (P=< 0.00001), angiovascular invasion (P= 0.004356) and advanced TNM tumor stage (P=0. 004077).

Conclusion: According to the current study, a higher stage and a bad prognosis may be linked to decreased or negative NAIF1 expression.

Keywords: Clinical, Colorectal Cancer, Expression, NAIF1, Pathological.

http://dx.doi.org/10.47832/2717-8234.12.22

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Introduction:

Colorectal cancer is the second most common reason for cancer-related mortality worldwide and the third most prevalent cancer globally (account for 10% of all malignancies in 2020).[1] Like other cancers, colorectal adenocarcinoma is caused by a number of genetic and epigenetic pathways that result in the transformation of healthy endothelium cells into cancer cells.[2]

Nuclear apoptosis-inducing factor 1(NAIF1), which derived from the DNA transposon by molecular domestication. NAIF1 is a nuclear protein that contains a Myb-like domain at its N-terminal region. NAIF1 is an apoptotic pathway gene that induces apoptosis in various human cancers. The relationship between NAIF1 and inhibition the progression of cancer by inducing apoptosis by two hypotheses according to Luo et al; 1st: NAIF1 may interact with some DNA binding protein, like histone, to help to change the configuration of DNA and then regulate some gene expression sequentially, inducing apoptosis, 2nd: NAIF1 plays an important role in control of the expression of some pivotal anti-cancer or apoptotsis-related genes in physiological level.[3]

NAIF1 has been found to inhibit, delayed growth and advancement of numerous human tumors. Overexpression of NAIF1 prevents prostate cancer cells from proliferating. It has been found that NAIF1 is downregulated or absent and inhibits the development of gastric cancer. Lung cancer cells had down-regulated NAIF1 expression, and when it was restored, it reduced the capacity of the cells to grow and survive in the absence of anchorage. However, it is downregulated in osteosarcoma tissue and cell lines and can stop the spread and invasion of these tumors.[3-11] This study used western blot and immunohistochemistry staining to analyze the proteomic expression of NAIF1 in colorectal cancer patient samples. The association between the patients' clinicopathological features and absence of expression of this protein was then investigated.

Material and Methods:

100 colorectal cancer formalin-fixed paraffin-embedded samples from patients who underwent surgery between 2008 and 2010 were gathered for this cross-sectional investigation. A checklist that comprised demographic data, clinical observations, and histopathological results was applied to all samples. Colorectal adenocarcinoma and appropriate pathology information were required for inclusion criteria. Lack of pathological information was the exclusion criteria. Patients' clinical and pathological characteristics were assessed. Age, gender, tumor location, tumoral size, pathological tumoral stage, tumoral differentiation, vascular and lymphatic invasions, existence of lymph node metastasis and TNM staging were among the stratification criteria. Two pathologists looked through the hematoxylin and eosin slides.

Western blot technique protein extraction:

Protein extractions from tissue samples: Cryostats were used to cut pieces of tissue samples maintained at -80°C. These tissue slices were processed in a manner similar to that described by Arnaoty et al.[8] to produce protein lysates. Using the Bradford technique, the protein extracts were quantified and stored at -20 °C.

Protein analysis utilising acrylamide gel electrophoresis:

Arnaoty et al.[8] previously published the method of protein analysis using acrylamide gel electrophoresis (SDS-PAGE). 50 micrograms of tissue-specific protein extracts were placed in each well of a polyacrylamide gel.

Western Blot:

Arnaoty et al.[8] previously provided a detailed explanation of the methodology. Using a 1:250 dilution of the primary antibody anti-NAIF1 from In Cell Art Nantes, France. Next, a secondary antibody (Amersham, GE Healthcare) coupled to goat anti-mouse IgG-HRP was incubated for an hour at room temperature. The membranes were then put through a chemiluminescence process to acquire images with the FUGI LAS4000 imager, which allowed for analysis (Amersham ECL Advance Western Blotting Detection Kit, GE Healthcare).

***Immunohistochemical technique*:**

To evaluate the level of NAIF1 gene expression, two formalin-fixed paraffin-embedded blocks were selected from each colorectal cancer patient and 5 µm thick slices were put on poly l-lysine slides. The slides were initially deparaffinized for 5 minutes three times in xylene after being exposed to 60°C for 15 minutes. The tissues were then given a 5-minute soak in distilled water and alcohols 70%, 90%, and 100% to rehydrate them. Utilizing sodium citrate buffer with a pH of 6 for 20 min at 97°C, antigen retrieval was carried out. After chilling, endogenous peroxidase activity was blocked for 15 minutes using a peroxidase 0.3% solution. To avoid background staining, the samples were twice washed in phosphate-buffered saline (PBS). Then, blocking solution was applied for 15 min, at room temperature. The tissues were incubated with NAIF1 antibody (In Cell Art, Nantes, France) for 1 hour at room temperature after being washed three times for 5 min each in PBS solution. Following PBS washing, the Goat anti Mouse IgG-HRP (*Amersham, GE Healthcare*) secondary antibody incubation and DAB staining were carried out. The normal process for tissue dehydration was followed after counterstaining with hematoxylin dye. The samples that had primary antibody incubation removed were regarded as negative controls.

NAIF1 Expression Analysis:

In a blinded manner, the patterns and intensities of immunological staining were evaluated on the slides by two expert pathologists. More than 98% of the cases, the outcomes were comparable. One interpretation was offered after re-examining the remaining samples. Each slide's stromal and inflammatory cells were rated as positive internal controls and given a score of +2, after which the staining intensity of the cancer cells was contrasted with those cells. If the staining intensity was equal to the positive control, it received a score of +2, +1 if it was inferior to the internal control, and a negative score if there was no immunostaining. Immunostaining that was more intense than positive control cells received a +3 rating.

When taking into account the heterogeneity of cancer cells, conditions were considered positive when staining of any intensity was seen in more than 10% of cancer cells figure1.

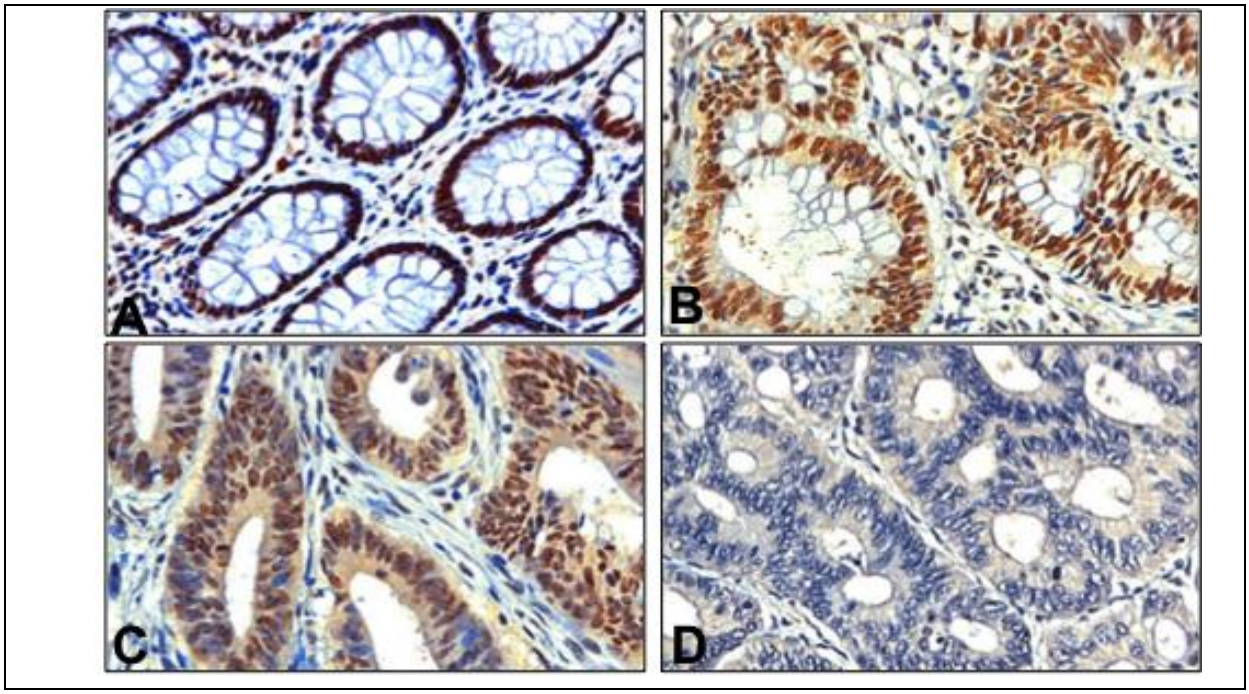

Figure1: Immunohistochemistry expression pattern of NAIF1 in normal mucosa and stages I–III colorectal carcinoma (CRC). A) Positive NAIF1 in epithelial nuclei of normal mucosa; B) Positive NAIF1 in cancer nuclei of stage I CRC; C) Positive NAIF1 in cancer cell nuclei of stage II CRC; D) Negative NAIF1 in cancer cell nuclei of stage III CRC.

Statistical Analysis:

Chi-square tests were used to determine the association between the expression of the NAIF1 gene and the patients' clinicopathological characteristics, such as gender, tumoral location, the histopathological subtype, the pathological tumoral stage, tumoral differentiation, lymphatic and vascular invasions, existence of lymph nodes metastasis, TNM tumoral stage. SPSS 20 (SPSS Inc., Chicago, IL, US) was used for the statistical analysis, and a P-value of 0.05 or less was regarded as statistically significant.

Results:

NAIF1 Expression by western blot:

NAIF1 primary antibody (In-Cell-Art, Nantes, France)[9] application revealed particular NAIF1 expression products in all samples evaluated (transfected Hela, normal colon tissue, and stage I–III colorectal cancer tissue) with a molecular weight of 35 kDa, which is the same as the NAIF1 transposase.[10] As demonstrated in figure 2, NAIF1 expression was higher in healthy colon tissue than in colorectal cancer, particularly as the cancer progressed from stage I to stage III.

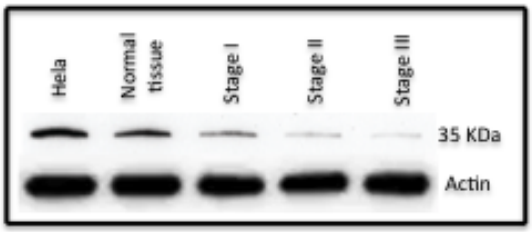

Figure 2: NAIF1 western blot analysis

Status of NAIF1 Expression By IHC and Clinicopathological Characteristics:

The immunohistochemistry assay was used to look for NAIF1 expression in all 100-tissue samples of colorectal cancer. Nuclear expression of NAIF1 has been identified in the normal and early stage cancer cells. NAIF1 was expressed negatively in 68 colorectal cancer samples and positively in 32 samples. There were other cases where NAIF1 was shown to have heteroexpression, meaning that although some cancer cells expressed NAIF1, others did not. These situations were viewed as positive. Table 1 displays the relationships between clinicopathological traits and NAIF1 expression. Loss of NAIF1 expression was connected to higher TNM stages (P=0.004077), lymphovascular invasion (P=< 0.00001) and angiovascular invasion (P= 0.004356) respectively. For all that, no additional association between NAIF1 expression and other factors such as gender, age, tumoral size, location, mucinous component, pathological tumoral stage, differentiation was discovered.

Table 1: Clinical and pathological characteristics of 100 colorectal adenocarcinomas were correlated with NAIF1 expression status.

Feature	***NAIF1 Expression***		***Clinico-pathologic* chi-square statistic**
Gender	Positive %	Negative %	The chi-square statistic is **0.1736**. The *p*-value is **.676922**. *Not* significant at $p < .05$
Male	23 (24) [0.04]	37 (36) [0.03]	
Female	17 (16) [0.06]	23 (24) [0.04]	
Age	Positive	Negative	The chi-square statistic is **0.5409**. The *p*-value is **.462074**. *Not* significant at $p < .05$.
< 55	21 (19.2) [0.17]	27 (28.8) [0.11]	
≥55	19 (20.8) [0.16]	33 (31.2) [0.1]	
Tumor location	Positive	Negative	The chi-square statistic is **0.9407**. The p-value is **.624794**. The result is not significant at p < .05
Proximal colon	14 (11.88) [0.38]	19 (21.12) [0.21]	
Distal colon	13 (14.76) [0.21]	28 (26.24) [0.12]	
Rectum	9 (9.36) [0.01]	17 (16.64) [0.01]	
Size of tumor (cm)	Positive	Negative	The chi-square statistic is **0.2525**. The p-value is **.615303**. Not significant at p < .05
< 5	15 (16.2) [0.09]	21 (19.8) [0.07]	
≥5	30 (28.8) [0.05]	34 (35.2) [0.04]	
PN stage	Positive	Negative	The chi-square statistic is **2.1355**. The *p*-value is **.143928**. *Not* significant at $p < .05$
PN0	32 (35.28) [0.3]	40 (36.72) [0.29]	
PN1-2	11 (14.28) [0.75]	17 (13.72) [0.78]	
PT stage	Positive	Negative	The chi-square statistic is **0.1266**. The *p*-value is **.721976**. *Not* significant at $p < .05$.
PT1-2	6 (6.66) [0.07]	12 (11.34) [0.04]	
PT3-4	31 (30.34) [0.01]	51 (51.66) [0.01]	
Differentiation	Positive	Negative	The chi-square statistic is **5.9228**. The p-value is **.051747**. The result is not significant at p < .05
Well	19 (23.52) [0.87]	37 (32.48) [0.63]	
Moderate	15 (13.86) [0.09]	18 (19.14) [0.07]	
Poor	3 (6.38) [1.79]	8 (4.62) [2.47]	
Mucinous component	Positive	Negative	The chi-square statistic is **0.3445**. The p-value is **.55722**. Not significant at p < .05
Absent	32 (33.18) [0.04]	47 (45.82) [0.03]	
Present	10 (8.82) [0.16]	11 (12.18) [0.11]	
Lymphovascular invasion	Positive	Negative	The chi-square statistic is **22.1612**. The *p*-value is **< 0.00001.** Significant at ***p* < .05**
Absent	10 (21) [5.76]	50 (39) [3.1]	
Present	15 (26) [4.65]	25 (14) [8.64]	

Angiovascular invasion	Positive	Negative	The chi-square statistic is **8.1289**. The *p*-value is **0.004356.** Significant a**t *p* < .05**
Absent	25 (31.5) [1.34]	45 (38.5) [1.1]	
Present	10 (16.5) [2.56]	20 (13.5) [3.13]	
TNM stage	Positive	Negative	The chi-square statistic is **8.2492**. The *p*-value is **0. 004077.** Significant at ***p* < .05**
I-II	20 (27) [1.81]	40 (33) [1.48]	
III-IV	15 (22) [2.23]	25 (18) [2.72]	

Discussion:

Identifying biomarkers that have an impact on clinical outcomes for patients is a crucial step in selecting a course of therapy that will be as effective as possible. Determining the markers that influence illness prognosis or result in treatment resistance is a goal of research on targeted medicines.[11] NAIF1 may considered as a tumor suppressor gene that is involved in a number of cellular functions, such as apoptosis, differentiation, genome stability, and survival.[3-11] The mechanism behind the progression of many cancers when NAIF1 expression drops or disappears totally is unclear, but its ability to trigger apoptosis provides one explanation.[3-11] According to our western blot analysis, the expression of NAIF1 reduced from healthy colon tissue to advanced colorectal cancer. This result is in line with earlier research on colorectal cancer tissue, it used a western blot to examine the expression of NAIF1 in healthy and cancerous tissue.[8] Results from immunohistochemistry in this investigation revealed that 68% of tumors had negative NAIF1 expression. There is no information available to compare our findings with about NAIF1 expression profile by immunohistochemistry in colorectal cancer. Additionally, we conducted this research for the first time and found no previous studies that demonstrated a relationship between NAIF1 expression and clinicopathological traits in colorectal cancer. Our research demonstrated a correlation between decreased NAIF1 expression and higher TNM stage, lympho and angiovascular invasions. This correlation is accountable in our research, because negative alterations in NAIF1 expression would promote cell growth, prevent apoptosis, and increase tumor aggressiveness.[3,5,7] Age, tumoral size, gender, tumoral location, cancer mucinous component, pathological tumoral stage and tumoral differentiation did not significantly affect the level of NAIF1 expression, according to our research. Several studies have shown that decreased synthesis of this protein in numerous malignancies is associated with an advanced stage of cancers which can lower patient survival, even if the NAIF1 gene's role as a prognostic marker is still controversial.

Conclusion

According to our research, lower NAIF1 expression is associated with a greater tumor stage and a poorer prognosis, and reduced or lost NAIF1 expression may be linked to tumor growth. In order to more clearly corroborate these findings, future studies need be broadened with bigger sample sizes.

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