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Diagnostic Value of IMP3 Expression in Colorectal Carcinoma and Adenoma

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ABSTRACT

Colorectal carcinomas are the cause of high mortality and morbidity and are common in the world and in our country. The main mechanism emphasized in colorectal carcinogenesis is the adenoma-carcinoma sequence. However, carcinoma does not develop from all adenomas.

Many factors are effective in the prognosis of colorectal carcinomas. The most important prognostic parameter is TNM staging. Another is lymph node metastasis. In our study, we planned to evaluate the expression of insulin-like growth factor II m-RNA binding protein 3 (IMP3) in colorectal pathologies and its relationship with prognostic factors.

The 30 colorectal adenocarcinomas (10 well differentiated, 10 moderately differentiated, 10 poorly differentiated), 30 colon adenomas (10 tubular, 10 villous, 10 tubulovillous) and 10 normal colonic mucosa were included in our study. The diagnostic value of the extent and intensity of staining and the relationship between stage and lymph node metastasis in patients stained with IMP3 was investigated.

There was a significant correlation between IMP3 and normal mucosa-adenoma and normal mucosa-carcinoma groups ($p < 0.05$), but no significant correlation was found between lymph node metastasis and stage ($p > 0.05$). Significant correlations were found between IMP3 staining intensity and adenoma-carcinoma groups and between villous adenomas and other adenoma groups ($p < 0.05$). There was no statistical significance between differentiation degrees of carcinomas in IMP3 staining intensity ($p > 0.05$).

As a result, it was seen that IMP3 could be used in diagnosis, but the relationship between these markers and lymph node metastasis and stage was not significant.

Keywords: Colorectal carcinoma, adenoma, IMP3, prognostic factor

Introduction

Colorectal carcinoma is more common in developed, industrial countries; with an estimated 1.849.518 new cases worldwide in 2018, is the second most common cancer in women and the third most common cancer in men. Incidence increases with age. Genetic predisposition is very important, the probability of developing colorectal carcinoma in people with first-degree close relatives' colorectal carcinoma is doubled compared to the normal population (1-4). Its incidence increases in those who are fed a diet that is high in calories, rich in animal fat and protein, and lacking in herbal ingredients (1). The mortality rate is similar in men and women (3).

Many factors with prognostic significance have been identified for colorectal carcinomas. Clinically; age, gender, and symptoms are important, and histopathologically, tumor stage, grade, lymph node metastasis, and DNA ploidy are prognostic factors (2).

Environmental factors, especially diet, age, adenoma presence, and family history with predisposing factors such as inflammatory bowel disease are effective in the development of colorectal cancer. Most cases develop from the background of adenomatous polyps (5). Adenomas are benign tumors containing dysplastic colon epithelium and supportive stroma. Dysplasia can be between a mild degree and carcinoma in situ. They can be single or numerous. Adenomas can be classified according to their size, macroscopic appearance (sessile, peduncle, flat), structural ratio (tubular, villous, tubulovillous), and degree of dysplasia (mild, medium, severe). The risk of developing adenomas into cancer is related to the diameter, number, histological type and degree of atypia. According to histological types, the incidence of cancer development is 10-18% in villous adenoma, 6-8% in tubular adenoma, and 2-3% in tubular adenoma (2, 3).

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Colorectal carcinoma and its progression mechanisms were evaluated with various immunohistochemical (IHC) markers. Among these, IMP3 is an oncofetal protein (6). IMP family proteins are released in the early stages of embryogenesis and play an important role in the movement and stabilization of RNA, cell growth and cell migration. IMP3 is secreted from immature tissue and placenta that develops during embryogenesis. However, it has been shown that it is secreted from adult tissues at undetectable low levels (7). IMP3 is expressed in many malignant tumors; such as pancreatic, lung, stomach, and colon cancers. It is not expressed in benign tissues adjacent to the tumor (8-13). Therefore, IMP3 is thought to have an important role in the regulation of cell proliferation and tumor invasion. (14-16). The main goal of our study is to determine IMP3 expression in colorectal adenomas and carcinomas and to evaluate its relationship with lymph node metastasis and stage.

Materials and Methods

5-year cases belonging to Pathology Department Laboratory were examined retrospectively. Our study was started with the approval of the Firat University Faculty of Medicine Ethics Committee on 5.01.2012 with reference number 12/02. Ten normal colon mucosa (NM), 30 colon adenomas, and 30 colon adenocarcinoma cases were randomly selected. The groups consisted of ten tubular (TA), tubulovillous (TVA), villose (VA) adenomas and ten well-differentiated (IDA), moderately differentiated (ODA) and poorly differentiated (ADA) adenocarcinoma cases. Archive preparations belonging to the cases were re-examined under a light microscope and 4 micrometer thick sections were taken from paraffin blocks for IHC application after confirming the diagnosis.

Sections obtained from the blocks of seventy cases that were re-evaluated were taken on poly-lysine coated slides. The slides were first kept in the oven at 60 °C for 10 minutes and then in the automated staining device (Ventana Medical System, SN: 712299, REF: 750-700, Arizona, USA) processed. After treatment with primary antibody, slides were washed in tap water and covered with ultramount. Placental tissue samples were used for IMP3 as a positive control.

Antibodies-stained slides were evaluated under an Olympus BX51 light microscope. Cytoplasmic staining for IMP3 was considered positive. Staining intensity and percentage were determined for each case. It was evaluated as staining over 70% (+++), staining between 41-70% (++), and staining between 10-40% (+). Less than 10% of the tumor cells in the samples were considered negative for IMP3 staining. Staining intensity was evaluated as weak (+), moderate (++) and severe (+++) similar to Kulaçoğlu et al. and scored as 1,2,3, respectively. (17). The values of the adenoma and carcinoma groups were formed by taking the mean of the subgroups. The differences between groups for each marker were evaluated using the Fisher Exact Chi-square test. Statistical Package for the Social Sciences version 20.0 (SPSS 20.0 Inc., Chicago, IL, USA) Windows package program was used for statistical evaluation. If the p-value was less than 0.05, it was considered statistically significant.

Results

Of the 70 cases included in the study, 18 (60%) of those diagnosed with adenoma and 20 (67%) of those diagnosed with carcinoma were male. There was no statistically significant difference between the groups in terms of age ($p>0.05$). The mean age was 65.90±11.83 in adenomas, 64.83±14.1 in carcinomas and 64.20±8.59 in the control group.

In the evaluation of the tumor stage of the cases, 5 cases were found to be stage 1, 11 cases stage 2a, 1 case stage 2b, 7 cases stage 3b, and 6 cases stage 3c. When the extensiveness and intensity of staining with the IMP3 marker we used in our study were compared with the stages, no significant difference was found ($p>0.05$).

In our study, lymph node metastasis was present in 12 (17.1%) of 30 cases with carcinoma. The relationship between the degree of differentiation and lymph node metastasis in carcinomas was found to be significant ($p<0.05$). There was no statistically significant difference between IMP3 prevalence and severity and lymph node metastasis ($p>0.05$).

When the normal mucosa, adenoma and carcinoma groups were compared, a statistically significant difference was observed in terms of IMP3 staining degree and severity ($p<0.05$) (Table 1-2).

When the intensity and extensiveness of IMP3 staining were compared between the adenoma and carcinoma groups, the staining intensity was higher in the carcinoma group, and the difference was statistically significant ($p<0.05$, Figure 1).

Table 1. Relationship between IMP3 staining intensity and diagnostic groups (DG). [normal colon mucosa (NM), tubular adenoma (TA) tubulovilloz adenoma (TVA), villous adenoma (VA), well-differentiated adenocarcinoma (IDA), moderately differentiated adenocarcinoma (ODA) and poorly differentiated adenocarcinoma (ADA)]

		DG							p
		NM	TA	TVA	VA	İDA	ODA	ADA	
IMP3 staining intensity	0	8	2	2	1	1	0	0	,000
		57,1%	14,3%	14,3%	7,1%	7,1%	0%	0%	
	1	2	7	8	0	4	2	3	
		7,7%	26,9%	30,8%	0%	15,4%	7,7%	11,5%	
	2	0	1	0	8	5	7	4	
		0%	4%	0%	32%	20%	28%	16%	
	3	0	0	0	1	0	1	3	
		0%	0%	0%	20%	0%	20%	60%	

Table 2. IMP3 staining extensiveness-diagnostic groups (DG) relationship. [Normal colon mucosa (NM), tubular adenoma (TA) tubulovilloz adenoma (TVA), villous adenoma (VA), well-differentiated adenocarcinoma (IDA), moderately differentiated adenocarcinoma (ODA) and poorly differentiated adenocarcinoma (ADA)

		DG							p
		NM	TA	TVA	VA	İDA	ODA	ADA	
IMP3 staining extensi.	0	8	2	2	1	1	0	0	,000
		57,1%	14,3%	14,3%	7,1%	7,1%	0%	0%	
	1	2	3	6	0	2	1	1	
		13,3%	20%	40%	0%	13,3%	6,7%	6,7%	
	2	0	4	2	2	3	4	4	
		0%	21,1%	10,5%	10,5%	15,8%	21,1%	21,1%	
	3	0	1	0	7	4	5	5	
		0%	0%	0%	0%	0%	0%	0%	

Discussion

Colorectal carcinomas are more common, especially in developed countries. Many factors such as diet, chronic inflammation, radiation and precursor lesions play a role in etiology (1). The main mechanism that is mostly emphasized is the adenoma-carcinoma sequence. It occurs with multiple clonal genetic changes, and the presence of adenoma is important because of the progression from normal epithelium to carcinoma (18). However, the emergence of adenomas and the time until carcinoma formation varies significantly. The important point is that not every adenomatous polyp will turn into carcinoma. Characteristics such as the type, number, and size of the adenoma determine the adenoma-carcinoma relationship (2).

As with all cancers, an important feature of colon carcinomas is their metastasis. Colon carcinomas usually make their metastases to regional lymph nodes first. The presence of metastases in regional lymph nodes negatively affects the prognosis of carcinoma, and the number of metastatic lymph nodes is considered an important prognostic factor (3).

Today, many different immunohistochemical markers are used to determine the prognosis and treatment of colorectal carcinomas. In our study, the relationship of IMP3, which has an important role in cell proliferation, tumor invasion and metastasis, with colorectal carcinoma and adenomas and prognostic factors, stage and lymph node metastasis was investigated.

IMP3 was first obtained from pancreatic carcinoma cells. However, in subsequent studies, its expression has been reported in many malignancies in humans (19, 20). IMP3 plays a very important role in tumor cell proliferation, adhesion, invasion and metastasis, and the increase of IMP3 in tissue has been associated with neoplastic cell proliferation (20). Studies have

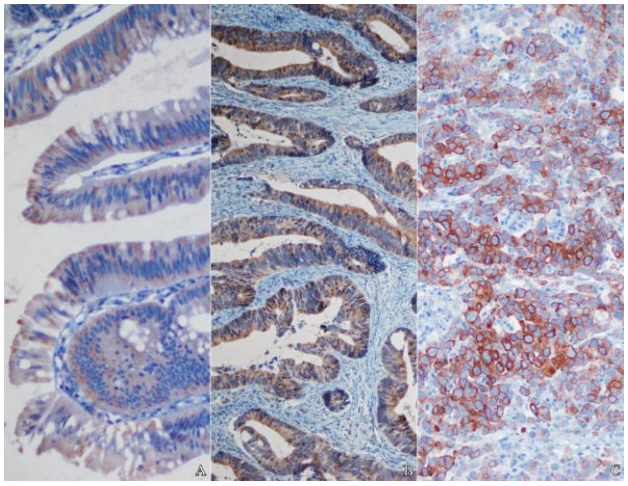

Fig. 1. IMP3 staining intensity a) staining in villous adenoma (++) (Immunperoxidase × 200) b) staining in moderately differentiated adenocarcinoma (++) (Immunperoxidase × 100) c) staining in poorly differentiated adenocarcinoma (+++) (Immunperoxidase × 200). IMP3 staining intensity did not differ significantly between well-moderately-poorly differentiated adenocarcinomas (p> 0.05). In villous adenomas, IMP3 staining intensity and extent were statistically significant compared to other adenomas (p <0.05).

reported that IMP3 is a useful marker in differentiating benign lesions of different organ systems, low-grade dysplasia, high-grade dysplasia, and invasive carcinoma (19).

There are few studies in the literature on the relationship between colorectal carcinomas and IMP3. In these studies, IMP3 positivity in colorectal carcinomas ranged between 65-74% (8, 21, 22). Nechifor et al. (23), in their study to evaluate IMP3 release in various tissues, found IMP3 negative in normal colon mucosa and diffuse (+++) in adenocarcinoma. Similarly, Wei et al. (8) found that there was no IMP3 expression in normal mucosa and hyperplastic polyps and diffuse expression in colorectal carcinomas in their study on biopsy specimens. In our study, IMP3 was not stained in 80% of cases in the normal colonic mucosa. However, staining of varying extent and intensity was observed in adenomatous polyps and adenocarcinomas. Intense (+++) staining with IMP3 was 3.3% in adenomatous polyps; and 13.3% in carcinomas.

Li et al. (21) studied IMP3 expression in groups with normal mucosa, carcinoma and lymph node metastases and found that IMP3 was higher and the difference was significant in lymph node metastasis compared to the other two groups. In the same study, a significant difference was observed between the groups in the Ki-67 proliferation index. A statistically significant increase in metastasis rates after curative colectomy was reported in patients with IMP3 positive. In this study, a significant decrease was found in patients who were IMP3 positive in 5-year surveillance. A study conducted by Wei and colleagues found a strong association between lymph node metastasis, TNM stage and tumor budding and IMP3 expression (24). In our study, there was no significant association between lymph node metastasis and stage and IMP3. But the possible reason for this may be the limited number of samples in our current study.

In a review by Wang et al. (25) on the oncogenic functions of IMP3 in carcinomas, numerous studies show that IMP3 communicates with many types of uncoded RNAs and proteins to promote malignant proliferation and metastasis and inhibit malignant cell apoptosis. They concluded that IMP3 could aid in cancer staging and be a potential molecular marker for the detection of malignancy due to its high expression in advanced cancers and poor overall survival rates of patients. Similarly, in our study, IMP3 expression was not observed in normal mucosa, but increased IMP3 expression was observed in adenoma and carcinoma groups.

Modern treatment of colorectal carcinomas is developing towards multidisciplinary management. In the treatment of colorectal carcinomas, preoperative chemotherapy and perhaps radiotherapy may become routine treatments. (26-30) It is thought that anti-IGF2BP3 drugs or monoclonal antibodies can be used as new therapeutic methods in the treatment in the near future (25). Therefore, preoperative correct staging of colorectal carcinomas is required in multidisciplinary treatment management. Microscopic examination of colonoscopic biopsy specimens is a critical diagnostic test for carcinoma diagnosis and is frequently used in routine daily treatment planning. Some microscopic features are important criteria for diagnosis, but they may not always be present in the biopsy sample. Of these, submucosal invasion (SMI) and desmoplastic stroma (DS) are considered key diagnostic features in distinguishing carcinoma from a high-grade dysplasia adenoma on biopsies (31, 32). However, because the biopsy material is very small and superficial or some artificial reasons, the distinction between carcinoma and adenoma cannot be always evaluated clearly. In our study, when the intensity and extent of IMP3 staining were compared between the adenoma and carcinoma groups, the staining intensity was

higher in the carcinoma group and the difference was statistically significant ($p< 0.05$). Considering that the importance of preoperative treatment is increasing day by day, this is a sign that IMP3 may be a reliable aid in cases of difficulty in diagnosis. Especially in endoscopic biopsies, the association of IMP3 with histopathological findings maybe facilitate the diagnosis of colorectal carcinoma.

References

1. Who Classification of tumors Editorial Board. Digestive system tumors Lyon (France): İnternational agency for research on cancer; 2019 (WHO classification of tumours series, 5th ed.; vol1). 157-192
2. Kumar V, Abbas AK, Aster JC. Small intestine and colon. Robbins & Cotran Pathologic Basis of Disease, 10th Edition. Philadelphia, Elsevier, 2021; 780-820.
3. Fenoglio-Preiser CM, Noffsinger AE, Stemmermann GN, Lantz PE, Isaacson PG. Epithelial Neoplasms of the Colon. Gastrointestinal Pathology, An Atlas and Text. 4'th ed, Philadelphia, Lippincott Williams&Wilkins 2017; 2071-2255.
4. Rosai J. Gastrointestinal Tract Large Bowel. Houston M. (editor) Rosai and Ackerman's Surgical Pathology. 11'th ed, China, Mosby Company 2018; 668-720.
5. Scott, C. M., Wong, E. M., Joo, J. E., Dugue, P. A., Jung, C. H., O'Callaghan, N., et al. Genome-wide DNA methylation assessment of 'BRCA1-like' early-onset breast cancer: data from the australian breast cancer family registry. Exp.Mol. Pathol 2018; 105: 404-410.
6. Schmiedel, D., Tai, J., Yamin, R., Berhani, O., Bauman, Y., and Mandelboim, O. The RNA binding protein IMP3 facilitates tumor immune escape by downregulating the stress-induced ligands ULPB2 and MICB. eLife 2016 5:e13426.doi: 10.7554/eLife.13426
7. Palanichamy J. K, Tran T. M, Howard, J. et al. RNA-binding protein IGF2BP3 targeting ofoncogenic transcripts promotes hematopoietic progenitor proliferation. J. Clin.Invest. 2016, 126, 1495–1511. doi: 10.1172/jci80046
8. Wei, Q., Zhou, H., Zhong, L., Shi, L., Liu, J., Yang, Q., et al. IMP3 expression in biopsy specimens as a diagnostic biomarker forcolorectal cancer.Hum. Pathol. 2017, 64, 137–144. doi: 10.1016/j.humpath.2017.03.013
9. Xu, W., Sheng, Y., Guo, Y., Huang, Z., Huang, Y., Wen, D., et al. IncreasedIGF2BP3 expression promotes the aggressive phenotypes of colorectal cancer cells in vitro and vivo.J. Cell. Physiol 2019; 234: 18466-18479.
10. Shaalan, Y. M., Handoussa, H., Youness, R. A., Assal, R. A., El-Khatib, A. H.,Linscheid, M. W., et al. Destabilizing the interplay between miR-1275and IGF2BPs by Tamarix articulata and quercetin in hepatocellular carcinoma. Nat. Prod. Res 2018; 32: 2217-2220.
11. Chen, H., Kong, Y., Yao, Q., Zhang, X., Fu, Y., Li, J., et al. Three hypomethylated genes were associated with poor overall survival in pancreatic cancer patients 2019; Aging11, 885-897.
12. Bi, R., Shen, X., Zhang, W., Cheng, Y., Feng, Z., Cai, X., et al. Clear cell carcinomas of the ovary: a mono-institutional study of 73 cases in China withan analysis of the prognostic significance of clinicopathological parameters andIMP3 expression. Diagn. Pathol 2016; 11:17.
13. Findeis-Hosey, JJ., Xu, H. Insulin-like growth factor II-messenger RNA-binding protein-3 and lung cancer. Biotech. Histochem 2012; 87: 24-29.
14. Yuan G, Tianping L, Xiwu O, Chunfu Z, Junqiang Z, Xihu Q. IGF2BP3 and miR191-5p synergistically increase HCC cell invasiveness by altering ZO-1 expression. Oncology Letters 2020; 20: 1423-1431.
15. Sitnikova L, Mendese G, Liu Q, Woda BA, Lu D, Dresser K et al. IMP3 Predicts aggressive superficial urothelial carcinoma of the bladder. Clin Cancer Res 2008; 14: 1701-1706
16. Zhao, W., Lu, D., Liu, L., Cai, J., Zhou, Y., Yang, Y., et al. (2017). Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) promotes lung tumorigenesis via attenuating p53 stability. Oncotarget 8, 93672–93687. doi: 10.18632/oncotarget.21280
17. Kulaçoğlu S, Erkılınç G. Imp3 Expression in Benign and Malignant Thyroid Tumors and Hyperplastic Nodules. Balkan Med J 2015 Jan; 32(1): 30–37. Published online 2015 Jan 1. doi: 10.5152/balkanmedj.2015.15547
18. Leslie A, Carey F.A. Partt NR, Steele. The colorectal adenoma-carcinoma sequence. R.J.C. Br J Surg 2002; 89: 845-860.
19. Jennifer J, Hosey F, Xu H. The use insulin like-growth factor 2 messenger RNA binding protein-3 in diagnostic pathology. Hum Pathol 2010; 6: 1-12.
20. Hanley KZ, Facik MZ, Bourne PA, Yang Q, Spaulding BO, Bonfiglio TA, et al. Utility of anti-L523S antibody in the diagnosis of benign and malignant serous effusions. Cancer 2008; 114: 49-56.
21. Li D, Yan D, Tang H, Zhou C, Fan J, Li S, Wang X, Xia J, Huang F, Qiu G, Peng Z.

IMP3 is a novel prognostic marker that correlates with colon cancer progression and pathogenesis. Ann Surg Oncol 2009; 16: 3499-3506.

22. Yuan RH, Wang CC, Chou CC, Chang KJ, Lee PH, Jeng YM Diffuse expression of RNA-binding protein IMP3 predicts high-stage lymph node metastasis and poor prognosis in colorectal adenocarcinoma. Ann Surg Oncol. 2009; 16: 1711-1719.
23. Nechifor-Boila A, Nechifor-Boila A, Loghin A, Catana R, Borda A. Expression of insulin-like growth factor-2 mRNA binding protein 3 (İMP3) in various normal and neoplastic human tissues. Revista Romana de Medicina de Laborator 2012; 20: 163-172.
24. Wei Q, Huang X, Fu B, Liu J, Zhong L, Yang Q, Zhao T. IMP3 expression in biopsy specimens of colorectal cancer predicts lymph node metastasis and TNM stage. Int J Clin Exp Pathol 2015; 8: 11024-11032.
25. Wang P.F, Wang X, Liu M et al. The Oncogenic Functions of Insulin-like Growth Factor 2 mRNA-Binding Protein 3 in Human Carcinomas. Curr Pharm Des 2020; 26: 3939-3954.
26. Kataoka K, Kanazawa A, Nakajima A, et al. Feasibility and potential benefit of preoperative chemotherapy for colorectal liver metastasis(CLM): a single-centered retrospective study. Surg Today 2013; 43: 1154-1161.
27. Qiu B, Ding PR, Cai L, et al. Outcomes of preoperative chemoradiotherapy followed by surgery in patients with unresectable locally advanced sigmoid colon cancer. Chin J Cancer 2016; 35: 65.
28. Arredondo J, Baixauli J, Pastor C, et al. Mid-term oncologic outcome of a novel approach for locally advanced colon cancer with neoadjuvant chemotherapy and surgery. Clin Transl Oncol 2017; 19: 379-385.
29. Foxtrot Collaborative Group. Feasibility of preoperative chemotherapy for locally advanced, operable colon cancer: the pilot phase of a randomized controlled trial. Lancet Oncol 2012; 13: 1152-1160.
30. Ahmed S, Johnson K, Ahmed O, Iqbal N. Advances in the management of colorectal cancer: from biology to treatment. Int J Color Dis 2014; 29: 1031-1042.
31. Cerar A, Zidar N, Vodopivec B. Colorectal carcinoma in endoscopic biopsies; additional histologic criteria for the diagnosis. Pathol Res Pract 2004; 200: 657-662.
32. Hirose M, Fukui H, Igarashi Y, et al. Detection of desmoplastic reaction in biopsy specimens is useful for predicting the depth of invasion of early colorectal cancer: a Japanese collaborative study. J Gastroenterol 2010; 45: 1212-1218.