ANTICANCER RESEARCH *41*: 543-547 (2021)
doi:10.21873/anticanres.14806

Over-expression of CEP55 Predicts Favorable Prognosis in Colorectal Cancer Patients With Lymph Node Involvement

REN-HUNG HUANG[1], WEN-KANG YANG[2], CHEUK MAN WU[2], CHUNG-MIN YEH[1,3] and WEN-WEI SUNG[2,4,5]

[1]*Department of Pathology, Changhua Christian Hospital, Changhua, Taiwan, R.O.C.;*
[2]*School of Medicine, Chung Shan Medical University, Taichung, Taiwan, R.O.C.;*
[3]*Department of Medical Technology, Jen-Teh Junior College of Medicine, Nursing and Management, Miaoli, Taiwan, R.O.C.;*
[4]*Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, R.O.C.;*
[5]*Department of Urology, Chung Shan Medical University Hospital, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: To characterize the potential roles of CEP55 in colorectal cancer development and assess its eligibility as a prognostic diagnosis tool for colorectal cancer. Patients and Methods: Immunohistochemical (IHC) analysis of CEP55 immunoreactivity in 166 cancer specimens from colorectal cancer patients. Results: CEP55 was not found to statistically significantly affect different patient clinical parameters. Multivariate analysis illustrated that patients with N stage (1+2) colorectal cancer and high CEP55 expression had a significantly lower five-year survival rate than patients with N stage (1+2) colorectal cancer and low CEP55 expression. Conclusion: There is a correlation between CEP55 and advanced N-stage colorectal cancer. Thus, CEP55 may be a potential diagnostic biomarker for colorectal cancer patients.*

Colorectal cancer holds the fourth highest incidence rate of all cancers in the USA and was estimated to account for 8.4%, or the second highest majority, of deaths due to cancers in 2019 (1). Though cancer screening policy contributes to early diagnosis and favorable clinical outcomes, cancer treatment remains a critical clinical challenge, especially for those with advanced stage cancers. When comparing the localized, regional, and distant stages of colon cancer, the five-year survival rate drops significantly between the prognostic and diagnostic stages of the disease, from 90% to 71%, and even as low as 14% (1). Therefore, using cancer staging or molecular prognostic markers to identify disease progression can provide important information for therapeutic decision making.

Some molecular markers are already viewed as early indicators of colorectal carcinogenesis (2). By using these discoveries, the present research sought to identify possible tumor markers that can be used to identify patients whose cancer is likely to relapse or metastasize. This would enable practitioners to schedule more intense follow-ups or treatments and thus, improve tumor outcomes. Cancer occurs under special conditions, and many potential tumor markers are based on the special characteristics of tumor growth and abnormal immunity. Abnormal cell cycles are important signifiers of these unique characteristics (3).

As part of a cell's life cycle, cell abscission involves parallel pathways, including centriolin and midbody assembly (4). Many structural and regulatory proteins are required for midbody assembly, such as Aurora B, MKLP2, PRC1, and ECT2 (4). These proteins are targeted by centrosomal protein 55 (CEP55), which is normally strongly restricted to the testis, thymus, and placenta (5-7). These proteins are either absent or mislocalized from the midbody microtubules in CEP55-knockdown cells (4). During abscission, CEP55 at the midbody, recruits proteins that are directly involved in membrane fission, such as TSG101 and ALIX (8). This feature can make both the up-regulation and down-regulation of CEP55 result in unsuccessful cytokinesis, and even aneuploidy, which is a common characteristic of tumor cells (9). In cells with depleted CEP55, an increased proportion of multinucleation is observed (8). The up-regulation of CEP55 has also been reported in many types of cancer, such as bladder cancer, head and neck squamous cell carcinoma, gastric cancer, and colon cancer (6, 10-13).

Although CEP55 expression is a potential diagnostic biomarker for colorectal cancer, its prognostic role remains unclear (2, 6, 10). As such, this study aimed to investigate

Correspondence to: Wen-Wei Sung, School of Medicine, Chung Shan Medical University, No.110, Sec.1, Jianguo N. Rd., Taichung City 40201, Taiwan, R.O.C. Tel: +886 424739595 ext.*10646, e-mail: flutewayne@gmail.com; sungww@csmu.edu.tw

Key Words: Centrosomal protein 55, CEP55, lymph node, prognosis, colorectal cancer.

the potential association between CEP55 expression and the clinical outcomes of colorectal cancer patients.

Patients and Methods

Study subjects and ethics statement. This study included a total of 166 patients who received pathologically confirmed CRC diagnoses at Changhua Christian Hospital between 1997 and 2000. Patients with histories of other malignancies or missing clinical data were excluded. Cancers were staged according to the American Joint Committee on Cancer (AJCC) Colon Cancer Staging, 7th edition. Clinical data, including gender, age, stage, T, N, and M stages, and follow-up information, were obtained from an established database (14). This study was approved, and consent was waived by the Institutional Review Board and the Ethics Committee of the Changhua Christian Hospital, Changhua, Taiwan (IRB No. 121008).

Immunohistochemistry staining of CEP55. Immunohistochemistry (IHC) staining was performed at the Department of Surgical Pathology, Changhua Christian Hospital, Changhua, Taiwan, as previously described (15, 16). IHC staining were performed on tissue microarray sections (4 μm) of formalin-fixed, paraffin-embedded, pre-chemotherapy primary colorectal tumors. The antibody used was anti-human CEP55 (CEP55 antibody, sc-374051, 1:50 dilution, Santa Cruz Biotechnology, Dallas, TX, USA).

Evaluation of CEP55 immunoreactivity. Immunoreactivity was analyzed by pathologists using a previously described scoring system (14, 15). In brief, Immunoreactivity scores were defined as cell staining intensity (0=nil; 1=weak; 2=moderate; and 3=strong) multiplied by the percentage of stained cells (0-100%), resulting in scores ranging from 0 to 300 (15).

Statistical analysis. The Student *t*-test, and the χ^2 test were applied for continuous or discrete data analysis. The associations between the CEP55 expression and patient survival outcome were estimated using the Kaplan–Meier method and assessed using the log-rank test. Potential confounders were adjusted by Cox regression models, with the CEP55 expression fitted as indicator variables. Overall survival time was defined as the interval between the date of surgery and the date of last follow-up or death. All statistical analyses were conducted using the SPSS statistical software program (version 15.0) (SPSS, Inc., Chicago, IL, USA). All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

Results

CEP55 expression is not significantly associated with clinical parameters. This study analyzed a total of 166 patients with colorectal cancer, including 71 females and 95 males. Representative IHC staining is shown in Figure 1. The average age of the patients was 64 years (SD: 13.4). At least 19% of the patients were diagnosed in the M1 stage, and 13% of the patients were diagnosed in stage 1. We stratified cases into different groups according to clinical parameters, such as age, gender, stage, T value, N value, and M value. There are no significant differences in CEP55 expression levels related to these parameters (Table I).

CEP55 is an independent prognostic marker for patients with advanced N-stage cancer. We used univariate analysis to determine the prognostic role of CEP55. As illustrated in Table II, patients with stage II, III, or IV cancer had lower overall five-year survival rates than patients with stage I cancer, as expected (stage II, III, and IV: 40%, stage I: 73%, $p=0.05$). However, CEP55 expression was not statistically significant as a prognostic marker (Table II and Figure 2A). The result did not change under multivariate analysis (HR=1.317, 95%CI=0.893-1.943, $p=0.165$, Table III). We further verified the prognostic role of CEP55 according to the subgroup of clinical parameters. As shown in Table III, CEP55 played no significant role under most of the subgroups. Nevertheless, in patients with advanced N stage cancer, lower CEP55 levels were associated with significantly poorer clinical outcomes (HR=1.896, 95%CI=1.034-3.475, $p=0.039$, Table III and Figure 2B).

Discussion

As a centrosome-associated protein, CEP55 is capable of homodimerization (7). It transfers from the centrosome to the midbody when the cell cycle enters the cytokinesis stage (4). The midbody is regulated by proteins, such as Aurora B, MKLP2, PRC1, and ECT2. In addition, it has been reported that CEP55 plays an important role in the membrane fission event of cytokinesis by regulating these proteins (7). The up-regulation of CEP55 can result in aneuploidy and thus, an increase in the number of multinucleated cells, which may lead to tumorigenesis (13). Over-expression of CEP55 has been found in hepatocarcinoma, oral squamous cell carcinoma, lung cancer, ovarian carcinoma, and colon carcinoma (10, 17-20). Furthermore, CEP55 expression levels vary significantly in size and degree (13, 17, 21). This variation can also be found in patients with different stages of lymph node metastases (17, 20, 21). Since research has already identified correlations between lymph node metastases, CEP55, and many types of cancers, the present study sought to determine if there are any similar correlations between these factors and colorectal cancer.

To our knowledge, this is the first study to uncover the relationship between CEP55 expression and advanced N stage colorectal cancer by using multi-variate analysis of 166 patients, as previous research has only indicated that CEP55 is over-expressed in patients with colorectal cancer (22). This discovery may prove fundamental to the application of CEP55 expression as a prognostic tool for N-stage colorectal cancer. Furthermore, because we determined that the presence of CEP55 expression can affect the survival rate of patients with lymph node metastases, future studies should aim to establish the mechanisms behind this phenomenon. No relationships were determined in the comparisons of the collected data. With CEP55 expression, the five-year survival

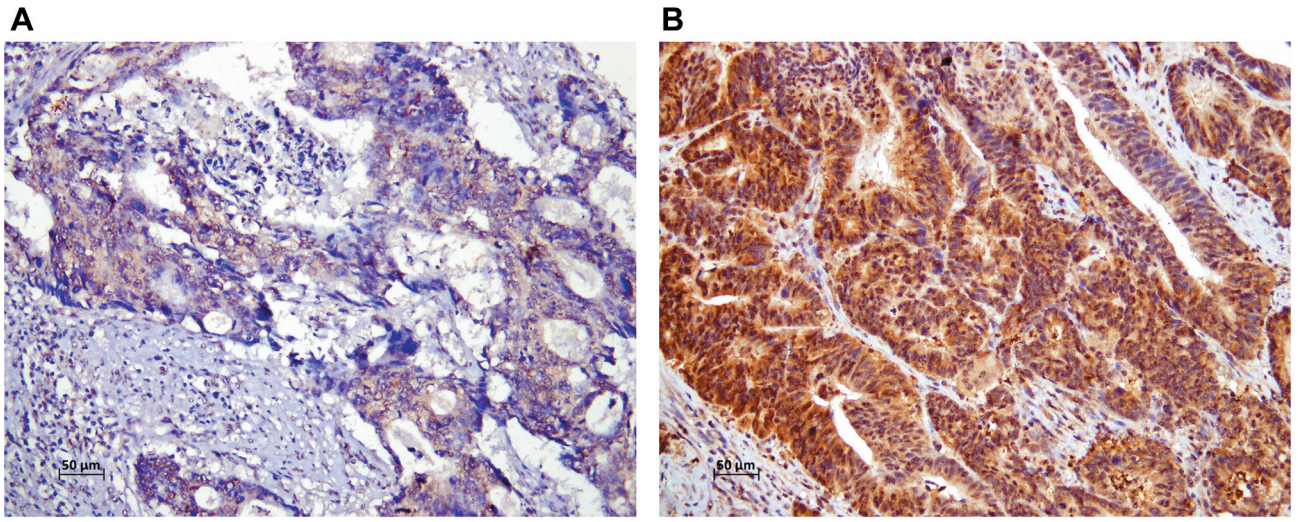

Figure 1. *Representative immunostaining of (A) low and (B) high expression of CEP55 in colorectal cancer specimens.*

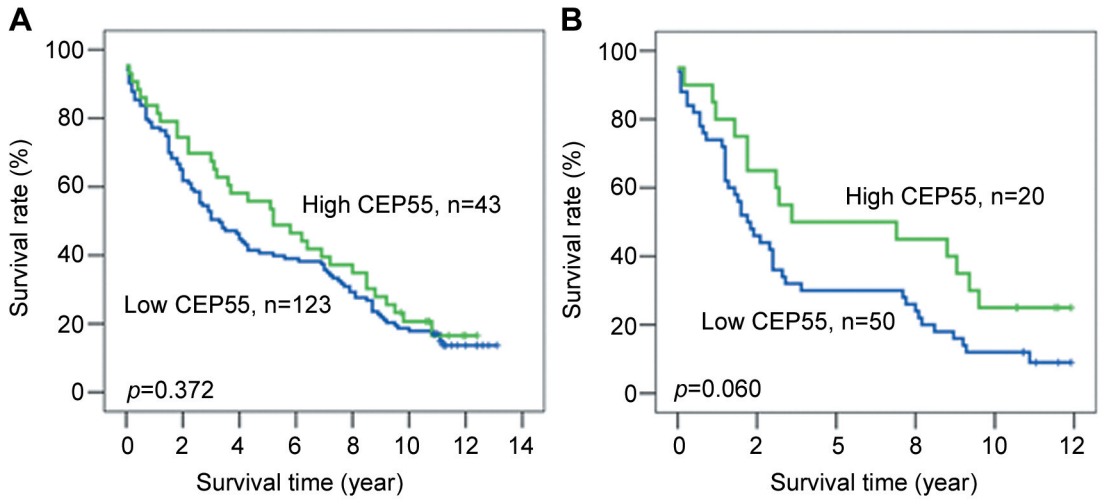

Figure 2. *Kaplan–Meier actuarial analysis of CEP55 expression with respect to overall survival among (A) all patients and (B) patients with N=1+2.*

rate dropped significantly for colorectal cancer patients with lymph node metastases (Table III). This finding is consistent with the results of previous studies of other types of cancers (17, 20, 21). However, due to the lack of statistical significance (p-value=0.372, Table II), the extent of CEP55 expression does not appear to impact survival rate.

On the other hand, our research identified no similar connections in patients with M0 or M1 stages of cancer. This may be because patients in the M0 stage of cancer can present with mild sickness or more serious symptoms. This diversity in disease presentation may decrease the correlation between CEP55 and the M0 stage of cancer. In addition, because M1 is classified as the worst prognostic status of cancer with the lowest survival rate, it may mask the impact of CEP55 expression on M1 patients' clinical outcomes. Indeed, the present study's results showed that CEP55 had no impact on this group.

This study had several limitations. First, the statistical results indicated that CEP55 expression had no impact on long-term survival when using the 12-year survival rate as an example (Figure 1). A potential cause of this factor may have been the limited number of patients included in this study. Follow-up research studies with more patients may provide the answer. Second, because this study only enrolled patients from Taiwan, which may have resulted in selection bias, subsequent research should consider selecting patients

Table I. *Relationships of CEP55 expression with clinical parameters in colorectal cancer patients.*

Parameters	Case number	CEP55 expression		*p*-Value
		Low	High	
Age (year)		63.2±12.6	66.5±15.1	0.165
Gender				
Female	71	52 (73.2)	19 (26.8)	0.828
Male	95	71 (74.7)	24 (25.3)	
Stage				
I	22	18 (81.8)	4 (18.2)	0.651
II	64	45 (70.3)	19 (29.7)	
III	51	37 (72.5)	14 (27.5)	
IV	29	23 (79.3)	6 (20.7)	
T value				
1	5	5 (100.0)	0 (0.0)	0.098
2	20	15 (75.0)	5 (25.0)	
3	124	87 (70.2)	37 (29.8)	
4	17	16 (94.1)	1 (5.9)	
N value				
0	96	73 (76.0)	23 (24.0)	0.797
1	63	45 (71.4)	18 (28.6)	
2	7	5 (71.4)	2 (28.6)	
M value				
0	97	97 (71.9)	38 (28.1)	0.168
1	26	26 (83.9)	5 (16.1)	

Table II. *Univariate analysis of the influence of various parameters on overall survival in colorectal cancer patients.*

Parameter	Overall survival			
	5-year survival (%)	HR	95%CI	*p*-Value
Age, y				
≥65/<65	44.3/44.9	1.220	0.870-1.713	0.249
Gender				
Male/Female	44.2/45.1	1.173	0.837-1.643	0.354
Stage				
II+III+IV/I	40.3/72.7	1.665	1.001-2.771	0.050
CEP55 expression				
Low/High	40.7/55.8	1.188	0.810-1.743	0.378

Table III. *Multivariate analysis of the influence of CEP55 expression according to clinical parameters on overall survival in colorectal cancer patients.*

Parameter	Overall survival[1]			
	5-year survival (%)[2]	HR	95%CI	*p*-Value
All cases[2]	40.7/55.8	1.317	0.893-1.943	0.165
Age (year)				
<65	40.4/66.7	1.619	0.785-3.337	0.192
≥65	40.9/51.6	1.212	0.760-1.933	0.420
Gender				
Female	44.2/47.4	1.271	0.689-2.346	0.442
Male	38.0/62.5	1.368	0.826-2.266	0.223
Stage				
I	72.2/75.0	0.514	0.145-1.823	0.303
II+III+IV	35.2/53.8	1.416	0.939-2.136	0.097
T value				
1+2	70.0/80.0	0.782	0.221-2.765	0.703
3+4	35.0/52.6	1.353	0.897-2.039	0.149
N value				
0	47.9/60.9	1.016	0.603-1.710	0.953
1+2[3]	30.0/50.0	1.896	1.034-3.475	0.039
M value				
0	46.4/60.5	1.233	0.808-1.881	0.331
1	19.2/20.0	1.274	0.398-4.078	0.683

[1]Adjusted for age, gender, and stage; [2]Adjusted stage: HR=1.785, 95%CI=1.068-2.986, *p*=0.027; [3]Adjusted stage: HR=1.146, 95%CI=0.153-8.602, *p*=0.895.

from various regions. Third, this study used a hazard ratio for analysis, which can only show instantaneous risks and may have resulted in selection bias due to the imbalanced number of patients with low and high CEP55 expression and the varying survival rates (23). Therefore, it may prove useful for future studies to establish other parameters, such as the relative risk and odds ratios.

In conclusion, our research shows that the expression of CEP55 in colorectal tumor patients is associated with lymph node metastases and, therefore, a poorer five-year survival rate. Based on this correlation, CEP55 should undergo further investigation as a potential prognostic marker.

Conflicts of Interest

The Authors declare that they have no competing interests relative to this study.

Authors' Contributions

RHH and CMY performed the experiments. RHH and WSS designed the study. WWS performed the statistical analyses. CMY and WWS analyzed and interpreted the data. WKY and CMW drafted the manuscript. RHH and WWS critically revised the manuscript. All authors read and approved the final manuscript.

References

1 Howlader N NA, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ and Cronin KA: SEER Cancer Statistics Review, 1975-2016. National Cancer Institute, 2019. Available at: https://seer.cancer.gov/csr/1975_2017/ [Last accessed on December 3, 2020]

2 Hauptman N, Skok DJ, Spasovska E, Boštjančič E and Glavač D: Genes CEP55, FOXD3, FOXF2, GNAO1, GRIA4, and KCNA5 as potential diagnostic biomarkers in colorectal cancer. BMC Med Genomics *12(1)*: 54, 2019. DOI: 10.1186/s12920-019-0501-z

3 J.Sherr C: Cancer cell cycle. Science *274(5293)*: 1672-1677, 1996. DOI: 10.1126/science.274.5293.1672

4 Zhao WM, Seki A and Fang G: Cep55, a microtubule-bundling protein, associates with centralspindlin to control the midbody integrity and cell abscission during cytokinesis. Mol Biol Cell *17(9)*: 3881-3896, 2006. PMID: 16790497. DOI: 10.1091/mbc.e06-01-0015

5 Inoda S, Hirohashi Y, Torigoe T, Nakatsugawa M, Kiriyama K, Nakazawa E, Harada K, Takasu H, Tamura Y, Kamiguchi K, Asanuma H, Tsuruma T, Terui T, Ishitani K, Ohmura T, Wang Q, Greene MI, Hasegawa T, Hirata K and Sato N: Cep55/c10orf3, a tumor antigen derived from a centrosome residing protein in breast carcinoma. J Immunother *32(5)*: 474-485, 2009. PMID: 19609239. DOI: 10.1097/CJI.0b013e3181a1d109

6 Inoda S, Morita R, Hirohashi Y, Torigoe T, Asanuma H, Nakazawa E, Nakatsugawa M, Tamura Y, Kamiguchi K, Tsuruma T, Terui T, Ishitani K, Hashino S, Wang Q, Greene MI, Hasegawa T, Hirata K, Asaka M and Sato N: The feasibility of Cep55/c10orf3 derived peptide vaccine therapy for colorectal carcinoma. Exp Mol Pathol *90(1)*: 55-60, 2011. PMID: 20950610. DOI: 10.1016/j.yexmp.2010.10.001

7 Jeffery J, Sinha D, Srihari S, Kalimutho M and Khanna KK: Beyond cytokinesis: the emerging roles of CEP55 in tumorigenesis. Oncogene *35(6)*: 683-690, 2016. PMID: 25915844. DOI: 10.1038/onc.2015.128

8 Carlton Jez G and Martin-Serrano J: Parallels between cytokinesis and retroviral budding: A role for the ESCRT machinery. Science *316(5833)*: 1908-1912, 2007. PMID: 17556548. DOI: 10.1126/science.1143422

9 Doxsey S: Duplicating dangerously: linking centrosome duplication and aneuploidy. Mol Cell *10(3)*: 439-440, 2002. PMID: 12408813. DOI: 10.1016/s1097-2765(02)00654-8

10 Sakai M, Shimokawa T, Kobayashi T, Matsushima S, Yamada Y, Nakamura Y and Furukawa Y: Elevated expression of C10orf3 (chromosome 10 open reading frame 3) is involved in the growth of human colon tumor. Oncogene *25(3)*: 480-486, 2005. PMID: 16170351. DOI: 10.1038/sj.onc.1209051

11 Singh PK, Srivastava AK, Rath SK, Dalela D, Goel MM and Bhatt MLB: Expression and clinical significance of Centrosomal protein 55 (CEP55) in human urinary bladder transitional cell carcinoma. Immunobiology *220(1)*: 103-108, 2015. PMID: 25178936. DOI: 10.1016/j.imbio.2014.08.014

12 Waseem A, Ali M, Odell EW, Fortune F and Teh MT: Downstream targets of FOXM1: CEP55 and HELLS are cancer progression markers of head and neck squamous cell carcinoma. Oral Oncol *46(7)*: 536-542, 2010. PMID: 20400365. DOI: 10.1016/j.oraloncology.2010.03.022

13 Tao J, Zhi X, Tian Y, Li Z, Zhu Y, Wang W, Xie K, Tang J, Zhang X, Wang L and Xu Z: CEP55 contributes to human gastric carcinoma by regulating cell proliferation. Tumour Biol *35(5)*: 4389-4399, 2014. PMID: 24390615. DOI: 10.1007/s13277-013-1578-1

14 Sung WW, Lin YM, Wu PR, Yen HH, Lai HW, Su TC, Huang RH, Wen CK, Chen CY, Chen CJ and Yeh KT: High nuclear/cytoplasmic ratio of Cdk1 expression predicts poor prognosis in colorectal cancer patients. BMC Cancer *14*: 951, 2014. PMID: 25511643. DOI: 10.1186/1471-2407-14-951

15 Sung WW, Wang YC, Cheng YW, Lee MC, Yeh KT, Wang L, Wang J, Chen CY and Lee H: A polymorphic -844T/C in FasL promoter predicts survival and relapse in non-small cell lung cancer. Clin Cancer Res *17(18)*: 5991-5999, 2011. PMID: 21807637. DOI: 10.1158/1078-0432.CCR-11-0227

16 Sung WW, Wang YC, Lin PL, Cheng YW, Chen CY, Wu TC and Lee H: IL-10 promotes tumor aggressiveness *via* upregulation of CIP2A transcription in lung adenocarcinoma. Clin Cancer Res *19(15)*: 4092-4103, 2013. PMID: 23743567. DOI: 10.1158/1078-0432.CCR-12-3439

17 Zhang W, Niu C, He W, Hou T, Sun X, Xu L and Zhang Y: Upregulation of centrosomal protein 55 is associated with unfavorable prognosis and tumor invasion in epithelial ovarian carcinoma. Tumour Biol *37(5)*: 6239-6254, 2016. PMID: 26615423. DOI: 10.1007/s13277-015-4419-6

18 Chen CH, Lu PJ, Chen YC, Fu SL, Wu KJ, Tsou AP, Lee YC, Lin TC, Hsu SL, Lin WJ, Huang CY and Chou CK: FLJ10540-elicited cell transformation is through the activation of PI3-kinase/AKT pathway. Oncogene *26*: 4272-4283, 2007. PMID: 17237822. DOI: 10.1038/sj.onc.1210207

19 Chen CH, Lai JM, Chou TY, Chen CY, Su LJ, Lee YC, Cheng TS, Hong YR, Chou CK, Whang-Peng J, Wu YC and Huang CY: VEGFA upregulates FLJ10540 and modulates migration and invasion of lung cancer *via* PI3K/AKT pathway. PLoS One *4(4)*: e5052, 2009. PMID: 19337377. DOI: 10.1371/journal.pone.0005052

20 Chen CH, Chien CY, Huang CC, Hwang CF, Chuang HC, Fang FM, Huang HY, Chen CM, Liu HL and Huang CY: Expression of FLJ10540 is correlated with aggressiveness of oral cavity squamous cell carcinoma by stimulating cell migration and invasion through increased FOXM1 and MMP-2 activity. Oncogene *28(30)*: 2723-2737, 2009. PMID: 19525975. DOI: 10.1038/onc.2009.128

21 Jiang W, Wang Z and Jia Y: CEP55 overexpression predicts poor prognosis in patients with locally advanced esophageal squamous cell carcinoma. Oncol Lett *13(1)*: 236-242, 2017. PMID: 28123547. DOI: 10.3892/ol.2016.5414

22 Hauptman N, Jevšinek Skok D, Spasovska E, Boštjančič E and Glavač D: Genes CEP55, FOXD3, FOXF2, GNAO1, GRIA4, and KCNA5 as potential diagnostic biomarkers in colorectal cancer. BMC Med Genomics *12(1)*: 54, 2019. PMID: 30987631. DOI: 10.1186/s12920-019-0501-z

23 Stensrud MJ, Aalen JM, Aalen OO and Valberg M: Limitations of hazard ratios in clinical trials. Eur Heart J *40(17)*: 1378-1383, 2019. PMID: 30500891. DOI: 10.1093/eurheartj/ehy77

Received December 10, 2020
Revised December 20, 2020
Accepted December 21, 2020