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Expression of protein regenerating liver-3 (prl-3) and E-cadherin in colorectal cancer

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Background: Colorectal cancer is the third most common malignant neoplasm worldwide. PRL-3 (phosphatase of regenerating liver-3/PTP4A3) was reported participates in the progression of colorectal cancer and play a role in epithelial to mesenchymal transition by down regulate the expression of E- cadherin.

Aims: To clarify the molecular mechanisms that involved in colorectal cancer development and progression, we investigate the expression of PRL-3 and E-Cadherin in colorectal cancer and correlate the expression with the clinicopathologic parameters.

Methods: Expression of PRL-3 and E-Cadherin in 76 colorectal cancer specimens were detected by immunohistochemistry.

Results: Among colorectal cancer specimens examined, there were 30 (39.5%) well differentiated, 36 (47.4%) moderately differentiated and 10 (13.2%) poorly differentiated CRC. There were significant correlation between histological grading and PRL-3 expression (p=0.044), and also with E-cadherin expression (p=0.039). The expression of PRL-3 was significantly correlated the expression of E cadherin in colorectal cancer (p=0.003). The result showed that the more higher PRL-3 expression the more lower the expression of E-cadherin in colorectal cancer.

Conclusions: These studies strongly suggest that PRL-3 may play a role to down regulate the expression of E cadherin in the development and progression of colorectal cancer.

Key words: PRL-3, E-cadherin, colorectal cancer, immunohistochemistry

INTRODUCTION

Colorectal cancer is the third most common malignant neoplasm worldwide[1] and the second leading cause of death due to cancer in the United States.[2] In Makassar Indonesia (2005-2014) colorectal cancer also the third common cancer, according to pathology based data. The prognosis of colorectal cancer patients especially with distant metastasis still remains poor, although recent advances in diagnostic and therapeutic agent have been established. For those reason, it is important to clarify the molecular mechanism involved in development of colorectal cancer and to identify the specific biomarkers of colorectal cancer metastasis. To identify the genetic alterations related with the transition from primer tumor of colorectal to liver metastasis, serial analysis of gene expression was done by Saha and defined that PRL-3 was frequently over expressed in the liver metastasis, with low expression in primary colorectal cancer and non tumor colorectal epithelium.[3]

Protein tyrosine phosphatases play a crucial role in regulating several proteins that involved in many aspect of physiological and pathogenic cellular processes.[4] The PRL-PTP family consist of three members isoforms, PRL1, PRL2, and PRL3 which has a unique motif of COOH-terminal prenylation and specific active site CX5R as sequence signature.[5,6] PRL phosphatase proteins were reported to be associated with the early endosome and the plasma membrane in their prenilated state, meanwhile these enzymes may switched to nuclear localization when absence of prenylation.[7] PRL proteins have about 22 kDa according to amino acid sequences, and share about 76-87% amino acid sequence similarity with the isoforms. In another study, human PRL-1 and PRL_2 were detected using an in vitro prenylation method.[5] PRL-1 as the first member of PRL phosphatases, was originally detected as an immediate early gene in regenerating rat liver and mitogen-treated cells. High expression of PRL-1 and PRL-2 has been identified to transform mouse fibroblasts and epithelial cells of pancreas in culture cells. PRL-1 and PRL-2 are also promote tumor growth in nude mice, and this indicate that these proteins involved in cancer development.[7,8] However, from review of some studies, comparing with others PRL family, PRL-3 is more prominent associated cancer.

PRL-3 was detected to be the only consistent gene expressed at high mRNA levels in all colorectal cancer metastasis studied. Since then, high expression of PRL-3 has been recognized to be associated with cancer development of many cancer including metastatic ability and poor prognosis. PRL-3 located at chromosomal loci 8q24 and expressed low in the pancreas and mainly detected in normal skeletal muscle and heart. This expression pattern is different from the pattern of PRL-1 and PRL-2.[9] PRL-3 has been identified to promote growth of fibroblast in embryonic kidney of human. By

using CHO cells, PRL-3 transfected cells recognized can enhance motility and invasiveness, promote migration and increase metastatic ability.[11] Deleted of PRL-3 gene was reported suppress growth of gastric cancer cells and ovarian cancer cells. These suggest that PRL-3 phosphatase is a key alteration contributing in progression and metastatic tumor cells, but what are the molecules interact with PRL-3 still need to clarify.

E-cadherin is a transmembrane protein, which, in normal epithelium, is responsible for intracellular interactions. Its cytoplasmic domain interacts with β-catenin or γ-catenin. In cancers, dysfunction of E-cadherin can be induced by lack of the cytoplasmic domain resulting no interaction with catenins or induced by lack of the extracellular domain and resulting no interaction with adjacent cells and made an accumulation of catenins. These disorders and mutations can lead to the detachment of cancer cells from the primary tumor mass and increase their invasiveness and metastatic abilities[12,13]

Consistent with an oncogenic mechanism of PRL-3 expression, transfection of PRL-3, causes cell transformation, promotes tumor growth and induces metastatic tumor formation in mice. PRL-3 could also enhance Epithelial Membrane Transition in SW480 model of colorectal cancer with deficient E-cadherin expression in vitro and in vivo.[11] PRL-3 promotes EMT by regulating directly E-cadherin. PRL-3 down regulated of both E-cadherin and CHH22. In the present study, we want to examine the expression of PRL-3 and E-cadherin in human colorectal cancer to know the significance expression of PRL-3 and E-cadherin in the progression of CRC.

MATERIALS AND METHODS

Tissue samples

A total of 76 formalin-fixed, paraffin-embedded specimens of primary colorectal cancers were collected from Department of anatomical Pathology, Hasanuddin University Hospital. They consisted of 40 men and 36 women with an age range of 25-84 years and mean 52.92 years. Histopathological type was graded as follows: well-differentiated, moderately differentiated and poorly differentiated adenocarcinoma. The grading of colorectal cancer based on the proportion of tumor glands relative to that of displaying solid sheets of the tumor area as presented in the WHO grading system **.**[17]

Immunohistochemistry

Serial sections from each block were cut for hematoxylin and eosin staining to define histological diagnoses and the remaining sections were prepared for the immunohistochemical study. Immunohistochemical staining was performed using the LSAB kit from Dako. The slides were placed in the container jar filled with 10 mmol/L citrate buffer (pH 6.0), and boil in microwave for 5 min. The slides were placed at room temperature for 60 min before being immersed for 15 min in 0.3% hydrogen peroxidase to block endogenous peroxidase. The primary polyclonal rabbit anti PRL-3 antibody, (1:100 dilution, Attogen Bio) and the primary monoclonal

anti-E-Cadherin antibody (1:100 dilution, Dako) were applied to slides and incubated 16 hours at 4°C. The slides were incubated with secondary antibody for 30 min and then streptavidin conjugated to horseradish peroxidase (DAKO) for 30 min. Chromogenic agent using the solution of 3,3-diamino-benzidine tetrahydrochloride (DAB) for 10 minutes until a distinct colour was detected microscopically. The slides were then counterstained with Mayer's hematoxylin. Negative control slides were incubated without both primary antibody.

Immunoreactivity of anti-PRL-3 and anti-E-Cadherin antibody were evaluated as follows: negative/0, almost no cells stained positive; +1, less than 50% of tumor cells showed weak expression; +2, less than 50% of tumor cells showed strong expression; +3, over 50% of tumor cells showed strong immunoreactivity. Smooth muscle fibers for PRL-3 which have strong expression were used for internal controls of positive immunoreaction. Immunostaining was scored independently by independent researchers (UM, MHC) who were not aware with the clinical and histopathogical diagnosis. The statistical analyses using chi-square test to evaluate the relathionship between the immunohistochemical study and clinicopathological data, $p < 0.05$ was regarded as statistically significant.

RESULTS

Immunohistochemical staining of PRL-3, E-cadherin and histological grading of colorectal cancer

We evaluated the localization and expression of PRL-3 and E-cadherin in 76 cases of colorectal cancer immunohistochemically. Immunostaining examples of PRL-3 and E-cadherin are shown in Fig. 1. PRL-3 protein was mainly located in the cytoplasm. Smooth muscle cells which showed strong expression of PRL-3, were used for positive internal control. As shown in Table 1, PRL-3 immunoreactivity was detected in 51 cases (67.1%) of 76 cases examined. The distribution of positivity rate between histological grading was as follow; of the 30 cases of well differentiated carcinoma, 15 were positive (50%); of the 36 cases of moderately differentiated carcinoma, 28 were positive (77.7%); and of the 10 cases of poorly differentiated carcinoma, eight were positive (80%). There was a significant correlation between PRL-3 and histological grading of CRC (P=0.004). The distribution of expression found more frequent in poorly differentiated than moderately and well differentiated. Weak expression was found in non-neoplastic colorectal mucosa surrounding tumor tissue. Moderate expression which detected in fibroblast and in some inflammatory cells was defined as internal control.

We also analyzed the relationship between the expression of E-cadherin protein and histological grading of CRC.

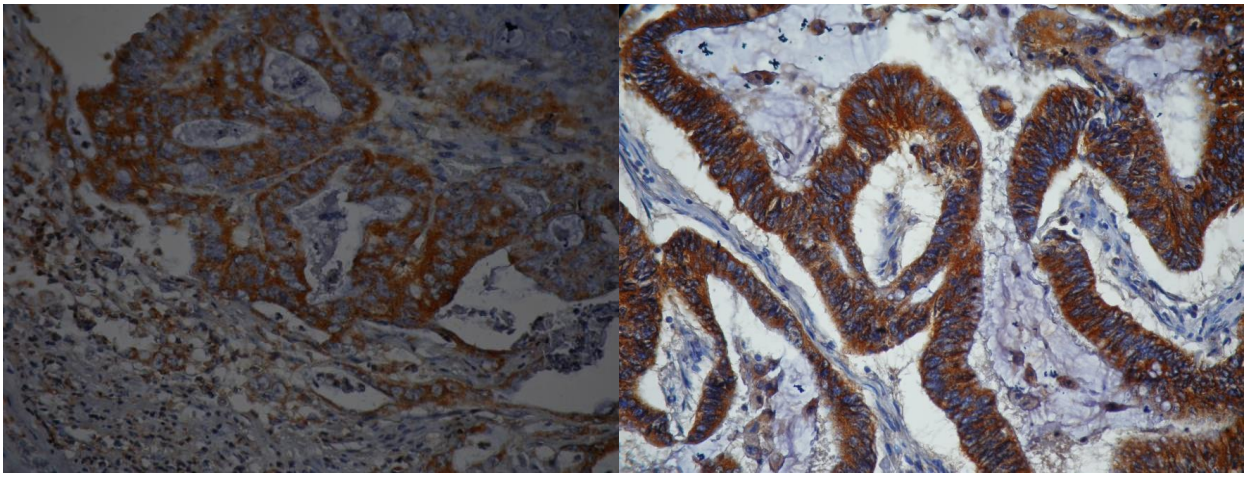

Figure 1 Representative example of immunohistochemical staining of PRL-3 (A) and E-cadherin (B) in colorectal cancer. Both are positive in cytoplasm and cytoplasmic membrane.

A summary of E-cadherin expression is shown also in Table 1. Negative and weak expression of E-cadherin was significantly associated with poorly differentiated tumor (*P*=0.039). Strong expression in non-neoplastic colorectal mucosa was used as positive internal control. No significant correlation between PRL-3 expression and gender, age, neither did E-cadherin.

Relationship between PRL-3 and E-cadherin expression in colorectal cancer

We evaluated the correlation between PRL-3 and E-cadherin in colorectal cancer. As shown in Table 2**.** There was a significant correlation between PRL-3 and E-cadherin expression (*P*=0.003). The result showed negative correlation between PRL-3 and E-cadherin, its mean the more frequent of PRL-3 expression in colorectal cancer the lower E-cadherin expression in colorectal cancer and vice versa.

DISCUSSIONS

Protein tyrosine phosphatase PRL-3 has been reported to be associated with human colorectal cancer progression. The phosphatase of regenerating liver 3 (PRL-3) protein belongs to the family of tyrosine phosphatases, together with PRL-1 and PRL-2. These proteins have unique COOH-terminal prenylation motif, and involved in a major reaction for the cell, i.e., dephosphorylation of tyrosine residues deactivating enzymes. Although its physiological role is poorly investigated, literature data suggest that PRL-3 takes part in development of tumor and in neoformation, i.e., migration, metastasizing, and angiogenesis.[4-6]

Table 1 Expression of PRL-3 and E-cadherin in different histological grading of colorectal cancer

	Histological Grading of CRC								
	76 Cases		Well diff.		Moderately		Poorly diff.		P value
Protein expression	n	%	n: 30	%	n:36	%	n:10	%	
PRL-3 expression									
Negative	25	32.9	15	50	8	22.2	2	20	0.044
Weak	19	25	7	23.3	11	30.6	1	10	
Moderate	20	26.3	7	23.3	10	27.7	3	30	
Strongly	12	15.8	1	3.3	7	19.4	4	40	
E- cadherin									
Negative	19	25	4	13.3	11	30.6	4	40	0.039
Weak	18	23.7	4	13.3	10	27.7	4	40	
Moderate	25	32.9	12	40	11	30.6	2	20	
Strongly	14	18	10	33.3	4	11.1	0	0	

CRC, colorectal cancer: diff, differentiated

Table 2 Correlation between PRL-3 and E-cadherin expression in colorectal cancer

Immunostaining	E-cadherin expression								P value
	Negative		Weak		Moderate		Strongly		
	n	%	N	%	n	%	n	%	
PRL-3 expression									
Negative	2	2.63	3	3.9	12	15.8	8	10.5	0.003
Weak	4	5.2	3	3.9	7	9.2	5	6.6	
Moderate	10	13.1	6	7.8	3	3.9	1	1.3	
Strongly	3	3.9	6	7.8	3	3.9	0	0	
TOTAL	19		18		25		14		

However, factors that regulate PRL-3 expression as well as its enzymes are not well known, and researchers are still searching for pathways and processes associated with the protein involvement. Still a few studies have revealed a link between PRL-3 and proteins responsible for cytoskeleton rebuilding. Regulation of cell adhesion is another mechanism of the protein in the promotion of cancer cell growth and invasion.[11,18,19] In this study, we detected the expression of PRL-3 had higher rates of positive expression in poorly differentiated than moderately and well differentiated. Although not just poorly can be metastasized, but this tumor grading reflect the worse prognosis than well or moderately differentiated. We found the significant correlation between high expression of PRL-3 and histological grading of CRC. These results suggest that PRL-3 may play a crucial role in development and differentiation of colorectal cancer.

E-cadherin, a transmembrane adhesion molecule has a function to maintain intercellular adhesiveness. Abnormal expression or decreased expression of E-cadherin cause loss of cell to cell contact, can lead to the detachment of cancer cells from the primary tumor mass and thus increase their invasiveness to stroma and vessels. In this study, expression of E-cadherin was abnormal and decreasing in cancer area comparing with non neoplastic area. E-cadherin positivity rate was more frequent decrease in tumor with poorly differentiated than moderately and well differentatied. It suggests that abnormal and decreased expression of E-cadherin play a role in progression and tumor differentiation.

PRL-3 seems to be particularly responsible for the development and migration of cancer cells. Wang et al. have been the first to suggest the involvement of PRL-3 in epithelial mesenchymal transition (EMT).[20] They have put forward the hypothesis that PRL-3 activates the Akt pathway through direct inhibition of PTEN (inhibitor for PI3K), which results in GSK-3β inactivation. Then, Liu et al. have presented evidence for PRL-3 involvement in EMT via cadherin-related signaling pathway. Most likely, PRL-3 plays a major role in direct inhibition of the expression of E-cadherin and CDH22 [21]. In our study, we analyzed the immunohistochemical correlation between the expression of PRL-3 and E-cadherin in colorectal cancer and observed a correlation between increased PRL-3 expression and abnormal E-cadherin expression ($P = 0.003$), which indicates that they may interact. We revealed a correlation of positive PRL-3 and abnormal E-cadherin with colorectal cancer. It is likely that an abnormal expression of

E-cadherin and an overexpression of PRL-3 are associated with the loss of cell junctions and loosening of cells in this histological type. Thus, PRL-3 and E-cadherin seem to exert an extremely significant effect on the progression of colorectal cancer. Our findings are compatible with previous studies.[20-22]

In conclusion, the present results strongly suggest that PRL-3 and E-cadherin have a role in tumor progression. There is a significant correlation between PRL-3 protein and E-cadherin expression showed mutual participate in the development of colorectal cancer. It suggest that PRL-3 may play a role to down regulate the abnormal expression of E-cadherin in the development and metastatic ability of colorectal cancer. PRL-3 might be considered as a new indicator for malignant potential and as a potential therapeutic target in cases of colorectal cancer.

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