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Original article

Expressions of *ZEB1* of patients with colorectal cancer in Indonesia: Correlations with clinicopathological characteristics and liver metastasis profile

Arum Linangkung[1]*, Adeodatus Yuda Handaya[2], Hendra Susanto[3], Nurcahya Setyawan[2], Imam Sofii[2], Agus Barmawi[2]

[1] Department of Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, Yogyakarta, Indonesia
[2] Digestive Division, Department of Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, Yogyakarta, Indonesia
[3] Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Indonesia

Abstract

Zinc finger E-box binding homeobox 1 (*ZEB1*) and *ZEB2* in the *ZEB1/2* isoform complex are known to play an important role in the progression, invasion, and metastasis of colorectal cancer (CRC). In cancer cells, *ZEB1* regulates the epithelial-mesenchymal transition (EMT). This study aimed to identify the *ZEB1* expression in CRC and its correlation with clinicopathological and metastatic status. Using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), we compared different *ZEB1* expressions in fresh-frozen tissue from non-tumor tissue (NT), primary tumor tissue without metastasis (PT), and primary tumor tissue with liver metastasis (PTLM). The correlation between *ZEB1* expression and clinicopathological characteristics was also investigated. Statistical analysis revealed that *ZEB1* expression is significantly higher in the primary tumor tissues (PT and PTLM) than in NT (p<0.05), and high *ZEB1* expression is associated with PTLM (p<0.05). *ZEB1* expression is significantly correlated with white blood cells (p=0.005), blood glucose (p=0.017), tumor invasion (p=0.001), pathological grading (p=0.002) and metastasis profile (p=0.001). There was no significant correlation between *ZEB1* expression and age, body mass index, hemoglobin, albumin, platelet count, alanine aminotransferase, aspartate aminotransferase, carcinoembryonic antigen, and tumor location. These findings suggest that *ZEB1* expression has diagnostic value in predicting tumor progression and detecting liver metastases.

Keywords: Colorectal cancer, predictive factor, zinc finger e-box binding homeobox 1, liver metastasis

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Introduction

Colorectal cancer (CRC) is the third most prevalent cancer worldwide (The Global Cancer Observatory, 2020). In 2020, CRC incidence reached over 1.9 million cases, with 935,173 deaths, and the distribution of cases between males (55%) and females (45%) was comparable. Among other continents, Asia has the highest prevalence of 50%. Of these, 75% of cases are reported in East Asia, with 10% of cases reported in Southeast Asia (Arnold et al., 2017). Indonesia has the highest estimated number of new cases (32%; 34.189 cases), followed by Thailand.

These high numbers of cases should be governed by enhancing the diagnosis of CRC cases and facilitating additional medical treatments. Numerous cases of CRC are frequently diagnosed at extremely advanced stages, with liver metastases. In the early stages of distant metastasis, radiologic assessment and macroscopic intraoperative appearance of liver metastasis are not always detectable. Identification of metastatic gene expression may yield clinical insights into the condition of liver metastasis.

CRC is caused primarily by sporadic factors such as age, obesity, low physical activity, alcohol consumption, and smoking (Marmol et al., 2017). Nearly 70% of CRC cases begin with an adenoma and progress to carcinoma. Hereditary factors, such as Lynch syndrome and Familial Adenomatous Polyposis (FAP), can also induce CRC (American Cancer Society, 2020). According to AlDubayan et al. (2018), a small portion of CRC cases is caused by mutations in oncogenes, tumor suppressor genes, and other genes involved in the DNA repairing mechanism. CRC-related mutations have been identified in *APC*, *MLH1*, and *BRAF* (Pino & Chung, 2010; Yamane, 2014).

CRC progression accelerates when the tumor is capable of metastasis dissemination via epithelial-mesenchymal transition (EMT) (Wang et al., 2020), the process by which epithelial cells differentiate and transform into mesenchymal cells. This change occurs due to the absence of adhesion between epithelial cells, increasing proliferation and metastasis in the CRC cells (Wu et al., 2018). Several master EMT-inducer genes, including *ZEB1, Twist, Snail,* and *Slug*, can activate EMT in cancer progression (Fenouille et al., 2012; Lindner et al., 2020; Petrova et al., 2016). EMT results in the loss of basement membranes and indicates the metastatic process. The overexpression of EMT-related genes in tumor tissue may assist in the earlier detection of liver metastasis.

Zinc finger E-box binding homeobox 1 (*ZEB1*) and *ZEB2* in the *ZEB1/2* isoform complex are primary inducers of chemoresistance and radio resistance, two major issues in cancer treatment outcomes (Zhang et al., 2018). Wang et al. (2017) reported that *ZEB1*

*Corresponding Author:
Arum Linangkung
Department of Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr, Sardjito Hospital, Yogyakarta, Indonesia
E-mail: arum.linangkung@gmail.com

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suppression is critical in DNA double-strand break (DSB) repair by increasing the ubiquitin-specific peptidase 7 (*USP7*) in checkpoint kinase 1 (*CK1*), as a DNA repair target. *USP7* and *CK1* are essential genes in the DNA damage response (DDR) pathway (Ye et al., 2021), and their activation allows the DDR pathway to suppress tumor development (de Barrios et al., 2019). *ZEB1* is also involved in cell invasion induced by *STAT3* (Burandt et al., 2021). As a result, *ZEB1* expression accelerates CRC progression and metastasis from adenoma to carcinoma. The increasing number of liver metastasis cases of CRC is affecting not only the Western population, but also Asian countries, such as Indonesia. The high expression of *ZEB1* in patients diagnosed with CRC in Indonesia has not been demonstrated yet in the previous study. Therefore, this study aimed to determine the expression of *ZEB1* in CRC and its significant correlation with clinical parameters.

Methods

Patients and Tumor Tissue Specimens

The observational study employed qualitative approach. In addition, quantitative RT-PCR (RT-qPCR) was used to measure the *ZEB1* expressions of 45 samples, including 13 samples of non-tumor tissue (NT), 20 samples of primary tumor tissue without metastasis (PT), and 12 samples of primary tumor tissue with liver metastasis (PTLM). This study was performed from September 2021 to February 2022. The CRC tissue samples were collected during surgery in Dr. Sardjito General Hospital, Yogyakarta, Indonesia. Samples were classified based on histopathological confirmation. In the NT group, only samples without tumor involvement were included. All primary tumor tissue samples were confirmed to be adenocarcinoma with three distinct differentiation patterns (well, moderate, and poor). The primary tumor tissue with confirmed liver metastasis pathology was assigned to the PTLM group.

All tissue samples were stored in the Biobank of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada and then transported to the Central Laboratory of Advanced Minerals and Materials, Universitas Negeri Malang, and Central Laboratory of Biological Sciences, Universitas Brawijaya for further molecular analysis. This study attained ethical clearance from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada-Dr. Sardjito General Hospital, Yogyakarta (KE/FK/0938/EC/2021). Patients with a diagnosis of CRC who underwent surgery and provided informed consent were included in this study. Patients with peritoneal metastasis, pulmonary metastasis, incomplete data, or refusal to participate were excluded. Clinicopathological information was extracted from patients' medical records, including age, gender, body mass index (BMI); laboratory findings, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and carcinoembryonic antigen (CEA), tumor location, tumor invasion, histopathological type, and liver metastasis profile.

Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA isolation process was extracted from the fresh-frozen tissue samples (100 mg) using a synthesis kit (TRISURE™/ Qiazol, BIOLINE, UK), and stored at -20°C before further analysis (e.g., conversion process to cDNA). We used a reverse transcription kit by ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan). The qPCR process was performed on an Analytic Jena qTower Machine with a mix solution made from the SensiFAST SYBR Green No-ROX kit (BIOLINE, UK). *ZEB1* primers were as follows: forward 5′ TTACACCTTTGCATACAGAACCC 3′ and reverse 5′ TTTACGATTACACCCAGACTGC 3′ (Li et al., 2017). The housekeeping gene used *β-actin*, with forward 5'- CATGTACGTTGCTATCCAGG -3' and reverse 5'- CTCCTT AATGTCACGCACGAT -3' (Zhang et al., 2018). The RT-qPCR was programmed to use the Kit SYBR Green 2-step cycling protocol (separate reverse transcription stage with qPCR). The thermal cycle was conducted with initial denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 10 s, and annealing/extension at 60ºC for 30 s. *ZEB1* expression was determined by measuring the mRNA level and quantifying the level of expression in the comparative cycle threshold (CT). The mRNA levels were quantified using the $2^{\Delta Ct}$ formula and relative quantification. The qPCR results were processed on JMP.6 software for analysis (SAS Institute, Chicago, IL; Cary, NC, USA).

Statistical analyses

The Kolmogorov-Smirnov test was used to determine the normality of the data. *ZEB1* expression levels from the NT, PT, and PTLM groups were compared using one-way ANOVA continued by Tukey post hoc test. The data from the PT and PTLM groups were compared using the unpaired T-test. To identify the correlation in *ZEB1* expression between sample groups, univariate correlation using Pearson correlation, Pearson Chi-Square, and Chi-Square test were used. A significance level of 5%, or $p \leq 0.05$, indicated a statistically significant difference.

Results

Comparison of *ZEB1* expression and clinicopathological feature

ZEB1 expressions were evaluated by comparing them in non-tumor tissue (NT), primary tumor tissue without metastasis (PT), and primary tumor tissue with liver metastasis (PTLM). According to one-way ANOVA test followed by Tukey post hoc test, *ZEB1* expression levels were significantly different in primary tumor tissue without metastasis (PT=1.3) than in non-tumor tissue (NT=0.8) (p=0.02) and significantly higher in primary tumor tissue with liver metastasis (PTLM=4.5) than in non-tumor tissue (NT=0.8) (p=0.01). Furthermore, *ZEB1* expression levels were compared between primary tumor tissue without metastasis (PT) and primary tumor tissue with liver metastasis (PTLM), there was a significant association between the non-metastasis group (PT=1.3)

and the liver metastasis group (PTLM=4.5) (p=0.03) (Figure 1).

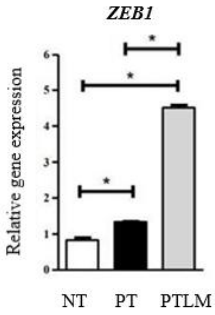

Figure 1. Comparison of *ZEB1* expression between non-tumor tissue (NT), primary tumor tissue without liver metastasis (PT), and primary tumor tissue with liver metastasis (PTLM). * Significant with $p \leq 0.05$ by one-way ANOVA test.

Our study included 32 patients with CRC from Dr. Sardjito General Hospital in Yogyakarta, Indonesia, consisting of 20 patients (62%) in the non-metastasis group and 12 patients (38%) in the metastasis group. The subjects' ages ranged from 29-75 years old, with a median of 54.50 ± 3.12 years in the non-metastasis and 57.76 ± 1.98 years in the metastasis group. In the non-metastasis group, the distribution of cases was similar between males and females. While in the metastasis group, the ratio of male to female was 1.31:1. The metastasis group had a lower BMI (19.40 ± 0.48) than the non-metastasis group (23.41 ± 1.19). the hemoglobin levels were comparable among the groups. The mean white blood cell (WBC) count in the metastasis group was higher (10.76 ± 0.88) than in the non-metastasis group (8.77 ± 0.84). Accordingly, there was an increase in blood glucose (142.33 ± 10.55) and decrease in albumin (3.14 ± 0.16) in the metastasis group, High serum levels of ALT (48.81 ± 2.83), AST (56.29 ± 2.83) and CEA (513.91 ± 2.26) were also elevated in the metastasis group. Based on tumor invasion, the majority of cases were predominantly T3 in the non-metastasis group (70%) and T4 in the metastasis group (92%). In the non-metastatic group, the majority of pathological grading was well-moderate differentiation (90%). According to tumor location, colon cancer (55%) was frequently observed in the non-metastatic group compared to rectal cancer (45%). In contrast, in the metastatic group, colon cancer (33%) was less frequent than rectal cancer (67%). The unpaired T-test revealed no significant differences in age, sex, hemoglobin, white blood cells, platelet count, albumin nor tumor location. However, there were significant differences between groups in BMI, blood glucose, ALT, AST, and CEA (Table 1).

Table 1. Comparison of clinicopathological characteristic in 32 patients with CRC.

Parameters	Group	
	Non-metastasis (n=20)	**Metastasis (n=12)**
Age (range: 29y-75y)	54.50 ± 3.12	57.76 ± 1.98
50y	5	1
51-65y	3	9
>65y	12	2
Sex		
Male	10 (50%)	7 (58%)
Female	10 (50%)	5 (42%)
BMI (kg/m^2)	23.41 ± 1.19	19.40 ± 0.48*
Underweight <18.50	2	4
Normal 18.50–24.99	13	8
Overweight 30> x ≥25	3	0
Obese ≥30.00	2	0
Hemoglobin (g/dL)	11.38 ± 0.47	11.56 ± 0.35
WBC (10^3/µl)	8.77 ± 0.84	10.76 ± 0.88
Blood glucose (mg/dL)	120.80 ± 10.28	142.33 ± 10.55*
Albumin (g/L)	3.63 ± 0.16	3.14 ± 0.16
PLT (10^3/µl)	284.10 ± 21.33	352.62 ± 20.92
ALT (U/L)	11.65 ± 1.74	48.81 ± 2.83*
AST (U/L)	21.35 ± 4.51	56.29 ± 2.83*
CEA (µg/L)	14.36 ± 5.51	513.91 ± 2.26*
Tumor Invasion		
T1	0	0
T2	2	0
T3	14	1
T4	4	11
Pathological grading		
Well, moderate	18	6
Poor	2	6
Tumor location		
Colon	11	4
Rectum	9	8

* Significant value of each parameter compared to the non-metastasis group ($p \leq 0.05$) by unpaired T-test. BMI: Body Mass Index, WBC: White Blood Cell, PLT: Platelet Count; ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, CEA: Carcinoembryonic Antigen, CRC: Colorectal cancer; well: well-differentiated, moderate: moderately differentiated, poor: poorly differentiated.

Correlation between *ZEB1* expression and clinicopathological features of CRC patients

Univariate analysis revealed significant correlations between *ZEB1* expression and white blood cells (p=0.005) and blood glucose (p=0.017) (Table 2). However, no significant correlation was found between *ZEB1* expression with age, BMI, hemoglobin, albumin, platelet count, ALT, AST, and CEA. In addition, the Pearson Chi-Square and Chi-Square test exhibited significant correlations between *ZEB1* expression and tumor invasion (p=0.001), pathological grading (p=0.002), and metastasis profile (p=0.001) (Table 3).

Table 2. Univariate correlations with *ZEB1* expressions in all participants.

Parameters	*ZEB1*	
	r	*p*-value
Age (years)	0.054	0.769
BMI (kg/m^2)	-0.206	0.258
Hemoglobin (g/dL)	-0.05	0.784
WBC (10^3/µl)	0.481	0.005*
Blood glucose (mg/dL)	0.419	0.017*
Albumin (g/L)	-0.251	0.166
PLT (10^3/µl)	0.141	0.44
ALT (U/L)	0.228	0.21
AST (U/L)	0.269	0.136
CEA (µg/L)	0.269	0.137

* Significant with $p \leq 0.05$ by Pearson product-moment correlation test. BMI: Body Mass Index, WBC: White Blood Cell, PLT: Platelet Count; ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, CEA: Carcinoembryonic Antigen, CRC: Colorectal cancer; *ZEB1*: zinc finger e-box binding homeobox 1.

Table 3. Correlation between *ZEB1* expression and clinicopathological features of CRC patients.

	ZEB1		Test value
	Low (0.8-1.3)	High (>1.3)	
Tumor invasion			
T1	0	0	0.001*
T2	2	0	
T3	14	1	
T4	4	11	
Pathological grading			
Well, moderate	18	6	0.002*
Poor	2	6	
Tumor location			
Colon	11	4	0.131*
Rectum	9	8	
Metastatic profile			
Non metastasis	20	0	0.001*
Metastasis	0	12	

* *Significant with $p \leq 0.05$* by Pearson Chi-Square and Chi-Square test. *ZEB1*: zinc finger e-box binding homeobox 1, CRC: Colorectal cancer; well: well-differentiated, moderate: moderately differentiated, poor: poorly differentiated.

Discussion

Colorectal cancer (CRC) is the leading cause of cancer-related deaths, with nearly half of patients developing liver metastases. In our study, elevations in *ZEB1* expression levels were investigated in patients with CRC as the predictive factors correlated with the risks of progression and liver metastatic profile. The obtained factors indicate the clinical status of the disease, whether local or systemic, which affects the management of CRC patients.

Our study discovered that 38% of patients had liver metastasis, with significant differences in BMI, blood glucose, ALT, AST, and CEA. The correlation between *ZEB1* expression and clinicopathological characteristics in CRC patients is unknown. However, it is worth mentioning that our study found the correlation between *ZEB1* expression and WBC, blood glucose, tumor invasion, and pathological grading.

The non-metastasis group had a mean BMI of 23.41±1.19, while the metastasis group had a mean BMI of 19.4±0.48. Previous studies have reported that an increase in BMI can increase the risk of CRC (Kroenke et al., 2016; Matsuo et al., 2012; Zheng et al., 2018). However, the BMI pattern began to decline in the metastatic group. This cancer-related condition developed as a chronic disease, resulting in weight loss. This condition was comparable with the previous study conducted by Aykan et al. (2018). Since the relationship between BMI and CRC progression is rarely reported, further investigation with a larger sample is urgently required to confirm the BMI trends in CRC patients.

Blood glucose levels were significantly different in patients with both liver metastasis and high *ZEB1* expression. In CRC patients, blood glucose levels provide a non-invasive assessment of tumor invasion (T status) and distant metastasis (M status) (Zhang et al., 2021). In the metastatic group, hyperglycemia was caused by the inflammatory process in the increased level of carcinogenesis (Wu et al., 2018; Wang et al., 2021). Hyperglycemia also hastens tumor progression by enhancing tumor cell proliferation, invasion, and migration (Li et al., 2019). The research on the impact of hyperglycemia and diabetes on CRC liver metastasis and its underlying mechanisms is still limited and needs to be expanded. Recent research, however, has shown that hyperglycemia promotes CRC liver metastasis by upregulating integrin αvβ6. These findings suggest that controlling glucose levels and inhibiting αvβ6 could reduce the risk of liver metastasis. Aside from assessing *ZEB1* expression levels, regularly testing blood glucose in non-diabetic patients with CRC has the additional benefit of detecting early liver metastasis.

The metastasis group had the highest ALT and AST levels, as the progression of the cancer was invading the liver. The elevation level increased nearly 4 folds in ALT and 2 folds in AST. In the metastasis group, the CEA level was significantly elevated, suggesting the spread of cancer from the local disease in the colorectal area to the distant metastasis of the liver (systemic disease) (Wu et al., 2010). The use of AST, ALT and CEA as biomarkers of liver function impairment, suggesting a liver metastasis, has been widely accepted.

The elevated WBC was also associated with the progression of cancer, as observed in the metastasis group (10.76 ± 0.88, p=0.005). The evaluation of WBC reveals alterations in the structure and function of immune cells. Additionally, the increase in WBC is

predictive of colon cancer incidence and mortality (Lee et al., 2006). WBC is a non-specific marker of inflammation, which is comparable with our correlation analysis. Since WBC are composed of granulocytes (neutrophils, eosinophils, and basophils) and non-granulocytes (lymphocytes, and monocytes), further investigation of each component is required to investigate its specific role and effect in metastasis process and elevation of *ZEB1* expression level.

The significant correlation between *ZEB1* expression with tumor invasion and pathological grading was also revealed. Since all of the tumor samples used were adenocarcinoma type, we used the 8th American Joint Committee of Cancer (AJCC) TNM system. The tumor invasion indicated direct penetration into the surrounding tissue (T status). Clinically, it is shown by its extension and size. The pathological grading reflects the appearance of cancer cells (G status). This grading system predicts the rate of tissue growth and proliferation. T and G status both help clinicians manage patients more effectively.

The high expression of *ZEB1* in various cancer cells has been investigated. These findings reveal that *ZEB1* plays a crucial role in the invasion and dissemination process. Consistent with the results of the previous studies, our research also confirmed that the expression levels of *ZEB1* in the tumor tissue samples were higher than in the non-tumor tissue samples. The highest level of expression was identified in the tumor tissue samples with liver metastasis. These results were consistent with the elevated *ZEB1* expression in hepatocellular carcinoma (Zhou et.al., 2012), but inconsistent with the gastric cancer study (Okugawa et al., 2011). Future research is required to investigate the various functions of in various tumors.

A significant correlation of *ZEB1* overexpression and liver metastasis in patients with CRC demonstrated indicated that a *ZEB*1 overexpression level is a molecular marker and predictive factor. It identified those patients are having higher risk of mortality and are candidates for more aggressive treatment and intensive follow-up.

In conclusion, expressions of *ZEB1* in the CRC fresh tissue samples were elevated. In addition, higher expression of *ZEB1* was noticed in the colorectal liver metastasis. Significant correlations were found between the *ZEB*1 overexpression with the clinicopathological parameters in WBC, blood glucose, tumor invasion, pathological grading and metastatic profile. Due to our single-centered study, multicenter research is required. *ZEB1* expression in tissue samples can also be coupled with the other biomarker genes and parameters to determine the expression pathway. This extensive study can help add to the diagnostic tools, prognostic information for patients, better treatment strategy and future investigations of CRC.

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References

AlDubayan, S. H., Giannakis, M., Moore, N. D., Han, G. C., Reardon, B., Hamada, T., Mu, X. J., Nishihara, R., Qian, Z., Liu, L., Yurgelun, M. B., Syngal, S., Garraway, L. A., Ogino, S., Fuchs, C. S., & Van Allen, E. M. (2018). Inherited DNA-repair defects in colorectal cancer. The American Journal of Human Genetics, 102(3), 401–414. https://doi.org/10.1016/j.ajhg.2018.01.018

American Cancer Society. (2020). Colorectal Cancer Facts & Figures 2020-2022. American Cancer Society.

Arnold, M., Sierra, M. S., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2017). Global patterns and trends in colorectal cancer incidence and mortality. Gut, 66(4), 683–691. https://doi.org/10.1136/gutjnl-2015-310912

Aykan, N. F., Artac, M., & Özatli, T. (2018). Body mass index and colorectal cancer. In (Ed.), Body-mass Index and Health. IntechOpen. https://doi.org/10.5772/intechopen.78617

Burandt, E., Lubbermever, F., Gorbokon, N., Buscheck, F., & Luebke, A. M. (2021). E-cadherin expression in human tumors: a tissue microarray study on 10,851 tumors. Biomarker Research, 9(1), 44–52.

de Barrios, O., Sanchez-Moral, L., Cortés, M., Ninfali, C., Profitós-Pelejà, N., Martínez-Campanario, M., Siles, L., del Campo, R., Fernández-Aceñero, M. J., Darling, D. S., Castells, A., Maurel, J., Salas, A., Dean, D. C., & Postigo, A. (2019). *ZEB1* promotes inflammation and progression towards inflammation-driven carcinoma through repression of the DNA repair glycosylase MPG in epithelial cells. Gut, 68(12), 2129–2141. https://doi.org/10.1136/gutjnl-2018-317294

Fenouille, N., Tichet, M., Dufies, M., Pottier, A., Mogha, A., Soo, J. K., Rocchi, S., Mallavialle, A., Galibert, M.-D., Khammari, A., Lacour, J.-P., Ballotti, R., Deckert, M., & Tartare-Deckert, S. (2012). The Epithelial-mesenchymal transition (EMT) regulatory factor SLUG (SNAI2) is a downstream target of SPARC and AKT in promoting melanoma cell invasion. PLoS ONE, 7(7), e40378. https://doi.org/10.1371/journal.pone.0040378

Kroenke, C. H., Neugebauer, R., Meyerhardt, J., Prado, C. M., Weltzien, E., Kwan, M. L., Xiao, J., & Caan, B. J. (2016). Analysis of body mass index and mortality in patients with colorectal cancer using causal diagrams. JAMA Oncology, 2(9), 1137. https://doi.org/10.1001/jamaoncol.2016.0732

Lee, Y.-J., Lee, H.-R., Nam, C.-M., Hwang, U.-K., & Jee, S.-H. (2006). White blood cell count and the risk of colon cancer. Yonsei Medical Journal, 47(5), 646. https://doi.org/10.3349/ymj.2006.47.5.646

Li, W., Zhang, X., Sang, H. *et al.* Effects of Hyperglycemia on the progression of tumor diseases. *J Exp Clin Cancer Res* 38, 327 (2019). https://doi.org/10.1186/s13046-019-1309-6

Lindner, P., Paul, S., Eckstein, M., Hampel, C., Muenzner, J. K., Erlenbach-Wuensch, K., Ahmed, H. P., Mahadevan, V., Brabletz, T., Hartmann, A., Vera, J., & Schneider-Stock, R. (2020). EMT transcription factor *ZEB1* alters the epigenetic landscape of colorectal cancer cells. Cell Death & Disease, 11(2), 147. https://doi.org/10.1038/s41419-020-2340-4

Malki, A., ElRuz, R. A., Gupta, I., Allouch, A., Vranic, S., & Al Moustafa, A.-E. (2020). Molecular mechanisms of colon cancer progression and metastasis: recent insights and advancements. International Journal of Molecular Sciences, 22(1), 130. https://doi.org/10.3390/ijms22010130

Marmol, I., Sánchez-de-Diego, C., Pradilla Dieste, A., Cerrada, E., & Rodriguez Yoldi, M. (2017). Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. International Journal of Molecular Sciences, 18(1), 197. https://doi.org/10.3390/ijms18010197

Matsuo, K., Mizoue, T., Tanaka, K., Tsuji, I., Sugawara, Y., Sasazuki, S., Nagata, C., Tamakoshi, A., Wakai, K., Inoue, M., & Tsugane, S. (2012). Association between body mass index and the colorectal cancer risk in Japan: pooled analysis of population-based cohort studies in Japan. Annals of Oncology, 23(2), 479–490. https://doi.org/10.1093/annonc/mdr143

Okugawa Y, Tiyama Y, Tanaka K. (2011). Clinical significance of Zinc Finger E-box Binding Homebox 1 (*ZEB1*) in human gastric cancer. J Surg Oncol, 106:280-285.

Petrova, Y. I., Schecterson, L., & Gumbiner, B. M. (2016). Roles for E-cadherin cell surface regulation in cancer. Molecular Biology of the Cell, 27(21), 3233–3244. https://doi.org/10.1091/mbc.E16-01-0058

Pino, M. S., & Chung, D. C. (2010). The chromosomal instability pathway in colon cancer. Gastroenterology, 138(6), 2059–2072. https://doi.org/10.1053/j.gastro.2009.12.065

Rim, C. H., Kim, C. Y., Yang, D. S., & Yoon, W. S. (2019). Clinical significance of gender and body mass index in Asian patients with colorectal cancer. Journal of Cancer, 10(3), 682–688. https://doi.org/10.7150/jca.28495

The Global Cancer Observatory. (2020). GLOBOCAN, Summary Statistics—Indonesia 2020.

Vasconcelos-dos-Santos, A., Loponte, H., Mantuano, N. *et al.* Hyperglycemia exacerbates colon cancer malignancy through hexosamine biosynthetic pathway. *Oncogenesis* 6, e306 (2017). https://doi.org/10.1038/oncsis.2017.2

Wang, B., Wang, S., Wang, W., Liu, E., Guo, S., Zhao, C., Niu, J., & Zhang, Z. (2021). Hyperglycemia promotes liver metastasis of colorectal cancer via upregulation of integrin αvβ6. Medical science monitor: international medical journal of experimental and clinical research, 27, e930921. https://doi.org/10.12659/MSM.930921

Wang, K., Song, K., Ma, Z., Yao, Y., Liu, C., Yang, J., Xiao, H., Zhang, J., Zhang, Y., & Zhao, W. (2020). Identification of EMT-related high-risk stage II colorectal cancer and characterisation of metastasis-related genes. British Journal of Cancer, 123(3), 410–417. https://doi.org/10.1038/s41416-020-0902-y

Wang, M., He, S.-F., Liu, L.-L., Sun, X.-X., Yang, F., Ge, Q., Wong, W.-K., & Meng, J.-Y. (2017). Potential role of *ZEB1* as a DNA repair regulator in colorectal cancer cells revealed by cancer-associated promoter profiling. Oncology Reports, 38(4), 1941–1948. https://doi.org/10.3892/or.2017.5888

Wu, J., Chen, J., Xi, Y., Wang, F., Sha, H., Luo, L., Zhu, Y., Hong, X., & Bu, S. (2018). High glucose induces epithelial-mesenchymal transition and results in the migration and invasion of colorectal cancer cells. Experimental and Therapeutic Medicine. https://doi.org/10.3892/etm.2018.6189

Wu, X. Z., Ma, F., & Wang, X. L. (2010). Serological diagnostic factors for liver metastasis in patients

with colorectal cancer. World Journal of Gastroenterology, 16(32), 4084–4088. https://doi.org/10.3748/wjg.v16.i32.4084

Yamane, L. (2014). Serrated pathway in colorectal carcinogenesis. World Journal of Gastroenterology, 20(10), 2634. https://doi.org/10.3748/wjg.v20.i10.2634

Ye, D., Wang, S., Huang, Y., Wang, X., & Chi, P. (2021). USP43 directly regulates *ZEB1* protein, mediating proliferation and metastasis of colorectal cancer. Journal of Cancer, 12(2), 404–416. https://doi.org/10.7150/jca.48056

Zhang, M., Yang, J., Jiang, H. et al. (2021). Correlation between glucose metabolism parameters derived from FDG and tumor TNM stages and metastasis-associated proteins in colorectal carcinoma patients. BMC Cancer 21, 258. https://doi.org/10.1186/s12885-021-07944-z

Zhang, X., Zhang, Z., Zhang, Q., Zhang, Q., Sun, P., Xiang, R., Ren, G., & Yang, S. (2018). *ZEB1* confers chemotherapeutic resistance to breast cancer by activating ATM. Cell Death & Disease, 9(2), 57. https://doi.org/10.1038/s41419-017-0087-3

Zheng, R., Du, M., Zhang, B., Xin, J., Chu, H., Ni, M., Zhang, Z., Gu, D., & Wang, M. (2018). Body mass index (BMI) trajectories and risk of colorectal cancer in the PLCO cohort. British Journal of Cancer, 119(1), 130–132. https://doi.org/10.1038/s41416-018-0121-y

Zhou YM, Cao L, Li B, et al. (2012). Clinicopathological significance of *ZEB1* protein in patients with hepatocellular carcinoma. Ann Surg Oncol 19:1700-1706