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R E S E A R C H   A R T I C L E

N-Cadherin as An Important Marker in Colorectal Cancer: An investigation of β-Catenin and Cadherin Expressions of SW-480 and HCT-116 Cell Lines

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Abstract

BACKGROUND: The absence of potential biomarkers to detect the metastatic process at an early stage will consequently delay colorectal cancer (CRC) treatment. Some biomarkers including β-Catenin, E-Cadherin and N-Cadherin have been suggested as potential markers. However, there were opposite reports regarding expressions of these markers. Therefore, current study was conducted using CRC cell lines for early stage (SW-480 cells) and late stage (HCT-116 cells) of CRC.

METHODS: SW-480 and HCT-116 cells were cultured and seeded on coverslip glasses for immunofluorescence staining to detect β-Catenin, E-cadherin, and N-cadherin. Expressions of β-Catenin, E-cadherin, and N-cadherin were observed and documented under a fluorescent microscope and analyzed with Image J software. Measured results were then statistically analyzed.

RESULTS: All β-catenin, E-Cadherin and N-Cadherin expressions were observed in SW-480 and HCT-116 cells. β-catenin MFI averages of SW-480 (47.157±3.479) and HCT-116 (47.240±4.107) cells were similar. E-Cadherin MFI average of SW-480 cells (45.104±4.107) was higher than the one of HCT-116 cells (40.191±3.702). N-Cadherin MFI average of HCT-116 cells (43.702±8.219) was significantly higher (p=0.009) than the one of SW-480 cells (72.506±5.297).

CONCLUSION: Taken together, N-Cadherin could be suggested as an important metastasis marker in CRC since the N-Cadherin expression was significantly higher in HCT-116 cells as the late-stage CRC model than SW-480 as the early-stage of CRC model. Further research is still needed by comparing several biomarkers from various clinical samples at all clinical stages of CRC.

KEYWORDS: CRC, β-Catenin, E-Cadherin, N-Cadherin, Metastasis, Biomarker

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Introduction

Colorectal cancer (CRC), a malignant tumor arising from the colon and rectum epithelium, is the 4th leading cause of death from all types of cancers.(1,2) In 2013, the prevalence of CRC worldwide was 9% of all types of cancer.(3,4) If the tumor remains localized, the five-year survival rate can reach 90%, but it decreases to 66% when transforming into regional metastasis.(5,6) The metastasis of 10% of

Duke's A stage and 15-30% of Duke's B stage CRC cells was reported as locoregional.(6) In fact, micrometastasis occurs in tumor cells sizing 0.2-2 mm and is frequently undetected in histopathological examinations.(7,8) Some markers are important to detect micrometastasis, including c-mesenchymal-epithelial transition factor (c-Met), universal melanoma antigen family (uMAGE), and β-human chorionic gonadotropin (β-HCG).(9)

There are proteins affect the adhesion between cells and the translocation of protein into the nucleus, such as Wingless-related integration site (Wnt)/β-Catenin.(8,9) Wnt/β-Catenin signal is a classic pathway involved in modulating the development of cancer cells, which include proliferation, resistance, differentiation, motility, adhesion, and apoptosis of cancer cells.(10) While β-catenin is a multifunctional protein with a central role in the homeostasis process in the human body. Wnt/β-Catenin signals and adhesion process are mediated by Cadherins, which are involved in both the embryonal development process and cancer progression. Among the Cadherins, there are E-Cadherin and N-Cadherin.

E-Cadherin is a complex existent in the cytoplasm of cancer cells that functions to form cytoskeleton for strengthening the adhesion between cells. E-Cadherin is responsible for maintaining cell polarity; therefore, it is considered as a marker of cancer cells that have not undergone metastasis.(11) Meanwhile, N-Cadherin is assumed as a marker for detecting micrometastasis in cancer as well.(12,13) N-Cadherin will experience escalating regulation in mesenchymal cells, in which these cells are more motile and less polarized than epithelial cells. N-Cadherin is expressed in several cell types, such as neuron cells, endothelial cells, stromal cells, and osteoblasts.(14,15)

In advanced stages of CRC, most will undergo a process called Epithelial Mesenchymal Transition (EMT).(11) In this process, Cadherin switching occurs, which means a reduction in E-Cadherin regulation followed by an escalation in N-Cadherin regulation.(14) However, in some circumstances, E-Cadherin expression does not change significantly, but the cells experience an increase in expression of N-Cadherin.(15) Moreover, in some types of cancer, E-Cadherin will switch to N-Cadherin, but for other types, N-Cadherin will switch to E-Cadherin.(14,16) Therefore, in this current study the micromestatic markers (β-Catenin, E-Cadherin and N-Cadherin) were investigated using stable cell model, SW480 and HCT-116 cell lines, which are known as the early (Duke's B) and late (Duke's D) stages of CRC, respectively.

Methods

Cell Culture

SW-480 cell line (CCL-228, ATCC, Gaithersburg, MD, USA) was cultured in L-15 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 2mM Glutamine (Sigma-Aldrich), 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich) and Antibiotic Antimycotic Solution (Sigma-Aldrich).

HCT-116 cell line (CL-247, ATCC) was cultured in McCoy′s 5a medium (Sigma-Aldrich), containing 2 mM Glutamine, 10% FBS and Antibiotic Antimycotic Solution. Both cell lines were cultured in humidified-37°C-5% CO_2 incubator. Upon reaching confluent, the cells were subcultured using 0.25% Trypsin-EDTA (Sigma-Aldrich).

Immunofluorescence

SW-480 and HCT-116 cells were seeded on coverslip glasses with their mediums in culture dishes overnight. Cells were rinsed with phosphate buffered saline (PBS) and fixed with fresly prepared 4% paraformaldehyde (Wako, Osaka, Japan) at neutral pH. Fixed-cell were then rinsed in PBS and permeabilized with 0.1% Triton X-100 (Merck Millipore, Burlington, MA, USA) in PBS. After rinsed with PBS, then the fixed-permeabilized cells were incubated in 1% Bovine Serum Albumin (BSA) in PBS. Primary antibodies used in this study were mouse monoclonal IgG β-Catenin (12F7) (sc-53488, Santa Cruz, Dallas, TX, USA), E-Cadherin (5F133) (sc-71007, Santa Cruz) and N-Cadherin (8C11) (sc-53488, Santa Cruz) antibodies. The primary antibodies were added on the cells overnight at 4°C. The cells were then rinsed and labelled with FITC-conjugated goat anti-mouse IgG (H&L) secondary antibody (610-1202-0500, Rockland, Limerick, PA, USA). Then the cells were rinsed and stained with 14.3 mM 4′, 6-diamidino-2-phenylindole (DAPI) solution (Sigma-Aldrich) and mounted with ProLong Gold Antifade Mountant solution (Thermo Fisher Scientific, Waltham, MA, USA) on slide glasses. Expressions of β-Catenin, E-Cadherin and N-Cadherin were observed and captured under an immunofluorescence microscope (Olympus, Tokyo, Japan). Each group with 8 images were analysed with ImageJ bundled with Java 1.8.0_172 software (NIH, Bethesda, MD, USA) with 8-bit color. Intensities of β-Catenin, E-Cadherin, N-Cadherin expressions were shown as Mean Fluorescence Intensity (MFI) unit.

Statistical Analysis

All calculated datas of β-catenin, E-Cadherin, and N-Cadherin were presented as mean±SD for three

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independent experiments. Statistical analyses were perfomed using SPSS for Windows software version 20 (IBM, Armonk, NY, USA). The significance level was set at confidence level of 95%, $p<0.05$ was significant.

Results

β-catenin Expressions of SW-480 and HCT-116 Cells

β-catenin expressions were observed in SW-480 and HCT-116 cells in cytoplasm and nucleus (Figure 1A). β-catenin MFI averages of SW-480 (47.157±3.479) and HCT-116 (47.240±4.107) cells were similar (Figure 1B) and not statistically different ($p=0.595$) with independent T-test.

E-Cadherin Expressions of SW-480 and HCT-116 Cells

E-Cadherin expressions were also observed in SW-480 and HCT-116 cells in cytoplasm (Figure 2A). E-Cadherin MFI average of SW-480 cells (45.104±4.107) was higher than the one of HCT-116 cells (40.191±3.702) (Figure 2B),

although it was not statistically different ($p=0.843$) with independent T-test.

N-Cadherin Expressions of SW480 and HCT-116 Cells

N-Cadherin expressions were clearly observed in SW-480 and HCT-116 cells in cytoplasm and nucleus. Especially for HCT-116, high N-Cadherin expression was observed as purplish white colour in the merged figure (Figure 3A). In accordance, N-Cadherin MFI average of HCT-116 cells (43.702±8.219) was significantly higher ($p=0.009$) than the one of SW-480 cells (72.506±5.297) (Figure 3B) with Mann-Whitney test.

Discussion

Translocation of cytoplasmic β-Catenin into nucleus in CRC was correlated with the change of benign to malignant.[17] Moreover, the loss of cytoplasmic β-Catenin expression was suggested to indicate a poor prognosis of CRC.[18]
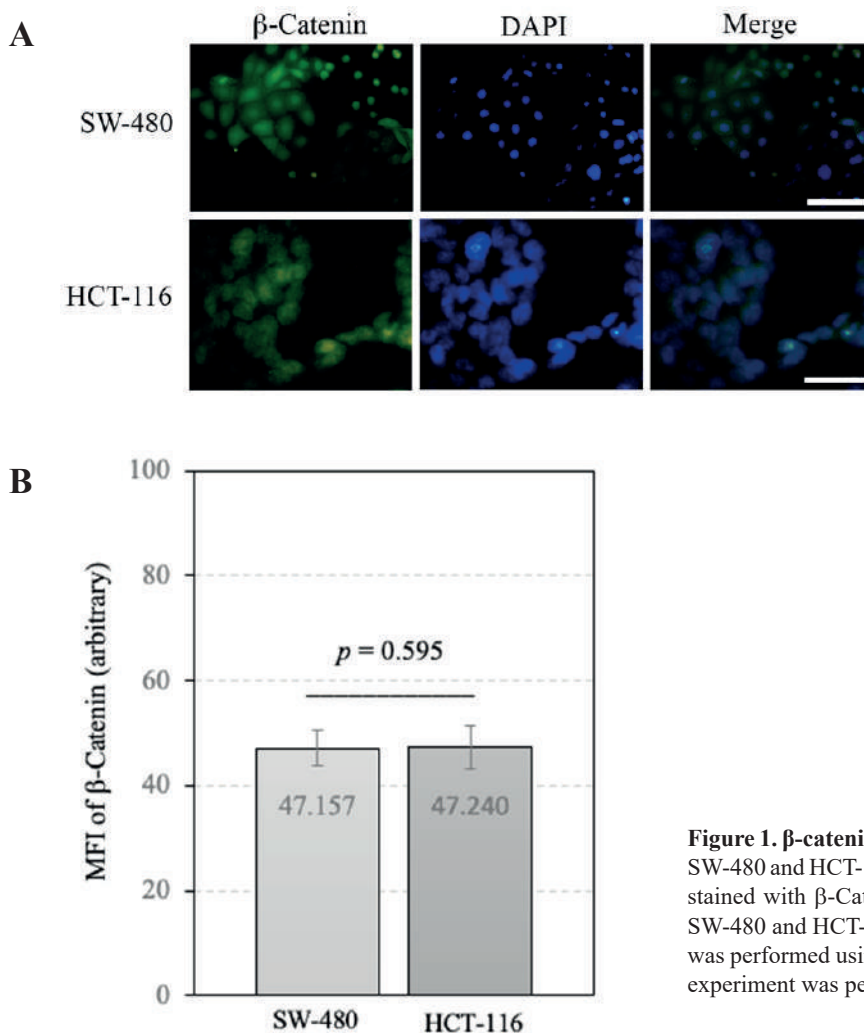

Figure 1. β-catenin expression of SW-480 and HCT-116 cells. A: SW-480 and HCT-116 cells were cultured and immunofluorescence stained with β-Catenin and DAPI. B: β-catenin MFI averages of SW-480 and HCT-116 cells were measured and statistical analysis was performed using independent T-test. White bar = 50 μm. Each experiment was performed in triplicate and conducted twice.
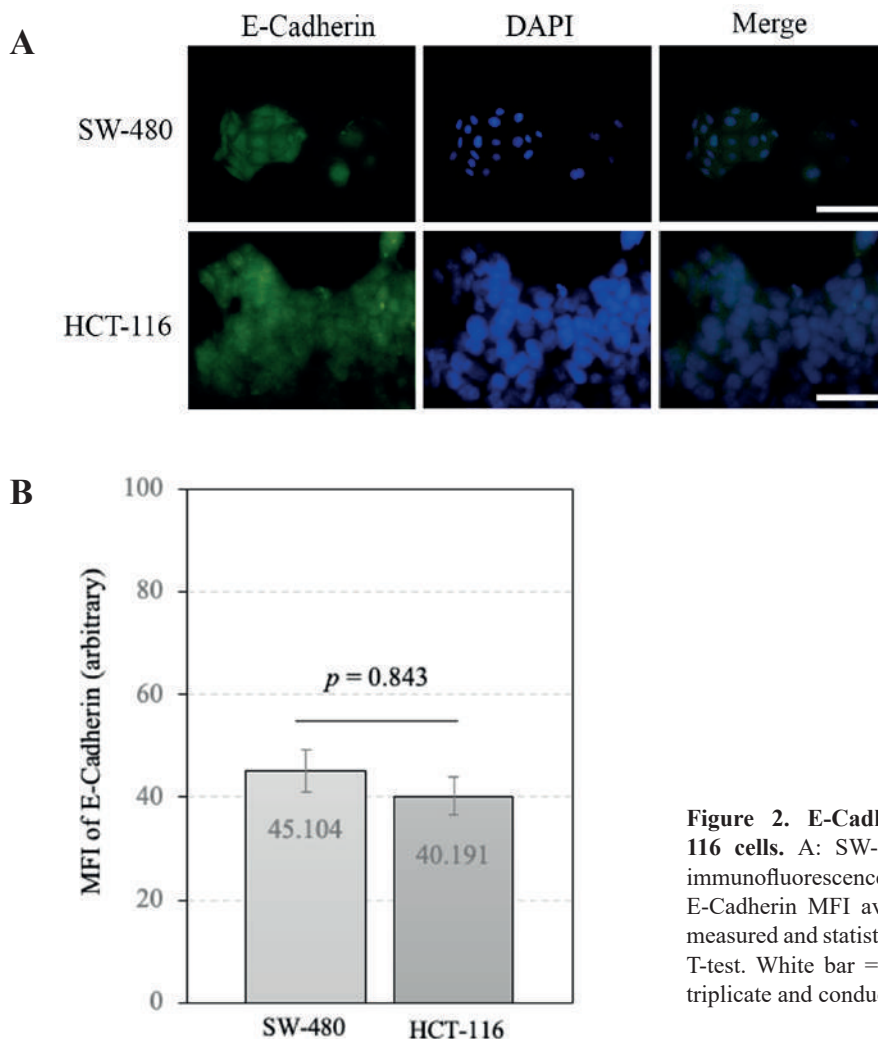
Figure 2. E-Cadherin expression of SW-480 and HCT-116 cells. A: SW-480 and HCT-116 cells were cultured and immunofluorescence stained with E-Cadherin and DAPI. B: E-Cadherin MFI averages of SW-480 and HCT-116 cells were measured and statistical analysis was performed using independent T-test. White bar = 50 μm. Each experiment was performed in triplicate and conducted twice.

However, in another study, β-catenin was reported to not correlated with CRC progressivity.(19) In our current results, β-Catenin expressions of both SW-480 as the early-stage of CRC model and HCT-116 cells as late-stage CRC model, were accordingly observed in the cytoplasm and nucleus. The expressions of β-catenin in SW-480 and HCT-116 cells were similar. Accordingly, both SW-480 and HCT-116 cells were CRC models with clear expressions of β-Catenin.

E-Cadherin is the main component to strengthening the bonds between epithelial cells and is vital for the development, differentiation, and defense of cancer tissue. (20) In CRC, E-Cadherin was reported as a tumor suppressor with opposite function to Wnt signals.(21) Decreased regulation of E-cadherin expression was associated with poor prognosis of CRC, and the loss of E-cadherin expression was associated with CRC cell metastasis.(22,23) However there are two forms of E-cadherin (membrane-tethered and soluble); as a result, E-cadherin has two properties, as an inhibitor of cancer progression and a trigger for metastasis. (16) E-cadherin can be expressed on several CRC cell lines, including HCT-116 and signet ring cell carcinoma (SRCC). (20) In accordance, in the current study, both SW-480 and HCT-116 cells expressed E-cadherin. E-cadherin expression of SW-480 cells was just slightly higher than E-cadherin expression of HCT-116 cells. Therefore, SW-480 as the early-stage CRC and HCT-116 cells as the late-stage of CRC, could not be determined clearly based on single marker of E-Cadherin.

N-cadherin expression was associated with the development of carcinoma.(15,16) In the opposite of E-cadherin, expression of N-Cadherin in CRC was higher at Duke's C/D stage than that of Duke's A/B stage.(24) Based on current results, HCT-116 cells, as the late-stage CRC model, expressed significantly higher N-Cadherin than SW-480 cells. However, since the SW-480 cells also expressed N-Cadherin, this suggested that the SW-480 might have low metastatic property as well. N-cadherin could be considered as a candidate biomarker along with E-cadherin as an independent promoter for EMT processes, such as Snail and Twist.(20,25)
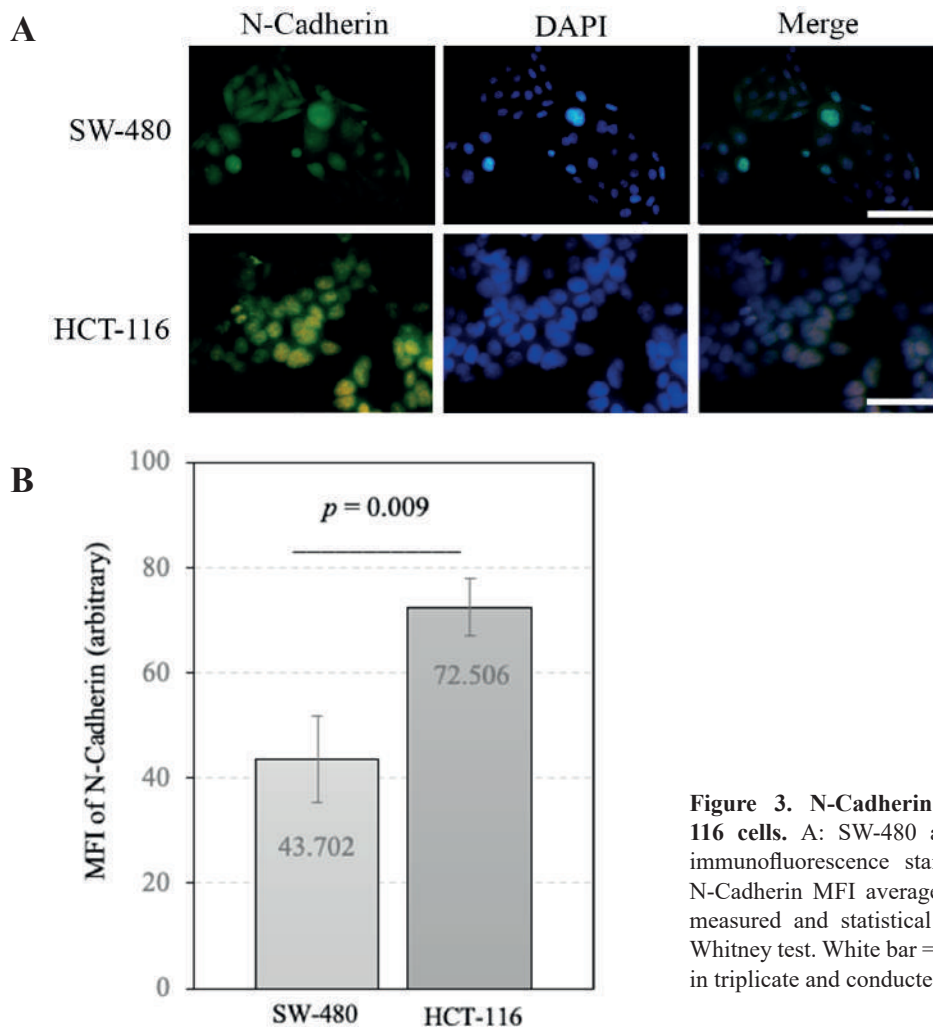
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Figure 3. N-Cadherin expression of SW-480 and HCT-116 cells. A: SW-480 and HCT-116 cells were cultured and immunofluorescence stained with N-Cadherin and DAPI. B: N-Cadherin MFI averages of SW-480 and HCT-116 cells were measured and statistical analysis was performed using Mann-Whitney test. White bar = 50 μm. Each experiment was performed in triplicate and conducted twice.

Conclusion

Since the N-Cadherin expression was significantly higher in HCT-116 cells as the late-stage CRC model than SW-480 as the early-stage of CRC model, it could be confirmed that N-Cadherin as an important metastasis marker in CRC. Meanwhile, β-catenin and E-Cadherin could be suggested as confirmative markers. Further research is still needed by comparing several biomarkers from various clinical samples at all clinical stages of CRC.

Acknowledgements

Authors Contribution

WL constructed research problem, baseline theory, hypothesis, methodology, doing research, discussion and conclusion. DWS contributed in the colorectal oncogenesis theory, methodological and statistical analysis and submission of this research. KM contributed in immunofluorescence and histopathologial theory and method and interpretation in this research. PR contributed in the transduction signaling (include Wnt/β-catenin, E-cadherin and N-cadherin) and construct the theory/research pathways. FS contributed in result and statistical analyses, interpretation and manuscript revision.

References

1. Wanandi SI, Lestari DR, Hilbertina N, Siregar NC, Jusman SW, Abdullah M. Secretomes of primary cancer-associated

fibroblasts upregulate the expression of stemness markers in HT-29 human colorectal carcinoma cells. Indones Biomed J. 2020; 12: 333-9.

2. Stanczak A, Stec R, Bodnar L, Olszewski W, Cichowicz M, Kozlowski W, Szczylik C, *et al.* Prognostic significance of Wnt-1, β-catenin and E-cadherin expression in advanced colorectal carcinoma. Pathol Oncol Res. 2011; 17: 955-63.

3. Ahmad A, Stromberg VH. Expression of β-catenin and E-cadherin, their clinical significance and association with complexity and index of colon carcinoma. Adv Genet Eng. 2016; 5: 1-10. doi: 10.4172/2169-0111.1000156.

4. Palaghia M, Mihai C, Lozneanu L, Ciobanu D, Trofin AM. E-cadherin expression in primary colorectal cancer and metastatic lymph nodes. Rom J Morphol Embryol. 2016; 57: 205-9.

5. Tedjasaputra TR, Hatta M, Massi MN, Natzir R, Patellongi I, Simadibrata M, *et al.* Risk assessment in hereditary colorectal cancer family by using APC and MSH2 mRNA gene expression and bayesian analysis. Indones Biomed J. 2020; 12: 368-75.

6. Kamiyama H, Noda H, Konishi F, Rikiyama T. Molecular biomarkers for the detection of metastatic colorectal cancer cells. World J Gastroenterol. 2014; 20: 8928-38.

7. Lips DJ, Koebrugge B, Jan Liefers G, van de Linden JC, Smit VT, Pruijt HF, *et al.* The influence of micrometastases on prognosis and survival in stage I-II colon cancer patients: the Enroute Study. BMC Surgery. 201; 11: [n.p.]. doi: 10.1186/1471-2482-11-11.

8. Yamamoto H, Murata K, Fukunaga M, Ohnishi T, Noura S, Miyake Y, *et al.* Micrometastatis volume in lymph nodes determines disease recurrence rate of stage II colorectal cancer: a prospective multicenter trial. Clin Cancer Res. 2016; 22: 3201-8.

9. Bilchick AJ, Nora DT, Saha S, Turner R, Wiese D, Kuo C, *et al.* The use of molecular profiling of early colorectal cancer to predict micrometastases. Arch Surg. 2002; 137: 1377-83.

10. Chen J, Xie ZR, Wu Y. Computational modeling of the interplay between cadherin-mediated cell adhesion and wnt signaling pathway. PLoS ONE. 2014; 9: 1-12. doi: 10.1371/journal.pone.0100702.

11. Wheelock MJ, Shintani Y, Maeda M, Fukumoto Y, Johnson KR. Cadherin switching. J Cell Sci. 2007; 121: 727-35.

12. Shang S, Hua F, Hu ZW. The regulation of β-catenin activity and function in cancer: therapeutic opportunities. Oncotarget. 2017; 8: 33972-89.

13. Menezes ME. The Wnt/β-catenin signaling pathway in epithelial mesenchymal transition. Post Doc Journal. 2014; 2: 1-9. doi: 10.14304/surya.jpr.v2n7.1.

14. Heuberger J, Birchmeier W. Interplay of cadherin-mediated cell adhesion and canonical wnt signaling. Cold Spring Harb Percpect Biol. 2010; 2: a002915. doi: 10.1101/cshperspect.a002915.

15. Mrozik KM, Blaschuk OW, Cheong CM, Zannettino ACW, Vandyke K. N-cadherin in cancer metastases, its emerging role in haematological malignancies and potential as a therapeutic target in cancer. BMC Cancer. 2018; 18: 939. doi: 10.1186/s12885-018-4845-0.

16. Loh C, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, *et al.* The E-cadherin and N-cadherin switch in epithelial to mesenchymal transition: signaling, therapeutic implications, and challenges. Cells. 2019; 8: 1-33.

17. Bhattacharya I, Barman N, Maiti M, Sarkar R. Assesment of beta-catenin expression by immunohistochemistry in colorectal neoplasm and its role as an additional prognostic marker in colorectal adenocarcinoma. Med Pharm Rep. 2019; 9: 246-52.

18. Yuan S, Tao F, Zhang X, Zhang Y, Sun X, Wu D. Role of Wnt/β-catenin signaling in the chemoresistance modulation of colorectal cancer. Biomed Res Int. 2020; 2020: 9390878. doi: 10.1155/2020/9390878.

19. Li S, Huang M, Liu Q, Wang D, Wu R, Zhang X, *et al.* Serum expression of β-catenin is a potential detection marker in patients with colorectal cancer. Dis Markers. 2019; 2019: 5070524. doi: 10.1155/2019/5070524.

20. Daulagala A, Bridges MC, Kourtidis A. E-cadherin beyond structure: a signaling hub in colon homeostasis and disease. Int J Mol Sci. 2019; 20: 2756. doi: 10.3390/ijms20112756.

21. Liang X, Xu X, Wang F, Chen X, Li N, Wang C, *et al.* E-cadherin knockdown increases β-catenin reducing colorectal cancer chemosensitivity only in three-dimensional cultures. Int J Oncol. 2015; 47: 1517-27.

22. He X, Chen Z, Jia M, Zhao X. Downregulated E-cadherin expression indicates worse prognosis in asian patients with colorectal cancer: evidence from meta-analysis. PLoS ONE. 2013; 8: e70858. doi: 10.1371/journal.pone.0070858.

23. Kim SA, Inamura K, Yamauchi M, Nishihara R, Mima K, Sukawa Y, *et al.* Loss of CDH1 (E-cadherin) expression is associated with infiltrative tumor growth and lymph node metastasis. Be J Cancer. 2016; 114: 199-206.

24. Yan X, Yan L, Liu S, Shan Z, Tian Y. N-cadherin, a novel prognostic biomarker, drives malignant progression of colorectal cancer. Mol Med Rep. 2015; 12: 2999-3006.

25. Ye Z, Zhou M, Tian B, Wu B, Li Z. Expression of lncRNA-CCAT1, E-cadherin and N-cadherin in colorectal cancer and its significance. Int J Clin Exp Med. 2015; 8: 3707-15.