Nidogen-2: A new biomarker in colon cancer patients

Ann Ital Chir, 2022 93, 1: 88-92
pii: S0003469X21034655
Online ahead of print 2021 - August 23
free reading: www.annitalchir.com

Remzi Kiziltan*, Öztekin Çikman*, Sermin Algül**, Mehmet Akif Aydin***, Özgür Kemik****

**Department of General Surgery, School of Medicine, Yuzuncu Yil University, Van, Turkey*
***Department of Physiology School of Medicine Yuzuncu Yil University, Van, Turkey*
****Department of General Surgery, School of Medicine, Altinbas University, Instanbul, Turkey*
*****Department of General Surgery, School of Medicine, Yuzuncu Yil University, Van, Turkey*

Nidogen-2: A new biomarker in colon cancer patients

AIM: *In the present study, we followed Nidogen-2 levels and clinicopathological parameters of patients with colon cancer.*
MATERIAL AND METHODS: *Eighty-eight patients (F/M, 43/45; Mean age ± SD, 57.86 ± 1.78 years) were included. The results of serum Nidogen-2 levels were shown with respect to stage, gender, age, and metastasis. Nidogen-2 levels in the sera of colon cancer patients and healthy donors were analyzed with ELISA.*
RESULTS: *The expression levels were significantly higher in patients (1010.8 ±184.36 pg/mL) than in healthy subjects (51.85 ± 1.44 pg/mL; p<0.001). Moreover, the Nidogen-2 expression significantly increased in the clinical stages of colon cancer (p<0.01). The Nidogen-2 levels did not vary by patient age or gender.*
DISCUSSION: *Under normal conditions, Nidogen-2 is a basal membrane protein. Nidogen-2 is primarily expressed in the extracellular matrix. Nidogen-2 has been defined as a major means to analyze the molecular pathways involved in cancer development and progression. Besides its important functions, it has been hypothesized that secreted Nidogen-2 may be a diagnostic biomarker for cancer detection.*
CONLUSION: *These findings suggest that increased expression of Nidogen-2 may have great pathological importance in the development of colon cancer and may also show a diagnostic value for colon cancer.*

KEY WORDS: Angiogenesis, Metastasis, Nidogen-2

Introduction

Colon carcinoma is one of the major causes of cancer-related deaths worldwide [1]. Despite the emergence of new markers and the use of different therapeutic agents, none of the treatment conditions is curative in patients with advanced cancer. The treatment of choice in the majority of colon cancer cases remains the radical surgical resection, with or without adjunctive chemotherapy [2].

The neoplastic conversion of human colorectal cells is a stepwise process from normal colonic epithelium to cancer [3]. Despite the accumulation of genetic evidence on the model of adenocarcinoma sequence [4], there are still no criteria that can predict adenoma progression to cancer. Tumor angiogenesis is an important step in the progress, metastatic extension, and progression of cancer. Nidogens, also known as entactins, are a family of sulfated monomeric glycoproteins located in the basal membrane[5]. Two nidogens have been defined in humans: nidogen-1 (NID1) (CA. 135 kDa) and nidogen-2 (NID2) (CA. 150 kDa) [6].
Nidogen also interacts with cell receptor molecules and controls cell polarization, migration, and invasion. Nidogen-2 is an expansive component of basement membranes underneath epithelia of most major organ systems [6].

Pervenuto in Redazione Agosto 2020. Accettato per la pubblicazione Ottobre 2020
Correspondence to: Remzi Kiziltan, Department of General Surgery, School of Medicine, Yuzuncu Yil University, Van, Turkey (e-mail: remzikiziltan@yahoo.com)

It has been detected that abnormal stromal microenvironment favors tumor formation and progression, and Nidogen-2 plays a key role in the maintenance of the structural entirety of basement membranes, which acts as a barrier for cell movement, migration, and invasion. Nidogen-2 defects have not been linked to human cancer. We also demonstrated the expression of Nidogen-2 in 88 colon cancer patients. In addition, we described the confidence interval and the superiority of Nidogen-2 in predicting tumor angiogenesis.

Materials and Methods

Patients

The patient characteristics are summarized in Table I. The study group comprised 88 patients who had received a diagnosis of colon carcinoma (45 male and 43 female, median age (range) 57.86 (54-61) years) at Yüzüncü Yıl University School of Medicine, Department of General Surgery, between November 2017 and November 2020. None of the patients received any chemotherapy or radiation therapy, nor they had a history of colonic resection prior to surgery. The samples were collected within 1 hour after resection. Serum samples were stored at -20°C until use. The pathological diagnosis was made on the basis of the tumor size, depth of infiltration, histological grade, lymph node status, and the presence of distant metastasis. All patients were classified according to the Union for International Cancer Control (UICC) stage classification using resected specimens. Written informed consent was obtained from all patients included in the study.

Elisa Test for Nidogen-2

ELISA test for Nidogen-2 was performed using commercially available kits according to the manufacturer's instructions (RayBiotech RayBio®, Catalog No: ELH-Nidogen-2). The patients' sera were transferred to wells of plates pre-coated with the primary antibody. Afterward, the recommended incubation period and washing period elapsed, and the substrate solution was added. The wells were added color-reagent and stop solution. Optical density was determined at a wavelength of 450 nm using automated optical densitometry. Each sample was run twice, and the mean value of the two measurements was used for analysis.
The minimum detectable dose of human Nidogen-2 was determined to be 49 pg/mL. This assay has high sensitivity and excellent specificity for detection of human Nidogen-2. No significant cross-reactivity or interference between human Nidogen-2 and analogs was observed. The detection range is 49-12000 pg/mL (Intra-Assay CV%: <10%; Inter-Assay CV%: <12%).

Statistical Analysis

The normality of the study data was tested using Shapiro Wilk and one sample Kolmogorov Smirnov tests, a histogram, a Q-Q plot, and a box plot graphics. The study data were expressed as mean, standard deviation, median, minimum, maximum, frequency, and percentage. The comparison of the study variables between the two groups was performed with independent samples t-test (for normally distributed variables) or Mann Whitney-U test (for non-normally distributed variables). Kruskal Wallis's one-sided analysis of variance was used for three or more groups. Multi comparisons were performed using the Dunn test. Nominal variables were compared using the Chi-square test with Yates correction. Statistical significance was set at a two-sided $p<0.05$. Statistical analyses were performed with NCSS 10 (2015. Kaysville, Utah, USA) statistical software.

Table I - *The clinicopathologic features of patients and controls.*

	Controls (n=40)	Patients (n=88)	p
Age (y)	58.15 ± 2.09 (54-62)	57.86 ± 1.78 (54-61)	<0.05
Gender (F/M)	19/21 47.25% / 52.5%	43/45 46.9% / 51.1%	
Tumor size			
<4cm	12		
>4cm	76		
TNM stage			
TI	12		
TII	14		
TIII	20		
TIV	42		
Invasion			
T1	12		
T2	14		
T3	22		
T4	40		
Lymph node metastasis			
N0	12		
N1	14		
N2	62		
Metastasis			
M0	20		
M1	68		

Table II - *Preoperative serum Nidogen-2 levels of the all patients and controls (Mean ± SD) (Min-Max).*

	Controls	Patients	p
Nidogen-2(pg/mL)	51.85 ± 1.44 (50.55)	1010.8 ± 184.36 (700-1350)	< 0.001

TABLE III - *Preoperative serum Nidogen-2 levels of the clinic-pathologic variables of the patients (Mean ± SD) (Minimum-Maximum).*

	Nidogen-2 (pg/mL) (Min-Max)
TNM stage	
TI	741.67 ± 19.4 (700-750)
TII	821.43 ± 25.67 (800-850)
TIII	957.5 ± 40.63 (900-1000)
TIV	1176.19 ±90.55 (1000-1350)
Invasion	
T1	741.67 ± 19.4 (700-750)
T2	821.43 ± 25.67 (800-850)
T3	957.5 ± 40.63 (900-1000)
T4	1176.19 ± 90.55 (1000-1350)
Lymph node metastasis	
N0	741.67 ± 19.46 (700-750)
N1	821.43 ± 25.67 (800-850)
N2	1105.65 ± 129.02 (900-1350)
Metastasis	
M0	765 ± 32.84 (700-800)
M1	1083.09 ± 143.15 (850-1350)

In 40 healthy subjects (21 men,19 women) with a median age of 58.15 (range 54-62 years) years. The study group included 88 patients with colon cancer (45 men and 43 women) with a median age of 57.86 (range 54-61 years) years (p>0.05). The postoperative TNM stages were TI, TII, TIII, and TIV in 12, 14, 20, and 42 patients, respectively.

Table II provides the serum Nidogen-2 levels of the patient and the control group. The two groups did not differ with respect to age (p>0.05) (Figs. 1, 2). In the healthy group, the mean ± SD serum Nidogen-2 level was 51.85 ± 1.44 pg/mL. The serum Nidogen-2 levels of the 88 patients with colon cancer were 1010.8 ± 184.36 pg/mL. The serum Nidogen-2 levels were significantly higher in patients with colon cancer than in the control group (p<0.001).

Table III provides the serum Nidogen-2 levels of patients with colon cancer according to the clinic – pathologic variables.

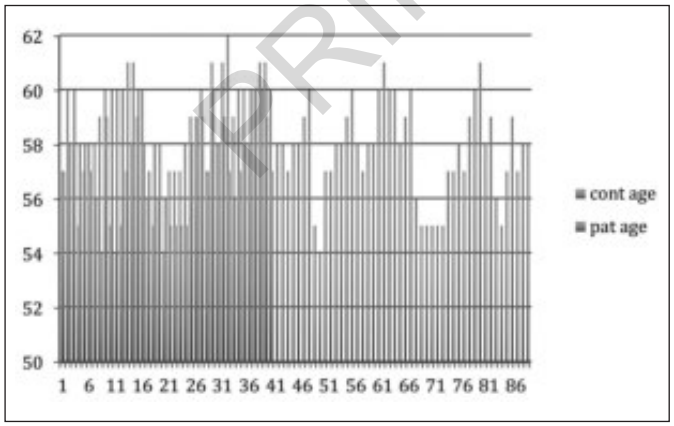

Fig. 1: The histogram of the age of the all samples.

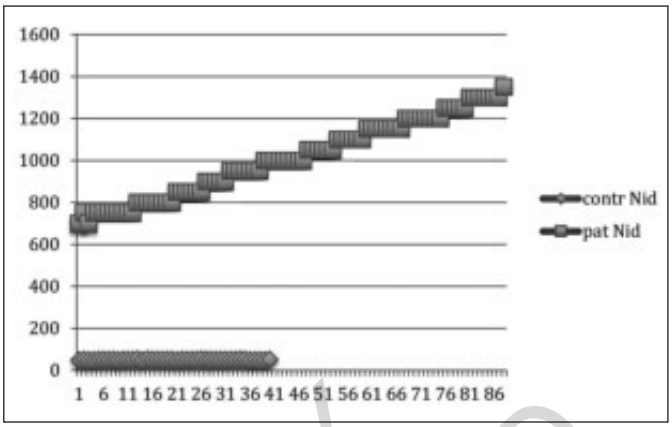

Fig. 2: The histogram of the Nidogen-2 levels of the all samples.

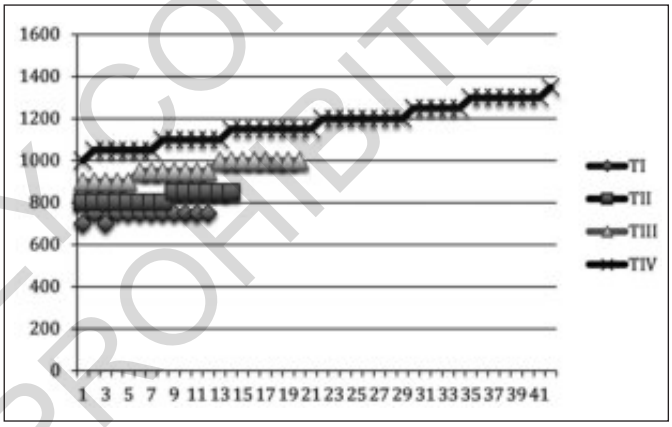

Fig. 3: The histogram of the TNM stages (TI-TIV stage).

The serum Nidogen-2 levels significantly increased from TI tumors to TIV tumors (p<0.01) (Fig. 3).

Additionally, serum Nidogen-2 levels were significantly higher in patients with colon cancer with increasing lymph vascular involvement, distant metastasis, lymph node metastasis, and TNM stage (p<0.01).

Lymph vascular involvement and distant metastasis by age were similar between the groups (p=0.714, p=0.632, p=0.558, respectively). Lymph node metastases and distant metastases by gender were similar between the groups (p=0.821, p=0.754, respectively). Gender is distributed homogenously between T stages and lymph node metastasis (p=0.765, p=0.801, respectively).

Discussion

As the stage of cancer has a definite impact on survival, diagnosis at earlier stages is highly critical [7].

Alteration of the basement membrane is essential for cell invasion [8]. In tumorigenesis, invasion through the basement membrane is a characteristic of malignancy [9]. Disruption of whole basement membrane design pro-

vides an invasion-permissive environment, which may promote cancer cell proliferation and invasion [10].
Nowadays, the only well-accepted colon cancer biomarker is CA125, which was introduced over 20 years ago. Besides its low sensitivity for early disease, CA125 is flawed by its low specificity as its levels remain at the same levels in advanced colon cancer. Thereby, new markers with higher sensitivity and specificity for colon cancer are required to enhance clinical outcomes.
Under normal conditions, Nidogen-2 is a basal membrane protein. Nidogen-2 is primarily expressed in the extracellular matrix. The expression of Nidogen-2 as a serum marker was designated with the surgical stage and grade even so since the number of our cases was relatively small.
Nidogen-2 has a major role as a tool to analyze the molecular pathways involved in cancer development and progression. Besides its significant functions, it is plausible that secreted Nidogen-2 may be a diagnostic biomarker for cancer detection. Nidogen-2 levels were significantly higher in colon cancer patients than the healthy control group. Our study demonstrated the diagnostic value of Nidogen-2 as a serum marker for colon cancer.
Briefly, attempts have been made to employ Nidogen in serum or plasma of patients as a diagnostic biomarker of a variety of cancers [11,12,13]. Nidogen biomarker may be a marker for the detection of various cancers as we may diagnose cancer by detecting products of cancer cells released into the bloodstream. Since Nidogen is very sensitive, we may prefer using this marker. It may ensure for the development of highly sensitive diagnostic strategies for swift and non-invasive monitoring of the pathological condition of cancer patients [14].

Conclusion

High levels of Nidogen-2 in colon cancer may assist greatly as important markers for patients with colon cancer. One of the limitations of this study is the expression of Nidogen-2 protein was not confirmed in colon cancer, therefore, advanced clinical and mechanistic analyses are lacking to confirm the results of the present study. An understanding of the molecular mechanisms of Nidogen-2 requires advanced research.

Riassunto

In questo studio abbiamo confrontato i livelli di Nidogen-2 e i parametri clinico-patologici su 88 pazienti con cancro al colon (F/M, 43/45; Età media 57,86 (54-61), Deviazione Standard = 1,78 anni).
I risultati del siero Nidogen-2 sono stati confrontati rispetto a stadio, sesso, età, metastasi. I livelli di nidogeno-2 nel siero di pazienti con cancro al colon e soggetti sani sono stati analizzati con ELISA.
Risultati: i livelli di espressione sono risultati significativamente più elevati nei pazienti (1010,8 punti 184,36 pg / mL) rispetto ai soggetti sani (51,85 punti 1,44 pg / mL; p <0,001). Inoltre, l'espressione di Nidogen-2 è aumentata in modo significativo nelle fasi cliniche del cancro del colon (p <0,01). I livelli di Nidogen-2 mom sono risultati risultati differenti con l'età e il sesso dei pazienti.
Dunque in una situazione normale Nidogen-2 è una proteina di membrana basale, come espressione di matrice extracellulare. Il Nidogen-2 si è dimostrato valido come mezzo per analizzare le vie molecolari coinvolte nello sviluppo e nella progressione del cancro. Inoltre, per le sue funzioni sostanziali, si presume che il Nidogen-2 secreto possa essere un biomarcatore diagnostico per il rilevamento del cancro
In conclusione questi risultati suggeriscono che una maggiore espressione di Nidogen-2 può avere una valenza patologica importante nello sviluppo del cancro del colon e può anche riflettere il valore diagnostico del cancro del colon.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: *Global cancer statistics*. Ca Cancer J Clin, 2011; 61(2): 69-90.

2. Pop MG, Bartoş DM, Fit AM, Vesa ŞC, Bartoş A, Corpadean AG, Nadim AH, Cosmin PI, Cornel J: *Detection of epithelial specific cell adhesion molecules in colon cancer and the correlation with clinical and pathological characteristics*. Ann Ital Chir, 2019; 90: 318-23.

3. Fearon ER, Vogelstein B: *A genetic model for colorectal tumorigenesis*. Cell, 1990; 61(5): 759-67.

4. Cho KR ,Vogelstein B: *Genetic alterations in the adenocarcinoma* sequence. Cancer, 1992; 70: 1727-731.

5. Umemori H, Hortsch M: *The sticky Synapse: Cell Adhesion Molecules and their role in synapse formation and maintenance.* New York NY: Springer, 2009; 66.

6. Miosge N, Holzhausen S, Zelent C, Sprysch P, Herken R: *Nidogen-1 and Nidogen-2 are found in basement membranes during human embryonic development.* The Histochemical J, 2001; 33(9-10): 523-30.

7. Sümer A, Demir A, Kemik A, Yavuz A, Suvak B, Dülger AC, Purisa S, Kemik O: *Serum vascular endothelial growth factor reseptor-3 levels in patients with esophageal squamous cell cancer*. Ann Ital Chir 2017; 88: 20-25.

8. Sherwood DR, Butler JA, Kramer JM, Sternberg PW: *FOS-1 promotes basement-membrane removal during anchor-cell invasion in C. elegans.* Cell, 2005; 121(6): 951-62.

9. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S: *Metastatic potential correlates with enzymatic degradation of basement membrane collagen.* Nature, 1980; 284: 67-68.

10. Kuk C, Gunawardana GC, Soosaipillai A, Kobayashi H, Li L, Zheng Y, Diamandis EP: *Nidogen-2: A new biomarker for ovarian cancer*. Clin Biochem, 2010; 43(4-5): 355-61.

11. Chai AWY, Cheung AKL, Dai W, Ko JMY, Lee NPY, Chan KT, Law SY, Lung ML: *Elevated levels of serum nidogen-2 in esophageal squamous cell carcinoma.* Cancer Biomark, 2018; 21(3): 583-90.

12. Torky HA, Sherif A, Abo-Louz A, Ali M, Ahmed A, Ali A: *Evaluation of serum nidogen-2 as a screening and diagnostic tool for ovarian cancer.* Gynecol Obstet Invest, 2018; 83(5): 461-65.

13. Cheng ZX, Huang XH, Wang Q, Chen JS, Zhang LJ, Chen XL: *Clinical significance of decreased nidogen-2 expression in the tumor tissue and serum of patients with hepatocellular carcinoma*. J Surg Oncol, 2012; 105(1): 71-80.

14. Ulazzi L, Sabbioni S, Miotto E, et al.: *Nidogen-1 and 2 gene promoters are aberrantly methylated in human gastrointestinal cancer*. Mol Cancer, 2007; 6:17.