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Original Article

The prognostic value of β-catenin and LEF-1 expression in patients with operable gastric carcinoma

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Abstract: Aim: The aim of this study is to evaluate the prognostic value of β-catenin and LEF-1 expression in patients with operable gastric cancer that receive adjuvant treatment and the relationship between demographic and histopathological variables. Material and method: In this study, 82 gastric cancer patients treated with adjuvant treatment after surgery and followed in the Medical Oncology Department of Kocaeli University were included. β-catenin and LEF-1 expression were examined by immunuhistochemical analysis in paraffin embedded tumor tissues of the patients. Results: Median age was 56 (26-81) and follow up was 19 months (4-61). Performance status (ECOG) were 0-1 in all patients. Male/female ratio was 53/29 (64.6/35.4%). The median disease free survival (DFS) time was 17 months (SE: 3 95% CI: 11-23) and 3 years DFS rate was 39.7%. The median overall survival (OS) time was 28 months (SE: 4 95% CI: 20-36) and 3 years OS rate was 41.2%. There was no statistical correlation between β-catenin and LEF-1 expression and age, gender, performance status, tumor localization, T and N stage, lymphovascular, perinoral invasion, grade and operation type (>0.005). According to univariate analysis, we did not find significant effect of age, gender, T stage, lymphovascular, perinoral invasion, grade and operation type on overall survival (p>0.005). Good performance status (ECOG 0), tumor infiltration without diffuse type like linitis plastica, and lower N stage had positive effect on survival respectively (p=0.04, 0.033 and 0.005). Conclusion: In this study group, we found that only N stage was an independent prognostic factor (<0.005). Demographic features of the patients, histopathological characteristics other than N stage, β-catenin and LEF-1 prognostic effects have not been shown.

Keywords: Gastric carcinoma, β-catenin, LEF-1

Introduction

Gastric cancer is the second most common cause of cancer-related death in the world. In Turkey, gastrointestinal tract cancers are second to respiratory tract cancers in males, third to breast and urogenital tract cancers in females, with gastric cancer having the highest incidence in all gastrointestinal tract cancers according to Health Ministry data [1]. The factors of early diagnosis, follow up and prognostic evaluation of gastric cancer have been studied. The positive and negative lymph node number, invasion of tumor depth, histopathological type, applied surgery, tumor grade, location of tumor, lymphatic and vascular invasion, patient age and sex have been detected as prognostic factors [2, 3]. In addition to these, the importance of some genetic alterations such as DNA ploidy, S-phase fraction, and P53 mutation have been studied [4-6]. Mutation of β-catenin is a frequent cause of the Wnt signaling pathway in gastric cancer [7]. β-catenin is translocated to the nucleus from cytoplasm by Wnt activation and binds to the members of TCF4 (T cell factor) and LEF-1 (lymphoid enhancing factor) as central mediators of transcription. In the carcinogenesis process, TCF4-β-catenin complex might regulate transcription of LEF-1 and it may lead to malign progression [8]. The correlation of LEF-1 and TCF4 with nuclear β-catenin and their tumoral distribution have been studied in colorectal cancers [9]. The Wnt signaling pathway has a role in the development of organs in lots of different species, but if activated abnormally, it is related to carcinogenesis [10].

The Wnt gene family code a group of glycoprotein signal molecules activated in different cancers. The Wnt signal pathway has an important role as its components have been shown to be

Table 1. Demographic and clinicopathologic features of the patients

Features	Patient number	%
Sex		
Female	29	35.4
Male	53	64.6
Age		
>60	34	41.5
≤60	48	58.5
Performance status		
ECOG 0	47	57.3
ECOG 1	35	42.7
Tumor localization		
Cardia	21	25.6
Corpus	21	25.6
Antrum	38	46.3
Diffuse invasion	2	2.4
T stage		
T1	1	1.2
T2	6	7.3
T3	69	84.1
T4	6	7.3
N stage		
N0	12	14.6
N1	20	24.4
N2	22	26.8
N3a	24	29.3
N3b	4	4.9
Dissection type		
D1	50	61.0
D2	29	35.4
D3	3	3.7
Vascular invasion		
Positive	14	17.1
Negative	68	82.9
Lymphatic invasion		
Positive	13	15.9
Negative	69	84.1
Perineural invasion		
Positive	2	2.4
Negative	80	97.6
Grade		
G1	32	39.0
G2	12	14.6
G3	38	46.3
Surgery border		
Negative	69	84.1
Positive	13	15.9

related to the clinical stage of some tumors and so it can be useful for prognostic purposes [11]. In addition, targeting of the agents that inhibit abnormally activated steps in carcinogenesis might be helpful in the development of chemopreventive and chemotherapeutic agents [12]. We aimed to study the prognostic importance of LEF-1 and β-catenin expression and the relation with histopathological variables in gastric cancer patients given adjuvant therapy after surgery in our clinic.

Material and method

Eighty-two patients with gastric cancer referred to the oncology clinic after surgery with curative intention were evaluated retrospectively. The demographic data, such as gender, age, and the surgical findings from the surgical reports were determined from the files of the patients. Pathological data such as histological type, tumor invasion depth, lymph node metastasis, vascular, lymphatic and perineural invasion status, localization and differentiation degree were also obtained from the patients records. The patients were evaluated according to performance status and histopathological findings after the operation. Adjuvant chemoradiotherapy (5FU 425 g/m^2 and FA 20 mg/m^2/28 day 5 days 5 cycles) or chemotherapy and radiotherapy alone were given according to performance status of the patients having T3N0 or a higher stage of disease by TNM classification with the pathological findings. Combined chemotherapy (Dosetaksel, Cisplatin, 5FU) 2 to 4 cycles after chemoradiotherapy were given to the stage four patients having T4 or N3 disease by pathological findings. After the completion of the treatment, patients were followed every 3 months. Palliative chemotherapy, radiotherapy, or surgery were administered to the patients that developed local recurrence or distant metastasis.

The tissue samples obtained from the Pathology clinic archives were reevaluated by the pathologist, reclassified according to WHO and Lauren classification and the results were confirmed. The pathological slides belonging to the cases were reexamined and the suitable ones for immunohistochemical staining were chosen from the paraffin block archives and stained. The immunohistochemical examination of the

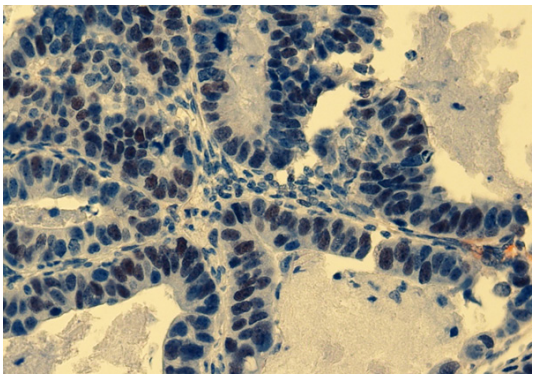

Figure 1. Intranuclear LEF-1 immunoreactivity in a tumor with tubular pattern (x400).

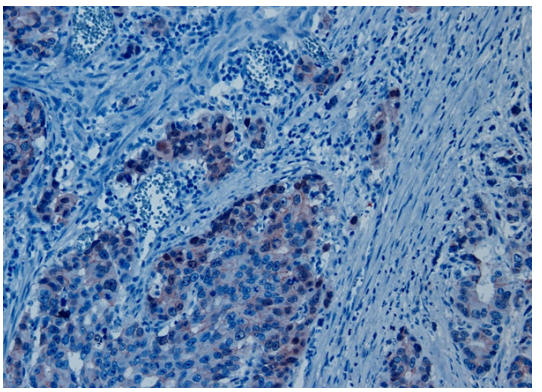

Figure 2. Focal membranous β-catenin immunoreactivity in a tumor with intestinal pattern (x100).

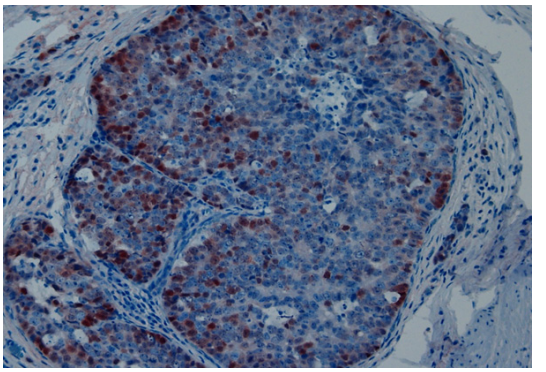

Figure 3. Intranuclear partial intracytoplasmic β-catenin immunoreactivity in a tumor with solid pattern (x100).

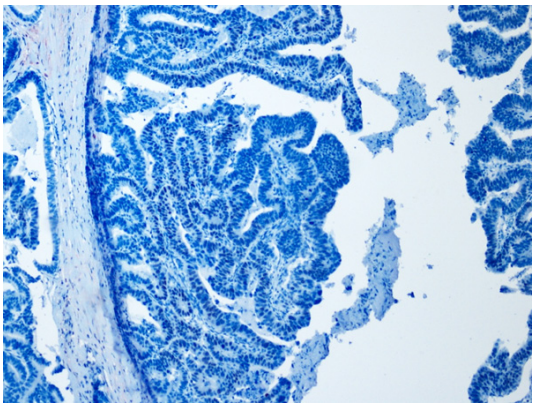

Figure 4. Negative control staining.

tissue samples were done in the Pathology Department unit. Five micrometer sections of the paraffin blocks were used for immunohistochemical staining. After the deparaffinization and rehydration process, the prepared sections were evaluated by using immunohistochemical examination with monoclonal antibody against β-catenin in 1/200 and LEF-1 in 1/150 dilution (Cell Signaling Technology, Inc, 9582, 2230, U.S).

The immunohistochemical antibody staining for β-catenin in tumor cells were evaluated as membranous, cytoplasmic and nuclear staining: (0) no staining, (1) diffuse membranous staining, (2) more than 10% membranous staining, (3) cytoplasmic staining, or (4) nuclear staining. β-catenin staining's were categorized as no staining, membranous staining, nuclear staining and compared with other parameters. The immunohistochemical antibody staining for LEF-1 in tumor cells were evaluated as (0) no nuclear staining and (1) positive nuclear staining.

The staining types of the obtained results were compared with the demographical, histopathological, and clinical characteristics of the patients. In addition, the effects of the staining pattern of β-catenin and LEF-1 on survival were calculated.

The statistical methods used were chi-square test for relationship between β-catenin and LEF-1 expression and patient features, Kaplan-Meier test for survival analysis acording between β-catenin and LEF-1 expression and patient features. In multivariate analysis, Cox regression test was used for prognostic factors. *p* values were accepted as significant at $p<0.05$.

Results

Eighty-two patients were included in this study. The median age was 56 (26-81). The median follow up time was 19 months (4-61). The demographic and clinicopathologic features are shown in **Table 1**.

Table 2. Relationship between LEF-1 expression and patient features

Patient features	LEF1 (-) n	LEF1 (+) n	p
Sex			0.284
Female	27	2	
Male	52	1	
Age			0.263
>60	34	0	
≤60	445	3	
Performance status			0.257
ECOG 0	44	3	
ECOG 1	35	0	
Tumor localization			0.260
Cardia	13	1	
Corpus	28	2	
Antrum	38	0	
T stage			0.899
T1	1	0	
T2	6	0	
T3	66	3	
T4	6	0	
N stage			0.427
N0	12	0	
N1	18	2	
N2	21	1	
N3a	24	0	
N3b	4	0	
Dissection type			0.935
D1	48	2	
D2	28	1	
D3	3	0	
Vascular invasion			0.423
Positive	14	0	
Negative	65	3	
Lymphatic invasion			0.444
Positive	13	0	
Negative	66	3	
Perineural invasion			0.780
Positive	2	0	
Negative	77	3	
Grade			0.059
G1	32	0	
G2	10	2	
G3	37	1	
Surgery border			0.444
Negative	66	3	
Positive	13	0	

As a result of immunohistochemical evaluation of the tumor tissue specimens, while there was no LEF-1 staining of 79 patients (96.3%) nuclear LEF-1 staining was positive in three patients (3.7%) (**Figure 1**).

β-catenin membranous staining in 36 (44%) of the patients (**Figure 2**) and β-catenin nuclear staining in 21 (26%) of the patients were found (**Figure 3**). There was no β-catenin staining in 25 (30%) of the patients (**Figure 4**).

Statistical significance was not found between LEF-1 expression and age, sex, performance status, tumor localization, T stage, N stage, vascular, lymphatic and perineural invasion, grade and dissection type ($p>0.05$). Relationship between LEF-1 expression and the patient features is summarized in **Table 2**.

The relationship between β-catenin expression and age, sex, performance status, tumor localization, T stage, N stage, lymphatic and perineural invasion, grade and dissection type was studied but statistical significance was not found ($p>0.05$). The relationship between β-catenin expression and the patient features is summarized in **Table 3**. In addition, there was no statistical significance between LEF-1 expression and β-catenin expression ($p=0.180$).

In 19 months of follow up, the median progression free survival period was 17 months (SE: 3 95% CI: 11-23) in all patient groups. Three years progression free survival ratio was 39.7%. Also in all patient groups, the median overall survival period was found to be 28 months (SE: 4% 95 CI: 20-36). Three years overall survival ratio was 41.2%.

In one variable analysis; there was no significant effect of age, sex, T stage, vascular, lymphatic and perineural invasion, grade and dissection type on overall survival ($p>0.05$).

Good performance status (ECOG 0), no diffuse tumor invasion like linitis plastica, low N stage of tumor according to TNM classification were found as positive factors on survival (respectively $p=0.04$, 0.033 and 0.005).

When the relationship between LEF-1 and β-catenin expression for survival was evaluated; the median overall survival was not reached in patients with LEF-1 expression while the median overall survival was found 27 months in patients with no LEF-1 expression ($p=0.126$).

While the median overall survival was found to be 28 months in the patients with β-catenin

Table 3. Relationship between β-catenin expression and patient features

Patient features	β-catenin Membranous Staining (n)	β-catenin Nuclear Staining (n)	β-catenin Staining (-) (n)	p
Sex				0.716
Female	13	6	10	
Male	23	15	15	
Age				0.051
>60	20	5	9	
≤60	16	16	16	
ECOG PS				0.194
ECOG 0	17	15	15	
ECOG 1	19	6	10	
Tumor localization				0.959
Cardia	7	3	4	
Corpus	12	9	9	
Antrum	17	9	12	
T stage				0.816
T1	1	0	0	
T2	2	1	3	
T3	30	18	21	
T4	3	2	1	
N stage				0.446
N0	3	6	3	
N1	9	3	8	
N2	10	6	6	
N3a	11	5	8	
N3b	3	1	0	
Dissection type				0.815
D1	22	13	15	
D2	12	7	10	
D3	2	1	0	
Vascular invasion				0.0870
Positive	7	3	4	
Negative	29	18	21	
Lymphatic invasion				0.972
Positive	6	3	4	
Negative	30	18	21	
Perineural invasion				0.097
Positive	0	0	2	
Negative	36	21	23	
Grade				0.160
G1	19	6	7	
G2	4	5	3	
G3	13	10	15	
Surgery border				0.389
Negative	32	18	19	
Positive	4	3	6	

membranous staining, 25 months in the patients with nuclear staining and 24 months in the patients with no staining (p=0.948). Although there is a positive trend on overall survival in the presence of LEF-1 expression, this trend could not reach to statistical significance (p=0.120).

Only N stage was found as an independent prognostic factor in multivariable cox regression analysis (p=0.05). Prognostic effects of patient demographic features, histopathological features apart from N stage, LEF-1 and β-catenin expression were not observed in our patient group.

Discussion

β-catenin, is a component of E-cadherin-catenin complex and has a crucial role in epithelial cell adhesion and maintenance of tissue structure [13, 14]. The abnormalities of this complex expression or functioning, result with adhesion loss between cells and may lead to cell transformation and cancer development [14, 15]. β-catenin expressions and gene mutations were investigated in many studies [14-18]. In gastrointestinal tumors, increased β-catenin expression was shown 75% in colorectal cancers, 56% in gastric cancer, 26.9% in hepatocellular cancer [19]. β-catenin expression is increased by way of Wnt signaling activation which can be found in about one third of gastric cancer cases. Wnt pathway dysregulation has a pivotal role in carcinogenesis. The overexpressed components of this pathway and downregulated or loss of Wnt inhibitors were discussed in development, progression and metastasis of gastric cancer. Different oncogenic signaling pathways might have interactions with Wnt regulators.

Consequently, chemotherapeutic approaches targeting Wnt/β-catenin pathway were evaluated [19].

In gastric cancer studies related to β-catenin expression conflicting results have been reported and the role of β-catenin mutations are not clear in gastric carcinogenesis [20, 21]. In a study of 157 gastric cancer cases by Nabais et al., it was reported that β-catenin expression decreased in diffuse and mixed type carcinomas while cytoplasmic and/or nuclear β-catenin expression increased in mixed type carcinomas. Nuclear localization of β-catenin was seen in 85.7% of the mixed tumors. In this study, cytoplasmic and/or nuclear β-catenin expression and lymphatic vessel invasion and lymph node metastases were associated significantly. They suggested that Wnt/β-catenin activation may effect this type of gastric cancer pathogenesis and this activation may be associated with worse outcomes in gastric carcinoma. No β-catenin mutation was seen in any of the cases. Therefore, the mechanisms underlying Wnt/β-catenin pathway in gastric carcinoma are not due to the β-catenin mutations and need to be clarified [20].

In another study by Grabsch et al., β-catenin expression was immunohistochemically investigated in a retrospective series of 401 R0-resected gastric carcinomas. In this study, in a subgroup of gastric carcinoma cases (53/401 case 13.2%) abnormal cytoplasmic or nuclear β-catenin expression has been shown and it was supposed that the Wnt signal pathway might be activated by β-catenin. But mechanisms of activation by β-catenin mediated signal pathway till now has not been explained. On the other hand, with the degree of membranous β-catenin expression or the type of staining (membranous vs cytoplasmic/nuclear) and the tumor grade, the histological tumor type as well as with the prognostic parameters pT, pN, category and vascular invasion were not significantly correlated. Additionally, there was no correlation between the presence of cytoplasmic/nuclear β-catenin expression and the loss of membranous β-catenin expression with tumor progression or prognosis [13].

In a study of 40 gastric cancer cases by Ramesh et al., membranous expression of β-catenin was decreased in 83% diffuse and 29% intestinal tumors (p=0.0014), and was associated with poor differentiation (p=0.0015) and short survival (p=0.032), but not due to age, sex, tumor size or nodal status. Nuclear expression of β-catenin was not common and in one third of the cases there was cytoplasmic expression but there were no correlations between cytoplasmic expression and histology, tumor grade or survival. Decreased membranous expression of β-catenin predicts poor prognosis in gastric cancer [21]. Ayed-Guerfali et al. investigated the expression of β-Catenin, adenomatous polyposis coli (APC) and E-cadherin in tumor tissues of the 80 Tunisian operated gastric cancer patients. In this study, membranous staining of tumor cells for β-catenin was accepted as normal expression while cytoplasmic, nuclear or no staining was accepted as abnormal expression. β-catenin expression was investigated both in combination with APC and E-cadherin or alone in gastric tumor cells. Membranous expression of β-catenin was detected in 61.3% of cases while cytoplasmic and or nuclear staining was seen in 38.7%. Only three of 80 cases exhibited nuclear expression alone. They have shown greater extent of lymph node metastasis, poor differentiation, and advanced T-stage in patients with abnormal β-catenin expression and correlated with a worse prognosis alone or in combination with loss of E-cadherin and APC expression [22].

In our study, we found β-catenin membranous staining in 36 (44%) of the patients while β-catenin nuclear and cytoplasmic staining in 21 (26%) of the patients. In 25 (30%) of the patients β-catenin staining was not observed. No statistical significance was found between β-catenin expression and age, sex, performance status, tumor localization, T stage, N stage, vascular, lymphatic and perineural invasion, grade or dissection type (p>0.05). With the evaluation of the relationship between β-catenin expression and overall survival, we found that median overall survival was 28 months in patients that have β-catenin membranous staining, 25 months in those who have nuclear staining and 24 months for those who have no staining (p=0.948). The factors affecting the results might be an insufficient number of patients and the high possibility of the selected group of patients. At the same time, there are some studies which have proven a relationship between β-catenin expression type and histopathological variables of the gastric

cancer while some studies do not show this relationship. So this relationship was not confirmed. In our study, β-catenin expression does not seem to be an independent prognostic factor in gastric cancer.

TCF4 and LEF-1 which are DNA binding proteins and transcription factors as nuclear β-catenin partners play a role in the wnt/β-catenin signal pathway in carcinogenesis. β-catenin translocates to the nucleus from the cytoplasm and binds to LEF-1 and TCF4 after Wnt signal pathway activation [15, 19]. In different solid tumors, LEF-1 expression was shown as a poor prognostic factor and associated with shorter survival [23, 24]. In colorectal carcinoma, LEF-1 expression had conflicting results in different studies. While Wang et al. found that LEF-1 expression was related with poor outcomes in colorectal carcinoma [25] and in a study by Lin et al., LEF-1 overexpression was shown to be associated with poor survival and increased risk for liver metastasis in colorectal carcinoma [26]. In contrast Kriegl et al. showed that LEF-1 expression was related with longer survival [9]. In a study of 214 patients with colorectal cancer, LEF-1, TCF4 and nuclear β-catenin analyzed immunohistochemically. There were no correlations between nuclear expressions of TCF4, LEF-1 and β-catenin. While nuclear β-catenin was positive in 75% of the cases, in contrast LEF-1 expression was positive only in 26% and TCF4 in 46% of colorectal carcinomas. In this study, while TCF4 expression was associated with shorter overall survival (p=0.02), LEF-1 expression was associated with longer overall survival (p=0.015). Comparing LEF-1, TCF4 and β-catenin expression, while some cases having nuclear β-catenin expression did not express LEF-1, TCF4 or both factors, other cases without β-catenin expression had LEF-1 and TCF4 expression. Therefore it was suggested that for the Wnt signal activation TCF4 or LEF-1 expression did not need to be with the nuclear β-catenin expression. Additionally, some other mechanisms different from Wnt signaling might be necessary for the regulation of TCF4 and LEF-1 expression as both of these factors were positive even when β-catenin expression was negative [9]. In a study by Radulescu et al. on mouse colonies, they activated the Wnt signaling pathway either by glycogen synthase kinase 3 (GSK3) or APC or by active β-catenin expression. Although in a very short time fundic gland polyp (FGP) formation and adenomatous change with parietal cell loss in the corpus and occurrence of adenomas in the antrum occurred, they could not succeedin displaying conversion to malignant gastric cancer either in the antrum or corpus. This suggests that Wnt signaling activation may not be enough to drive malignancy [27].

Finally, Kermanshahi et al. studied LEF-1 expression in 602 colorectal and other gastrointestinal tract neoplasms. In this study, only seven of 103 gastric carcinoma cases had positive LEF-1 expression in 15/175 (8%) of upper gastrointestinal tract adenocarcinomas while LEF-1 expression was detected in more than one third (89/241, 37%) of the colorectal carcinoma cases. Although moderate/strong LEF-1 expression levels had a trend of poor overall survival this could not reach to statistical significance (p=0.15) and there was no correlation with LEF-1 expression and clinicopathological findings in colorectal carcinoma [28].

In our study, we investigated the expression of LEF-1 in patients with gastric cancer which was not studied alone in gastric cancer as an isolated trial before. By the immunohistochemical analysis of tumor tissue specimens, LEF-1 expression was not found in 79 patients (96.3%) but was found only in three patients (3.7%). The median overall survival was 27 months in patients without LEF-1 expression while median overall survival time couldn't be reached in patients with LEF-1 expression (p=0.126). No relationship was found between LEF-1 expression and histopathological variables like β-catenin. There was no correlation found between β-catenin and LEF-1 expression as it was shown in colorectal cancers suggesting the presence of different effective mechanisms other than the Wnt signal pathway.

In the presence of LEF-1 expression, although there was a positive tendency in overall survival, it could not be reached to statistical significance. Actually, TGF-β/smad signaling pathway-could activate LEF-1 expression independent from the Wnt signaling pathway [29]. In colorectal cancer, tumor progression is mediated by inhibition of TGF β signaling [30, 31] and during cancer progression induction of growth arrest, differentiation and apoptosis being crucial events shown to be caused by inactivating mutations of the TGFβ pathway [32, 33]. TGFβ

signaling activation might be indicated by LEF-1 expression that inhibits tumor progression and development of metastasis. This type of mechanism might be effective in gastric cancer as well.

Disclosure of conflict of interest

None.

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References

[1] http://kanser.gov.tr/daire-faaliyetleri/kanser-istatistikleri/922-2009-kanser-insidansları.html.

[2] Adachi Y, Shiraishi N, Suematsu T, Shiromizu A, Yamaguchi K, Kitano S. Most important lymph node information in gastric cancer: multivariate prognostic study. Ann Surg Oncol 2000; 7: 503-507.

[3] Yokota T, Kunii Y, Teshima S, Yamada Y, Saito T, Takahashi M, Kikuchi S, Yamauchi H. Significant prognostic factors in patients with early gastric cancer. Int Surg 2000; 85: 286-290.

[4] Nesi G, Bruno L, Saieva C, Caldini A, Girardi LR, Zanna I, Rapi S, Bechi P, Cortesini C, Palli D. DNA ploidy and S-phase fraction as prognostic factors in surgically resected gastric carcinoma: a 7-year prospective study. Anticancer Res 2007; 27: 4435-4441.

[5] Starzynska T, Markiewski M, Domagala W, Marlicz K, Mietkiewski J, Roberts SA, Stern PL. The clinical significance of p53 accumulation in gastric carcinoma. Cancer 1996; 77: 2005-2012.

[6] Sanz-Ortega J, Steinberg SM, Moro E, Saez M, Lopez JA, Sierra E, Sanz-Esponera J, Merino MJ. Comparative study of tumor angiogenesis and immunohistochemistry for p53, c-ErbB2, c-myc and EGFR as prognostic factors in gastric cancer. Histol Histopathol 2000; 15: 455-462.

[7] Clements WM, Wang J, Sarnaik A, Kim OJ, MacDonald J, Fenoglio-Preiser C, Groden J, Lowy AM. Beta-Catenin mutation is a frequent cause of Wnt pathway activation in gastris cancer. Cancer Res 2002; 62: 3503-3506.

[8] Gurney A , Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, Fischer M, Chaudhari A, Ji M, Kapoun AM, Lam A, Lazetic S, Ma S, Mitra S, Park IK, Pickell K, Sato A, Satyal S, Stroud M, Tran H, Yen WC, Lewicki J, Hoey T. Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. Proc Natl Acad Sci U S A 2012; 109: 11717-11722.

[9] Kriegl L, Horst D, Reiche JA, Engel J, Kirchner T, Jung A. LEF-1 and TCF4 expression correlate inversely with survival in colorectal cancer. J Transl Med 2010; 8: 123.

[10] Giles RH, van Es JH, Clevers H. Caught up in aWnt storm: Wnt signaling in cancer. Biochim Biophys Acta 2003; 1653: 1-24.

[11] Ochoa-Hernández AB, Juárez-Vázquez CI, Rosales-Reynoso MA, Barros-Núñez P. WNT-β-catenin signaling pathway and its relationship with cancer. Cir Cir 2012; 80: 389-398.

[12] Surana R, Sikka S, Cai W, Shin EM, Warrier SR, Tan HJ, Arfuso F, Fox SA, Dharmarajan AM, Kumar AP. Secreted frizzled related proteins: Implications in cancers. Biochim Biophys Acta 2014; 1845: 53-65.

[13] Grabsch H, Takeno S, Noguchi T, Hommel G, Gabbert HE, Mueller W. Different patterns of beta-catenin expression in gastric carcinomas: relationship with clinicopathological parameters and prognostic outcome. Histopathology 2001; 39: 141-149.

[14] Huiping C, Kristjansdottir S, Jonasson JG, Magnusson J, Egilsson V, Ingvarsson S. Alterations of E-cadherin and beta-catenin in gastric cancer. BMC Cancer 2001; 1: 16.

[15] Karim R, Tse G, Putti T, Scolyer R, Lee S. The significance of the Wnt pathway in the pathology of human cancers. Pathology 2004; 36: 120-128.

[16] Ogasawara N, Tsukamoto T, Mizoshita T, Inada K, Cao X, Takenaka Y, Joh T, Tatematsu M. Mutations and nuclear accumulation of beta-catenin correlate with intestinal phenotypic expression in human gastric cancer. Histopathology 2006; 49: 612-621.

[17] Kolligs FT, Bommer G, Göke B. Wnt/beta-catenin/tcf signaling: a critical pathway in gastrointestinal tumorigenesis. Digestion 2002; 66: 131-144.

[18] Tsukashita S, Kushima R, Bamba M, Nakamura E, Mukaisho K, Sugihara H, Hattori T. Beta-catenin expression in intramucosal neoplastic lesions of the stomach. Comparative analysis of adenoma/dysplasia, adenocarcinoma and signet-ring cell carcinoma. Oncology 2003; 64: 251-258.

[19] Doucas H, Garcea G, Neal CP, Manson MM, Berry DP. Changes in the Wnt signalling pathway in gastrointestinal cancers and their prognostic significance. Eur J Cancer 2005; 41: 365-379.

[20] Nabais S, Machado JC, Lopes C, Seruca R, Carneiro F, Sobrinho-Simões M. Patterns of beta-catenin expression in gastric carcinoma: clinicopathological relevance and mutation analysis. Int J Surg Pathol 2003; 11: 1-9.

[21] Ramesh S, Nash J, McCulloch PG. Reduction in membranous expression of β-catenin and increased cytoplasmic E-cadherin expression predict poor survival in gastric cancer. Br J Cancer 1999; 81: 1392-1397.

[22] Ayed-Guerfali DB, Hassairi B, Khabir A, Sellami-Boudawara T, Gargouri A, Mokdad-Gargouri R. Expression of APC, β-catenin and E-cadherin in Tunisian patients with gastric adenocarcinoma: clinical significance. Tumour Biol 2014; 35: 1775-1783.

[23] Papagerakis P, Pannone G, Shabana AH, Depondt J, Santoro A, Ghirtis K, Berdal A, Papagerakis S. Aberrant beta-catenin and LEF1 expression may predict the clinical outcome for patients with oropharyngeal cancer. Int J Immunopathol Pharmacol 2012; 25: 135-146.

[24] Bleckmann A, Siam L, Klemm F, Rietkötter E, Wegner C, Kramer F, Beissbarth T, Binder C, Stadelmann C, Pukrop T. Nuclear LEF1/TCF4 correlate with poor prognosis but not with nuclear β-catenin in cerebral metastasis of lung adenocarcinomas. Clin Exp Metastasis 2013; 30: 471-482.

[25] Wang WJ, Yao Y, Jiang LL, Hu TH, Ma JQ, Ruan ZP, Tian T, Guo H, Wang SH, Nan KJ. Increased LEF1 expression and decreased Notch2 expression are strong predictors of poor outcomes in colorectal cancer patients. Dis Markers 2013; 35: 395-405.

[26] Lin AY, Chua MS, Choi YL, Yeh W, Kim YH, Azzi R, Adams GA, Sainani K, van de Rijn M, So SK, Pollack JR. Comparative profiling of primary colorectal carcinomas and liver metastases identifies LEF1 as a prognostic biomarker. PLoS One 2011; 6: e16636.

[27] Radulescu S, Ridgway RA, Cordero J, Athineos D, Salgueiro P, Poulsom R, Neumann J, Jung A, Patel S, Woodgett J, Barker N, Pritchard DM, Oien K, Sansom OJ. AcuteWNT signalling activation perturbs differentiation within the adult stomach and rapidly leads to tumour formation. Oncogene 2013; 32: 2048-2057.

[28] Kermanshahi TR, Jayachandran P, Chang DT, Pai R. LEF-1 is frequently expressed in colorectal carcinoma and not in other gastrointestinal tract adenocarcinomas: an immunohistochemical survey of 602 gastrointestinal tract neoplasms. Appl Immunohistochem Mol Morphol 2014; 22: 728-734.

[29] Nawshad A, Hay ED. TGFbeta3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol 2003; 163: 1291-1301.

[30] Ba Y, Tonoki H, Tada M, Nakata D, Hamada J, Moriuchi T. Transcriptional slippage of p53 gene enhanced by cellular damage in rat liver: monitoring the slippage by a yeast functional assay. Mutat Res 2000; 447: 209-220.

[31] Benson KF, Person RE, Li FQ, Williams K, Horwitz M. Paradoxical homozygous expression from heterozygotes and heterozygous expression from homozygotes as a consequence of transcriptional infidelity through a polyadenine tract in the AP3B1 gene responsible for canine cyclic neutropenia. Nucleic Acids Res 2004; 32: 6327-6333.

[32] van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 2002; 111: 241-250.

[33] Li F, Cao Y, Townsend CM, Ko TC. TGF-beta signaling in colon cancer cells. World J Surg 2005; 29: 306-311.