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Difference Expression of Galectin-3 in Various Depth of Invasion on Colorectal Adenocarcinoma in RSUP Sanglah Denpasar

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Abstract: ***Colorectal carcinoma is the fourth leading dead of malignancy in the world, including Indonesia. The depth of invasion is one of the major prognostic factors. Galectin-3 is one of the markers that are involved in anti-apoptosis, cell adhesion,and various mechanism, such as survival, angiogenesis, proliferation, transformation, and cell migration. The purpose of this study was to determine the role of galectin-3 in depth of invasion of colorectal carcinoma. This study used analytic cross-sectional method. Forty-five samples were selected from adenocarcinoma NOS by proportional stratified random sampling. Re-diagnosis was carried out to determine depth of invasion, followed by immunohistochemical staining of galectin-3 that performed at Sentra Diagnostik Patologi Bali Laboratory. Data were analyzed by One Way Anova and Chi-square test with a value of p<0.05. One WayAnova analysis showed significantly mean difference expression of galectin-3 in various depth of invasion (p=0.006) and the highest mean difference was found in pT4. Chi-square analysis showed significantly difference expression of galectin-3 with depth of invasion (p=0.039).This study proved that galectin-3 plays a role in the depth of invasion of colorectal adenocarcinoma not otherwise specified and can be considered as an adjuvant marker to predict prognosis.***

Keywords: galectin-3, depth of invasion, colorectal adenocarcinoma, immunohistochemistry

1. Introduction

Colorectal carcinoma (CRC) is a common malignancy in digestive tract system, especially in developing countries. The incidence is higher in developed countries and is associated with diet and lifestyle. This carcinomahas various prognostic factors, so tumors with different clinicopathological features will give different treatment results.

According to GLOBOCAN data in 2012, colorectal carcinoma is the fourth leading cause of death in cancer worldwide (694,000 cases or 8.5% of total cases) and is the third major malignancy in men (746,000 cases or 10,0 % of total cases) and second in women (614,000 cases or 9.2% of total cases) [1].Data recorded in 2011 showed that in Indonesia 1,200 new cases of colorectal carcinoma were found in men and 1,142 cases in women [2]. According to research data at Sanglah General Hospital Denpasar in 2014-2016, there were 75 cases (7.11%) of rectal cancer and 38 cases (3.60%) of colon cancer [3]. This shows that the CRC is still a serious health problem in Indonesia in general and in Bali in particular.

Galectin-3 is an ß-galactoside-binding protein that is involved in several biological behaviors, such as cell proliferation, apoptosis, adhesion, angiogenesis, invasion, and distant metastases. This shows that this protein plays an important role in the development and progression of human tumors, including colorectal cancer.Immunohistochemical examination can be done to measure galectin-3 expression levels and depth of invasion that influence the prognosis of patients [4], [5]. There are varied opinions regarding the expression of galectin-3 against the depth of the CRC invasion. According to Tao et al, Ibrahim et al, there was a significant correlation between galectin-3 expression and the depth of invasion in the CRC according to Duke stage, whereas according to Huang et al, Wu et al, there was a significant relationship between galectin-3 expression and the depth of invasion in the CRC according to AJCC. Gopalan et al, and Povegliano et al, stated that there was no significant relationship between galectin-3 expression and the depth of tumor invasion. Those studies found nosignificant relationship between galectin-3 expression with variables of age, sex, location, and tumor size [4], [5], [6], [7], [8], [9].

According to those controversial studies, we are interested to examine the differences galectin-3 expression at various depth of invasion on CRC.

2. Material and Methods

2.1 Specimens

Slides and paraffin embedded tissue blocks from 45 patient's colorectal adenocarcinoma nototherwise specified were retrievedfrom the histopathology archives in Anatomic PathologyLaboratory of Sanglah Hospital and Sentra Diagnostik Patologi Bali Laboratoryin Bali from 2016 to 2018. Clinical data were gathered from the medical reports and cancer registries.

2.2 Histopathology Evaluation

The slides from these cases were reviewed and histopathologic diagnoses in the histopathologic reports were confirmed independently by two pathologists and one resident. Tumour section of the deepest invasion was selected.

2.3 Galectin-3 Immunohistochemistry and Interpretation

The tissue section at 3 µm thickness from each case was prepared for immunostaining. After 30 minutes incubation in a 60°C oven, deparaffinizationand rehydration, the tissue sections were treated with 3% hydrogen peroxide for 10 minutes. This process is followed by incubation of the sections in blocking buffer for 30 minutes in room temperature. The slides then incubated with one of the following primary antibodies galectin-3mousemonoclonal (clone 9C4). The color was visualized by DAB as chromogen. Immunostaining were interpreted independently by two pathologists and one resident with eye ball estimation method.

Immunohistochemistry resultswere evaluated by asemiquantitative approach using Histo-score (H-score).The galectin-3 expression was assessed on thecytoplasmic staining of tumour cells throughout the invasive area. Theintensityscoreis givenby 0 (negative), 1(weak), 2(moderate)and 3(strong) (Figure 1). The percentage of cells at eachstaining intensity level is assigned using the followingformula: {[1 × (% cells 1+)] + [2 × (% cells 2+)] + [3 × (%cells 3+)]}. The H-score was obtained from the calculationwith a range of 0-300 and median value 175. The samples show low galectin-3 expression if H-score <175 and high galectin-3 expression if H-score ≥ 175[9].

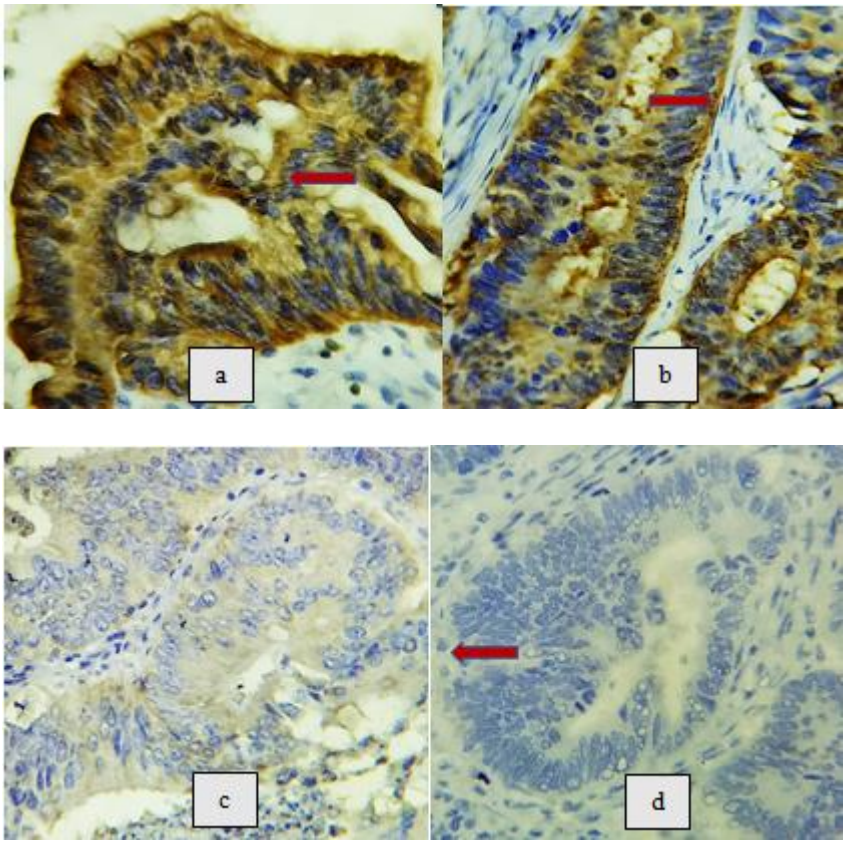

Figure 1: Galectin-3 immunohistochemistry staining intensity (a) strong (3+), (b) moderate (2+), (c) weak (1+), (d) negative (0) (x400)

2.4 Statistical analysis

The descriptive statistics then calculated and the Anova test was used to assess mean difference of galectin-3 expression H-score in various depth of invasion. Furthermore, Chi square test were used to assess the difference value galectin-3 expression withdepth of invasion of colorectal adenocarcinoma not otherwise specified. Thep-value of<0.05 was considered significant. All statistical analyses were performed using SPSS 20.0.

3. Result

The mean age of patients was 58.11 ± 13.4 year (range 31-88 year). Men was more frequent than women [25 (55.6%) were men and 20 (44.4%) were women]. Colon was slightly more frequent than rectal [23 (51.1%) colon and 22 (48.9%) rectal].

All of the histopathology diagnosis was colorectal adenocarcinoma not otherwise specified. There is no sample with stage 1 (pT1) based on TNM AJCC characteristics. Stage 2 (pT2) was 9 (20.0%), stage 3 (pT3) was 30 (66.7%) and stage 4 (pT4) was 6 (13.3%). Based on One WayAnova testshown in Table 1, there was a significant mean difference of galectin-3 expression H-score in various depth of invasion (p=0.006). From the results of LSD (Least Significant Differences), there was a significant difference in the mean expression of galectin-3 based on the depth of invasion between pT2 and pT4 with a result of 102.833 (p = 0.002) and between pT3 and pT4 with a result of 73.000 (p = 0.007), whereas between pT2 and pT3 there was no

significant difference with the results of 29.833 (p = 0.182) (Table 2).In Table 3, the Chi-square analysis showed significant difference expression of galectin-3 with depth of invasion (p=0.039).

Table 1: The difference mean H-Score of galectin-3 expression based on the depth of invasion

Depth of Invasion	Mean	*p* value
pT2	138.33±67.40	0.006
pT3	168.17±58.13	
pT4	241.17±34.34	

Table 2: Mean difference in various depths of tumor invasion

(I) Depth of Invasion	(J) Depth of Invasion	Mean Difference (I-J)	*p*value
pT2	pT3	-29,833	0.183
	pT4	-102,833	0.002
pT3	pT2	29,833	0.182
	pT4	-73,000	0.007
pT4	pT2	102,833	0.002
	pT3	73,000	0.007

Table 3: Differences expression of galectin-3 with depth of tumor invasion

		Depth of Invasion			Total	p value
		pT2	pT3	pT4		
Gal-3 Expession *(H-score)*	Low	4 (44.4%)	17 (56.7%)	0	21 (46.7%)	0.039
	High	5 (55.6%)	13 (43.3%)	6 (100%)	24 (53.3%)	
Total		9 (100%)	30 (100%)	6 (100%)	45 (100%)	

4. Discussion

Currently the clinical guidelines used to determine the management and prognosis of colorectal carcinoma are the classification of Tumor Node Metastasis (TNM) approved by the American Joint Commission on Cancer (AJCC). This system reports the extent of tumor spread (T), lymph node status (N), and metastasis (M). Other prognostic factors include: lymphovascular and perineural invasion, resection margin, histological subtype of CRC and patient response (angiogenesis, local inflammation and desmoplastic response). All of these factors are significant factors that determine the prognosis of patients [10], [11].

Samplecolorectal adenocarcinoma not otherwise specified in this study showed varies age, range from 31 years to 88 years (mean age 58.11 ± 13.4 years). This mean age is almost the same as CRC patients in another study, on sixth decade. The age range of most patients between 51-60 years, as many as 18 (40%) cases [10], [12], 13], [14]. In this study, the youngest age of colorectal adenocarcinoma not otherwise specified patient was 31 years male, with depth of invasion to pT4, with a high galectin-3 expression score. Whereas the oldest age is 88 years old male, with depth of invasion at pT3 and low galectin-3 expression score. There was no significant relationship between age and galectin-3 expression in colorectal adenocarcinoma not otherwise specified in this study.

The incidence of colorectal carcinoma sort by sex, is relatively higher in men, this is due to differences in diet and lifestyle between men and women, such as alcohol consumption and smoking. And there was no significant relationship between sex and galectin-3 expression in patients with colorectal carcinoma not otherwise specified.

Colorectal carcinoma mostly occurs in the sigmoid and rectum, but in recent research there has been a change of location with increasing proportions of carcinoma in the more proximal part with increasing age [4]. In this study, there were more patients with colorectal adenocarcinoma not otherwise specified located in the colon (51.1%) and in the rectum (48.9%). There was no significant relationship between tumor location with galectin-3 expression in colorectal adenocarcinoma not otherwise specified.

In a study which conducted by Huang et al, showed that there was a significant correlation between galectin-3 expression and depth of invasion (p = 0.027), this is related to galectin-3 as it was a member of the lectin family that has multi-functional oncogenic proteins involved in cell growth, anti-apoptosis, adhesion, angiogenesis and invasion [5]. Likewise, the study of Wu et al, found a significant correlation between galectin-3 expression and depth of invasion, where galectin-3 expression was higher in the later stages (TNM stage) compared to the initial stage or normal colon tissue [9].

In a study conducted by Gopalan et al found that galectin-3 was expressed more strongly in colorectal adenocarcinoma (95%) compare to adenoma (73%) and it was also said that galectin-3 was expressed more strongly in advanced colorectal cancer compare to its primary tumor. On the other hand, in perforated colorectal cancer or poor differentiation, galectin-3 is less expressed. Based on its stage (TNM) it was found that in stage I and II with a sample size of 22, there were 21 (95%) cases of strong expression, while stages III and IV with a sample of 51, there were 48 (94%) cases of strong expression and this result was not significantly statistically (p = 0.651), but if it is connected between pathological stages and mRNA expression from various galectin there is a significant difference. This associationwas explained by not all colorectal carcinoma followed galectin-mediated proliferation and metastasis [8].

Povegliano et al research found that galectin-3 was expressed in the cytoplasm on all samples. Forty-three (57.33%) were weak or negative and 32 (42.67%) were moderate or strong. This study was statistically not significant according to the pathology stage (p = 0.48) [6].

In a study conducted by Ibrahim et al, it was found that there was a significant correlation between galectin-3 expression and tumor location (p = 0.038), histology type (p <0.001), histological grading (p = 0.002), and modified Duke's stage (p = 0.01789), while age, gender, macroscopic features of the tumor, tumor size, depth of tumor invasion, metastases to lymph node, distant metastases and vascular invasion were found to be unsignificant [10]. Likewise, with the findings of Tao et al, there was a significant correlation between galectin-3 expression with tumor size, tumor differentiation, and Duke staging (p <0.05) [4].

Based on this study that writen on table 1 and table 2, it can be concluded that the mean difference of galectin-3 expression in pT2 is equal to pT3 or not statistically significant. While the mean difference of galectin-3 expression in pT2 and pT3 was smaller than pT4 or statistically significant.

Based on the depth of invasion it was found that there were differences in galectin-3 expression at various levels of depth of invasion and strong galectin-3 expression in the depth of invasion of pT4. This indicates that galectin-3 expression was increased in colorectal adenocarcinoma not otherwise specified with depth of invasion of pT4 compared to pT3 and pT2. This finding has reinforced the theory of the role of galectin-3 in cancer development such as anti-apoptosis, cell adhesion, and cell proliferation in colorectal adenocarcinoma not otherwise specified, which underlies tumor growth and invasion and is associated with metastasis and poor prognosis.

During the development of cancer and metastasis, galectin-3 affects cancer activity through several pathways. First, the intracellular (cytoplasmic) galectin-3 is anti-apoptotic providing survival advantage to cancer cells. Second, galectin-3 promotes tumor angiogenesis. Third, the extracellular galectin-3 is involved in homotypic aggregation. Fourth, tumor–endothelial cell interactions required for metastasis are believed to be mediated by endothelium-associated galectin-3 and cancer cell-associated TFD. Fifth, tumor cell secreted galectin-3 induces apoptosis of cancer-infiltrating T-cells possibly promoting immune escape during tumor progression. Thus, galectin-3 plays an important role in the development of cancer and metastasis [15].

To found the difference in galectin-3 expression with the depth of tumor invasion Chi-Square test was performed using a 2x3 cross table. This test showed a statistically significant difference between galectin-3 expression and depth of tumor invasion ($p = 0.039$) (Table 3).

Based on the results of this study, it was conclude that there were differences in galectin-3 expression at various levels of depth tumor invasion and higher expression was found at pT4. There were also a significant relationship between galectin-3 expression and depth of invasion in colorectal adenocarcinoma not otherwise specified.

References

[1] Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F. 2014. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 136(-): 359–386.
[2] Dirjen-Yanmed 2011. Kanker di Indonesia, Jakarta, Dirjen Yanmed Departemen Kesehatan Republik Indonesia.
[3] Anthonysamy, M.A., Maker, L.P.I.I., Gotra, I.M., Saputra, H. 2018. Prevalence of colorectal carcinoma based on microscopic type, sex, age and anatomic location in sanglah hospital. Intisari Sains Medis. 9(2): 1-19.
[4] Tao, L., Jin, L., Dechun, L., Hongqiang, Y., Changhua, K., Guijun, L. 2017. Galectin-3 Expression in Colorectal Cancer and its Correlation with Clinical Pathological Characteristics and Prognosis. Open Med (Wars). 12(-): 226-230.
[5] Huang, Z., Aia, Z., Lia, N., Xia, H., Gaoa, X., Wanga, F., Tanc, X and Liu, H. 2016. Over expression of galectin-3 associates with short-term poor prognosis in stage II colon cancer. Cancer Biomarkers 17 (2016): 445–455.
[6] Povegliano, L.Z., Oshima, C.T.F., Lima, F.D.O., Scherholz, P.L.A., Forones, N.M. 2011. Immunoexpression of Galectin-3 in Colorectal Cancer and its Relationship with Survival. Journal Gastrointest Cancer. 42(-): 217-221.
[7] Ibrahim, B.B., Helmy, O.D., El Sheikh, A.S., Mostafa, R.R. 2015. Immunohistochemical Expression of Galectin-3 in Colorectal Carcinoma. MEJSR. 23(4): 580-591.
[8] Gopalan, V., Saremi, N., Sullivan, E., Kabir, S., Lu, C.T., Salajegheh, A., Leung, M., Smith, R.A., Lam, A.K. 2016. The expression profiles of the galectin gene family in colorectal adenocarcinomas. Hum Pathol. 53(-): 105-113.
[9] Wu, K.L., Kuo, C.M., Huang, E.Y., Pan, H.M., Huang, C.C., Chen, Y.F., Hsiao, C.C., Yang, K.D. 2018. Extracellular galectin-3 facilitates colon cancer cell migration and is related to the epidermal growth factor receptor. Am J Transl Res. 10(8): 2402-2412.
[10] Hamilton, S.R., Nakamura, S.I., Bosman, F.T., Quirke, P., Boffetta, P., Riboli, E., Iiyas, M., Sobin, L.H., Morreau, H. 2010. Carcinoma of the colon and rectum. In: Bosman, F.T., Jaffe, E.S., Lakhani, S.R. &Ohgaki, H., editors. WHO Classification of Tumours of the Dygestive System.4thn. Ed. Lyon IARC Press. p. 134-146.
[11] Goldblum, J.R. 2018. Large Bowel. In: Goldblum, J.R., Lamps, L.W., Mc Kenney, J.K. &Myers, J.L., editors. Rosai and Ackerman's Surgical Pathology.Eleventh. Ed. Philadelphia:Elsavier. p. 648-685.
[12] Hornick, J.L., Odze, R.D. 2015. Polyps of the Large Intestine. In: Odze, R.D. &Goldblum, J.R., editors. Oldze and Goldblum Surgical Pathology of the GI Tract, Liver, Biliary Tract, and Pancreas.Third. Ed. Philadelphia:Elsevier Saunders. p. 607-655.
[13] Washington, M.K., Berlin, J., Branton, P., Burgart, L.J., Carter, D.K., Fitzgibbons, P.L., Halling, K., Frankel, W., Jessup, J., Kakar, S., Minsky, B., Nakhleh, R., Compton, C.C. 2010. Protocol for the Examination of Specimens From Patients With Primary Carcinoma of the Colon and Rectum. Arch Pathol Lab Med. 133(10): 1539-1551.
[14] Redston, M., Driman, D.K. 2015. Epithelial Neoplasms of the Large Intestine. In: Odze, R.D. &Goldblum, J.R., editors. Odze and Goldblum Surgical Pathology of the GI Tract, Liver, Biliary Tract, and Pancreas.Third. Ed. Philadelphia:Elsevier Saunders. p. 737-778.
[15] Ahmed, H., Guha, P., Kaptan, E., Bandyopadhyaya, G. 2014. Galectin-3: a potential target for cancer prevention. NIH Public Access. 3(2): 13–22.