RESEARCH ARTICLE

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Low Expression of Mucin 2, High Expression of Mucin 13, and High Expression of Nuclear Factor Kappa-Light-Enhancer of Activated B Cells were Significant Pathways in Colorectal Cancer Development

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Abstract

Objective: To prove the role of MUC2, MUC13, and NFκB protein expression as significant carcinogenesis pathways in colorectal cancer development. **Methods:** This is a nested case-control study conducted at the Udayana University, Sanglah General hospital, from September 2020 to 2021. All eligible subjects who visited the Digestive Surgery outpatient clinic with a colorectal surgery plan 2021 were included. The subjects were classified as case group (cancerous colonic mucosa) and control group (normal colonic mucosa), proved by histopathology examination. The parameters in this study were the expression of MUC2, MUC13, and NFκB by immunohistochemistry analysis. The data in this study will be collected and tabulated in SPSS 25.0 (Chicago, Illinois, USA). **Results:** A total of 36 subjects with colorectal cancer (case group) and 36 subjects with normal colonic mucosa (control group) were analyzed in this study. The cancerous colonic mucosa significantly had a lower MUC2, higher MUC13, and higher NFκB expression. After multivariate analysis for controlling the age variable, the result showed that only MUC2, MUC13, and NFκB expressions were still significant with $p<0.05$. The effect from MUC2, MUC13, and NFκB expression totally could assess up to 85.4% of the risk of developing colorectal cancer. **Conclusion:** There was a significantly lower MUC2, higher MUC13, and higher NFκB expression in the carcinogenesis of colorectal cancer, representing the influence of the inflammatory pathway and the abnormality of the protective barrier. Therefore, in the future, this result could remark a future early prediction or scoring system to assess colorectal cancer in clinical application.

Keywords: Mucin- colorectal cancer- carcinogenesis- NFkB

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Introduction

Colorectal cancer is still reported to have high incidence and mortality until now. Most people with colorectal cancer in Indonesia were in an advanced stage, whereas the cases were usually unresectable and had bad prognoses (Ferlay et al., 2015). The carcinogenesis of normal colonic mucosa developing into colorectal cancer was related to inflammation and abnormality of the protective barrier system in the intestinal mucosa (Raisch et al., 2014).

The main mucus layer that makes up the colonic mucosa is mucin types 2 and 13 (Kufe, 2009). In colorectal cancer, it was found that there was a decrease in MUC2 expression and an increase in MUC13 expression (Ahn et al., 2005). The relationship between inflammation and cancer has been studied intensively, and there is a role of the transcription factor NF-κB (Terzić et al., 2010). Thus, MUC2, MUC13, and NFκB were hypothesized to be involved in the pathways of colorectal cancer carcinogenesis. However, research results were still varied. There was no existing study in Indonesia that assessed those markers in colorectal cancer. Therefore, this study aimed to prove MUC2, MUC13, and NFκB protein expression's role as significant carcinogenesis pathways in colorectal cancer development.

Materials and Methods

Study Design

This is a nested case-control study conducted at the Division of Digestive Surgery, Department of Surgery, and the Department of Pathology of the Udayana University Medical School - Sanglah General Hospital. Data from medical records were collected from September 2020 to 2021.

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Study population

The population of this study was all patients who visited the Digestive Surgery outpatient clinic of Sanglah Hospital with a colorectal surgery plan from 2020 to 2021. The included subjects were the population with the official histopathology examination after surgery. If the result was colorectal carcinoma, subjects were put in the case group, and subjects were put in the control group if the result was normal. The exclusion criteria were the unavailable demographic information from the medical records, the error of paraffin block due to a lot of necrotic and bleeding tissue, or the remaining amount being insufficient for further analysis. From the results of the calculation of sample 2 proportions for case-control research, the required sample size in this study was 36 subjects for each group.

Study parameters

The parameters in this study were the expression of MUC2, MUC13, and NFκB. The paraffin blocks from the included subjects were collected in the Pathology Laboratory of Sanglah General Hospital. They were then sliced and processed for immunohistochemistry examination. The specimen was fixed with 10% buffered formalin and then painted with each monoclonal antibody kit product. The painting process includes peroxidase block, primary antibody or negative control reagent, peroxidase-labeled polymer, substrate – chromogen, Hematoxylin Counterstain (Optional), and mounting. The specimen object glass was then read under Olympus CX21 microscope.

The expression of MUC2, MUC13, and NfκB was interpreted as the sum of the intensity scores and the percentage scores. The intensity score was assessed based on the density of tumor cells stained positively with strong magnification (400x), scored negative (0), weak (+1), moderate (+2), and strong (+3). The percentage scores were interpreted as how much the tumor cells were stained in one field by the magnification of 40x, scored as no (0), <25% (+1), 26-50% (+2), 51-75% (+3), and >75% (+4). The cut-off point was determined from the median value of each variable. The determined median value for MUC was 6. If the score is less than 6, MUC2 or MUC13 expression is categorized as low and if the score is more than or equal to 6, the MUC2 or MUC13 expression is categorized as high. The determined median value for NFkB is 7. So if the score is less than 7, the expression of NFκB is categorized as low; if the score is more than or equal to 7, the expression of NFB is categorized as high.

The demographic data were age, gender, the habit of smoking, drinking alcohol, red meat consumption, and presence of family history, taken from medical records. The respondent age was calculated from the date of birth to the year the research was conducted, categorized as less or more than 50 years. Gender was categorized as male or female. The habit of smoking was defined as using at least one cigarette per day, every day, categorized as yes or no. The habit of drinking alcohol was categorized as consuming alcohol at least one drink per day, every day, categorized as yes or no. The habit of consuming red meat was defined as eating more than three servings of red meat per week, with a single meal of about 350-500 grams of meat, categorized as yes or no. Family history is defined as the presence or absence of a family history of colorectal cancer.

Statistical analysis

The data in this study will be collected and tabulated in SPSS 25.0 (Chicago, Illinois, USA). The categorical variables will be distributed in the form of narration and tables. The numerical variables were expressed in terms of median, mean, and standard deviation, while categorical variables were expressed in terms of frequency and percentage. A statistical test was performed with Chi-Square to determine the difference in the expression of MUC2, MUC13, and NFκB on the occurrence of colorectal cancer. To determine the magnitude of the risk, an Odds ratio was estimated. The bias from demographic variables will be controlled by multivariate logistic regression analysis. The Hosmer-Lemeshow test assessed the feasibility of the logistic regression model and the model's coefficient of determination was assessed by the Nagelkerke R square. The precision of the data was a 95% confidence interval, and the significance level was considered when p was less than 0.05.

Results

Characteristics of Subjects

A total of 36 subjects with colorectal cancer (case group) and 36 subjects with normal colonic mucosa (control group) were analyzed in this study. The majority of subjects with colorectal cancer were >50 years old (90.9%), male (61.1%), did not have a smoking habit (58.3%), did not have an alcohol consumption habit (75%), had a habit of consuming meat (94.4%), and had no family history of colorectal cancer (90.9%). The only significant

Table 1. Characteristics of Subjects

Data	Cases (Colorectal cancer)	Control (Colitis)	Total (n)	p
Age				
<50 years old	5	16	21	0.004*
>50 years old	31	20	51	
Gender				
Female	14	17	31	0.475
Male	22	19	41	
Smoking				
Yes	21	28	49	0.077
No	15	8	23	
Alcohol consumption				
Yes	27	22	49	0.126
No	9	4	13	
Meat consumption				
Yes	2	4	6	0.394
No	34	32	66	
Family history				
Yes	30	33	63	0.285
No	3	6	9	

*Significant

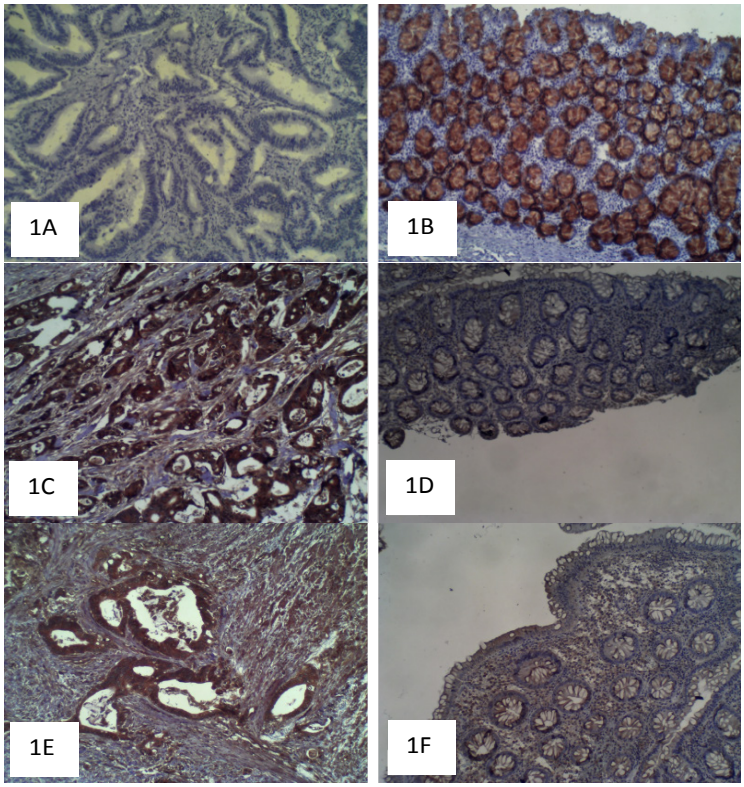

Figure 1. Immunohistochemical Analysis in Colon Mucosa. A, Low MUC2 expression; B, High MUC2 expression; C, High MUC13 expression; D, Low MUC13 expression; E, High NFκB expression; F, Low NFκB expression

Table 2. The Difference in MUC2, MUC13, and NFκB Expression of the Colorectal Mucosa on the Risk of Developing Colorectal Cancer

Data	Cancerous colonic mucosa	Normal colonic mucosa	OR	p
MUC2 expression				
Low	33	8	38.5	<0.001*
High	3	28	(95%CI 9.3-159.2)	
MUC13 expression				
High	33	6	55	<0.001*
Low	3	30	(95%CI 12.6-239.6)	
NFκB expression				
High	30	4	40	<0.001*
Low	6	30	(95%CI 10.3-155.8)	

*Significant

difference was found between the age groups >50 years o,d and <50 years old in the risk of developing colorectal cancer (p=0.004; Table 1).

The MUC2, MUC13, and NFkB expression on the normal and cancerous colonic mucosa

In this study, the cancerous colonic mucosa significantly had a lower MUC2 expression (p<0.001; OR 38.5 (95%CI 9.3-159.2)), a higher MUC13 expression (p<0.001; OR 55.0 (95%CI 12.6-239.6), and a higher NFκB expression (p<0.001; OR 40,0 (95%CI 10.3-155.8); Table 2; Figure 1).

To control the age variable, multivariate analysis was done. The result showed that only MUC2, MUC13, and

Table 3. Regression Logistic Model for Risk of Developing Colorectal Cancer

Variabel	B	SE	p	Exp (B)	95%CI
MUC2 expression	3.693	1.33	0.005	40.17	2.96-544.29
MUC13 expression	3.817	1.268	0.003	45.47	3.79-545.84
NFκB expression	3.202	1.179	0.007	24.58	2.44-247.66
Constant	-6.305	2.007	0.002	0.002	

NFκB expressions were statistically significant, with p<0.05. The effect from MUC2, MUC13, and NFκB expression totally could assess up to 85.4% of the risk of developing colorectal cancer. Of the three variables, MUC13 expression had the most significant effect compared to MUC2 and NFκB expressions (Table 3).

Discussion

The development of colorectal cancer is an interaction between genomic and environmental factors that continues to evolve, associated with inadequate protection (MUC2 downregulation and MUC13 upregulation) and transcriptional pathways (NFκB) (Terzić et al., 2010). The epithelial lining of the intestine is covered by mucous layers, which consist mainly of secreted mucin. MUC2 is a significant component of the protective mucus layer of the colon and plays a role in the development of colorectal cancer (Byrd and Bresalier, 2004).

Downregulation of MUC2 expression removes the barrier in epithelial cells so that bacteria can make contact with epithelial cells and trigger an inflammatory response (Hansson and Johansson, 2010). In cancer cells, the cytokines IL-4, IL-3, and TNF-alpha increased, resulting in the downregulation of MUC2. In addition, the carcinogenesis process directly triggers direct methylation of MUC2 in colorectal cancer cells in promoter area A (-289 and -274) and area C (-193 and -160), which in turn downregulate MUC2 and triggers transcription factors (Okudaira et al., 2010). In this study, it was shown that there was a significantly lower MUC2 expression in the cancerous colonic mucosa, which was consistent with other studies. Low MUC2 expression levels were significantly associated with poor, disease-free, or relapse-free survival (Al-Khayal et al., 2016).

MUC13 is a mucin normally expressed at low levels at the apical boundary of epithelial cells containing phosphorylation sites. In the pathogenesis of metaplasia, IL-6 is known to increase the JAK/STAT5 pathway, complex with MAPK p44/42, increase P-ERK expression, then form complex with the MUC13 promoter to increase its expression (Maher et al., 2011). MUC13 increases the expression of several proteins, including SHH, TERT, BMI-1, GATA1, HER2/MAPK signaling, and Bcl-xl, which are involved in the development and metastasis of colon cancer. In addition, the MUC13 promotes NF-B activation by interacting with TNFR1 and the E3 ligase, cIAP1, to increase RIPK1, which triggers cell proliferation (Sheng et al., 2017). MUC13 can also interact directly with GSK-3β or via other complex members, preventing its ability to initiate catenin degradation, leading to catenin accumulation, increased Wnt/β-catenin signaling, increased transcriptional activity, and promoting the development of colorectal cancer. Overall, MUC13 is associated with activating several pathways that are essential for cancer progression. In this study, it was found that in the age group, the majority of the population had MUC13 upregulation, following other studies which reported that increased MUC13 production by colorectal cancer cells correlated with the ability of colon cancer cells to metastasize to the liver (Byrd and Bresalier, 2004).

NF-κB factor is a transcription factor closely related to the process of carcinogenesis (Karin, 2006). In colorectal cancer cells, cancer stem cells trigger an increase in IL-6/STAT3 signaling, AMPK/mTOR activation, and NFκB directly. Epithelial cell NFκB activation directly promotes colitis progression to colorectal cancer. Cyclin D1 and cMyc induced by NFκB have important roles in cell growth and proliferation. In addition, various studies have shown that NFκB can block apoptosis by increasing anti-apoptotic proteins, such as inhibitors of apoptotic proteins (IAPs) and inhibiting activation (JNK). On the other hand, major angiogenic factors such as vascular endothelial growth factor (VEGF) and interleukin-8, directly or indirectly enhanced by NFκB activation, also play a role in cancer cell proliferation (Greten et al., 2004). This study found that there was an effect of high NFκB expression and the risk of colorectal cancer. From theory, the pathway that influenced a lot was the canonical pathway. Canonical pathways are associated with proinflammatory cytokines, IκBα phosphorylation, translocation to the nucleus, and promotion of transcription (Oeckinghaus et al., 2011). These results concluded that the expression of MUC2, MUC13, and NFκB plays an important role in the carcinogenesis pathway in colorectal cancer. In addition, this condition indirectly illustrates that the lower MUC2, higher MUC13, and high NFκB, the higher the cell proliferation and the more progressive cancer. MUC13 has the most decisive influence compared to others.

This study showed a relationship between the three independent variables, MUC2, MUC13, and NFκB, even after controlling for the control variables, on colorectal cancer carcinogenesis. The novelty of this study is that there has never been a study that has assessed the expression of MUC2 and NFκB in colorectal cancer in the Indonesian population. Previous studies had only assessed MUC2, MUC13, and NFκB separately. However, there were several limitations of this study. First, this study only takes a subject of the Balinese population. Second, the analysis of smoking habits and meat consumption seems lacking because of the limitations of a more detailed analysis of the quantity consumed. In the future, an early colorectal cancer risk stratification system can be developed from these variables, both with markers and scoring systems.

In conclusion, there was a significantly lower MUC2, higher MUC13, and higher NFκB expression in the carcinogenesis of colorectal cancer, representing the influence of the inflammatory pathway and the abnormality of the protective barrier. Therefore, in the future, this result could remark a future early prediction or scoring system to assess colorectal cancer in clinical application.

Author Contribution Statement

None.

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This study did not receive funding from a third party or pharmaceutical company.

Data availability

Data sharing is applicable upon request by contacting the corresponding author. We did not register the data due to the privacy of the participant's data.

Ethical Issue

Udayana University Local Ethical Committee has approved this study

Conflict of interest

The authors declare no potential conflict of interest.

References

Ahn DH, Crawley SC, Hokari R, et al (2005). TNF-alpha activates MUC2 transcription via NF-kappaB but inhibits via JNK activation. *Cell Physiol Biochem*, **15**, 29-40.

Al-Khayal K, Abdulla M, Al-Obaid O, et al (2016). Differential expression of mucins in Middle Eastern patients with colorectal cancer. *Oncol Lett*, **12**, 393-400.

Byrd JC, Bresalier RS (2004). Mucins and mucin binding proteins in colorectal cancer. *Cancer Metastasis Rev*, **23**, 77-99.

Ferlay J, Soerjomataram I, Dikshit R, et al (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*, **136**, E359-86.

Greten FR, Eckmann L, Greten TF, et al (2004). IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell*, **118**, 285-96.

Hansson GC, Johansson ME (2010). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Gut Microbes*, **1**, 51-4.

Karin M (2006). Nuclear factor-kappaB in cancer development and progression. *Nature*, **441**, 431-6.

Kufe DW (2009). Mucins in cancer: function, prognosis and therapy. *Nat Rev Cancer*, **9**, 874-85.

Maher DM, Gupta BK, Nagata S, et al (2011). Mucin 13: structure, function, and potential roles in cancer pathogenesis. *Mol Cancer Res*, **9**, 531-7.

Oeckinghaus A, Hayden MS, Ghosh S (2011). Crosstalk in NF-κB signaling pathways. *Nat Immunol*, **12**, 695-708.

Okudaira K, Kakar S, Cun L, et al (2010). MUC2 gene promoter methylation in mucinous and non-mucinous colorectal cancer tissues. *Int J Oncol*, **36**, 765-75.

Raisch J, Buc E, Bonnet M, et al (2014). Colon cancer-associated B2 Escherichia coli colonize gut mucosa and promote cell proliferation. *World J Gastroenterol*, **20**, 6560-72.

Sheng YH, He Y, Hasnain SZ, et al (2017). MUC13 protects colorectal cancer cells from death by activating the NF-κB pathway and is a potential therapeutic target. *Oncogene*, **36**, 700-13.

Terzić J, Grivennikov S, Karin E, et al (2010). Inflammation and colon cancer. *Gastroenterology*, **138**, 2101-14.e5.