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ORIGINAL ARTICLE

Expression of Discoidin Domain Receptor 2 in Colorectal Carcinoma: An Immuno Histochemical Study

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ABSTRACT

Background: Colorectal cancer (CRC) is considered a highly fatal disease. There is a strong need for novel markers to reach a proper outcome. Discoidin domain receptor 2 (DDR2) belongs to the family receptors of tyrosine kinases with a possible relation towards CRC, as DDR2 has a proved role in development of other cancers by enhancing proliferation and metastasis.

Methods: Forty-eight patients diagnosed as colorectal carcinoma were encountered in this study. We analyzed the immune-histochemical expression of discoidin domain receptor 2 and then assessed the correlation of its expression with clinic-pathological data.

Results: Discoidin domain receptor 2 expression is significantly associated with tumor size, infiltrating tumor border, presence of necrosis, tumor grade, tumor stage, lymphocytic infiltration and peritoneal spread (P=0.002, P=0.001, P=0.001, P=0.01, P=0.005, P=0.001, P=0.001, respectively), which all are associated with well-known indicators of poor prognosis.

Conclusions: Discoidin domain receptors 2 over expression, being correlated with poor prognostic factors in colorectal carcinoma, confirm the role of DDR2 in poor outcome.

Keywords: Colorectal carcinoma, CRC, discoidin domain receptor 2, DDR2, immune-histochemical, prognostic.



INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer and the fourth most common cause of cancer related deaths worldwide [1]. In Egypt, CRC represents about 33.8% of whole gastrointestinal tumors and 6.26% of total malignancies [2]. About 20% of patients with CRC already have distant metastasis at the time of initial diagnosis. Only 10% to 30% of those cases are fit for surgical resection of both primary and metastatic lesions. The prognosis greatly depends on stage, where the 5-year survival rate is 10% in cases with distant metastasis, 67% in cases with local lesions and 90% for those with cancer in situ [3]. Recently, advanced CRC has been treated with cytotoxic anticancer agents combined with chemotherapy, as well as with cyto-reductive surgery as a treatment for peritoneal dissemination. Unfortunately, those lines of treatment could not reach the expected cure rate or improve the prognosis. So, to improve the treatment outcome of CRC, an accurate biomarker is required [4]. Discoidin domain receptor 2 (DDR2) is derived from the family of tyrosine kinases receptors that is activated by extracellular collagen. These receptors are important in metabolism, differentiation and cell growth.

DDR2 gene is located on chromosome 1q23.3 and normally secreted in epithelial cells of gastrointestinal tract, lung, kidney and brain [5-7]. In different types of cancer, DDR2 has been incriminated in driving proliferation and metastasis such as small cell carcinoma in lung, urothelial carcinoma, hepatocellular carcinoma and gastric carcinoma. However, the biological roles of DDR2 in CRC remain not known [8]

METHODS

Patients and tissue specimens: This is a cross-sectional study that includes forty-eight paraffin-embedded and formalin-fixed tissue blocks which were collected from the archive of Pathology department, Faculty of Medicine, Zagazig University, and from private pathology laboratories. We obtained all the clinic pathological data from patients' archives. All tissue specimens were stained with hematoxylin and eosin (H&E) stains then were reviewed for confirmation of the histo pathological diagnosis. Only cases of conventional and mucinous adenocarcinoma subtypes with complete clinic pathological data were included in this study. Patients receiving chemotherapy or radiotherapy were excluded.

Immuno histochemistry of DDR2: For immune

histochemical assay, we used streptavidin-biotin complex technique on serial 5-µm sections, preceded by blockage of endogenous peroxidase activity by 0.5% H₂O₂ in methanol for half an hour, then we incubated all slides with the anti-DDR2 monoclonal mouse antibody (1:100 dilution, Clone:3B11E4, Santa Cruz Biotechnology). After that, the slides were diluted using the phosphate buffered saline at 4°C for one night. We incubated anti-rabbit IgG (1:100 dilution) and biotinylated rabbit anti-mouse IgG (1:100 dilution) at room temperature for one hour duration and observed with streptavidin-peroxidase complex. Hematoxylin stain was used as counterstain for the sections [9].

Interpretation and evaluation of immunostaining: We have evaluated the cytoplasmic expression of DDR2 semi-quantitatively regarding to two factors which are the intensity of stain and the extent of positively stained cancer cells. The grading used to assess the extent of positively stained cancer cells was as follows: score 0: positive stained cells ≤5%, score 1: 6-25%, score 2: 26-50%; score 3: 51-75% and score 4: >75%. The intensity of stain was graded as follows: negative stain, score 0; light brown stain, score 1; brown score 2; and dark brown, score 3. To reach the final stain score, we multiplied the scores of the extent and the intensity to reach a final score from 0-12. For statistical analysis, we have applied the cut point of 4 to divide the DDR2 expression into low expression below 4 and high expression from 4-12 [10].

STATISTICAL ANALYSIS

All collected data were both tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). Results with P-value < 0.05 were considered statistically significant, for p-value <0.001 was considered highly statistically significant, and results with p-value ≥0.05 were considered statistically

insignificant.

Ethical Considerations: Written informed consent was taken from all cases. The work has been executed in accordance with The Code of Ethics of the World Medical Association (Helsinki Declaration of 1975, revised in 2000) for studies involving humans. Institutional Review Board (IRB) of the faculty of Medicine Zagazig university confirmed the study protocol (No.4884).

RESULTS

The study was conducted on 48 cases with the mean age of 60.89 ± 10.1 years and a range between 35 and 77 years. Tumor size was obtained from retrospective examination of the radiological reports of the studied cases with 28/48 cases measuring ≥ 5cm. Most cases were grade II (45.8%) (Table1). There was a high statistically significant association between high DDR2 expression and both smoking and the presence of comorbid conditions mainly Diabetes mellitus and obesity (P=0.001). DDR2 expression showed a statistically significant association with tumor size (P= 0.002), infiltrating tumor border (P=0.001) and the presence of necrosis (P=0.001). All mucinous CRC revealed high DDR2 expression whereas in 81.8% of the conventional CRC high DDR2 expression was noticed. There was a statistically significant association between DDR2 expression and grade (P=0.01), with high expression in higher grades (Figures 1&2&3). Regarding lymph node metastasis, distant metastasis, DUKE C staging, and AJCC IV staging, a statistically significant association was found with DDR2 expression (P=0.001, P=0.01, P=0.006, P= 0.005, respectively) of the studied cases (Table2).

A statistically significant association was noticed between DDR2 expression and tumor budding (P=0.02), presence of lymphocytic infiltration (P=0.001) and presence of peritoneal spread (P=0.001) (Figure 4).

TABLE 1: Correlation between DDR2 expression and clinical data

Variable	Total	Low DDR2		High DDR2		χ ²	p-value
	No (48)	No(15)	%	No (33)	%		
Age						0.1	0.9
<62 years	25	8	53.3%	17	51.5%		
≥62 years	23	7	46.7%	16	48.5%		
Sex						0	1
Male	33	10	66.7%	23	69.7%		
Female	15	5	33.3%	10	30.3%		
Smoking						FET	0.001*
None	20	12	80.0%	8	24.2%		
Yes	28	3	20.0%	25	75.8%		

Variable	Total No (48)	Low DDR2 No(15) %	High DDR2 No (33) %	χ^2	p-value
Co-morbid conditions				19.9	
None	17	12 80.0%	5 15.2%		0.001**
Diabetes mellitus	18	3 20.0%	15 45.5%		
Obesity	11	00 0.00%	11 33.3%		
Others	2	00 0.00%	2 6.1%		
Family history				FET	
Absent		13 86.7%	22 66.7%		0.1
Present	35	2 13.3%	11 33.3%		
	13				
Previous history				FET	
Absent		12 80.0%	22 66.7%		0.3
Present	34	3 20.0%	11 33.3%		
	14				
Previous history of other cancers				FET	
Absent	41	14 93.3%	27 81.8%		0.2
Present	7	1 6.7%	6 18.2%		

FET= Fischer Exact test.* Statistically significant difference ($P \leq 0.05$) ** Statistically highly significant difference ($P \leq 0.001$)

TABLE 2: Correlation between DDR2 expression and clinic-pathological data, tumor budding, peri-neural invasion, lympho-vascular invasion and lymphocytic infiltrate.

Variable	Total No (48)	Low DDR2 No(15)	High DDR2 No (33)	χ^2	p-value
Initial site				1.2	
Rightcolon	38	13 86.7%	25 75.7%		0.5
Left colon	8	2 13.3%	6 18.2%		
Rectum	2	0.0 0.0%	2 6.1%		
Tumor size					
<5cm	20	14 93.3%	6 18.2%	FET	0.002*
≥ 5cm	28	1 6.7%	27 81.8%		
FAP				FET	
Absent	43	15 100.0%	28 84.8%		0.1
Present	5	0.0 0.00%	5 15.2%		
Polyp				FET	
Absent	35	13 86.7%	22 66.7%		0.1
Present	13	2 13.3%	11 33.3%		
Histo-pathological subtype				FET	
Conventional adenocarcinoma	42	15 100.0%	27 81.8%		0.07
Mucinous adenocarcinoma	6	0.0 0.00%	6 18.2%		
Necrosis				FET	
Absent (<10)	17	13 86.7%	4 12.1%		0.001**
Present (≥10)	31	2 13.3%	29 87.9%		
Tumor border				FET	
Pushing	14	12 80.0%	2 6.1%		0.001**
Infiltrating	34	3 20.0%	31 93.9%		
IBD				FET	
Absent	45	15 100.0%	30 90.9%		0.2
Present	3	0.0 0.00%	3 9.1%		

Variable	Total No (48)	Low DDR2 No(15)		High DDR2 No (33)		χ^2	p-value
Primary presentation		2	13.3%	8	24.2%	3.1	0.2
Obstruction	10	0.0	0.0%	4	12.1%		
Perforation	4	13	86.7%	21	63.7%		
Bleeding per rectum	34						
Tumor Grading	14	8	53.3%	6	18.2%	8.4	0.01*
Grade I							
Grade II	20	7	46.7%	13	39.4%		
Grade III	14	0.0	0.0%	14	42.4%		
LN metastasis						FET	0.001**
No	18	11	73.3%	7	21.2%		
Yes	30	4	26.7%	26	78.8%		
TNM staging						5.2	0.1
T1	5	3	20.0%	2	6.1%		
T2	9	4	26.7%	5	15.1%		
T3	20	3	20.0%	17	51.5%		
T4	14	5	33.3%	9	27.3%		
N staging						1.2	0.5
N0	18	11	73.3%	7	21.2%		
(N1+N2)	30	4	26.7%	26	78.8%		
M staging						FET	0.01*
M0	33	14	93.3%	19	57.6%		
M1	15	1	6.7%	14	42.4%		
DUKE staging						FET	0.006*
A	9	5	33.3%	4	12.1%		
B	9	6	40.0%	3	9.1%		
C	17	3	20.0%	14	42.2%		
D	13	1	6.7%	12	36.4%		
AJCC staging						12.8	0.005*
I	9	5	33.3%	4	12.1%		
II	9	6	40.0%	3	9.1%		
III	15	3	20.0%	12	36.4%		
IV	15	1	6.7%	14	42.4%		
Tumor budding						FET	0.02*
No	34	14	93.3%	20	60.6%		
Yes	14	1	6.7%	13	39.4%		
Peri-neural invasion						FET	0.1
No	32						
Yes	16	12	80.0%	20	60.6%		
		3	20.0%	13	39.4%		
Lympho-vascular invasion						FET	0.1
No							
Yes	28	11	73.3%	17	51.5%		
	20	4	26.7%	16	48.5%		
Stromal lymphocytic infiltrate						FET	0.001**
No	35	4	26.7%	29	87.9%		
Yes	15	11	73.3%	4	12.1%		
Peritoneal spread						FET	0.001**
No	28	14	93.3%	14	42.4%		
Yes	20	1	6.7%	19	57.6%		

FET= Fischer Exact test. * Statistically significant difference ($P \leq 0.05$) ** Statistically highly significant difference ($P \leq 0.001$) FAP: Familial adenomatous polyposis. IBD: Inflammatory bowel disease.

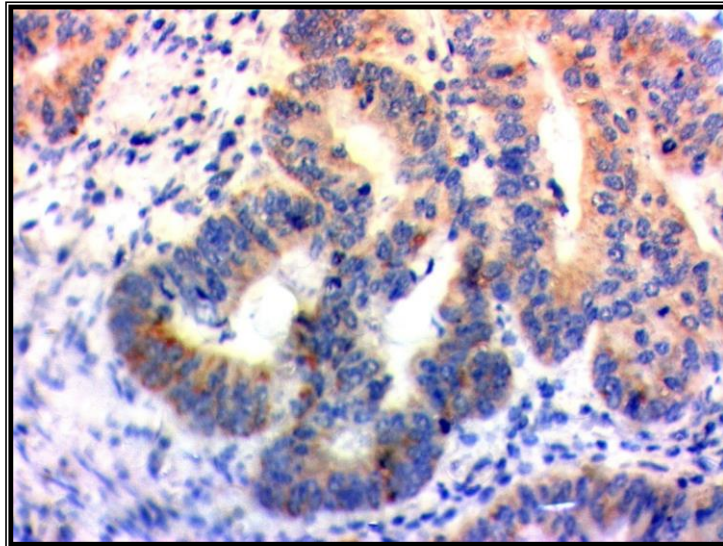


Figure1 A case of well differentiated adenocarcinoma showing low cytoplasmic DDR2 expression (score 3) in the epithelial lining of malignant acini, surrounded by marked lymphocytic infiltrate (IHC, original magnification X 400).

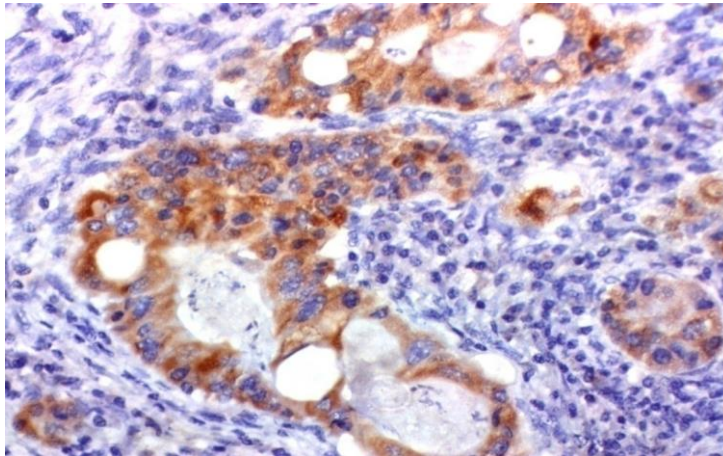


Figure2 A case of moderate differentiated adenocarcinoma showing high cytoplasmic DDR2 expression (score 9) (IHC, original magnification X 400).

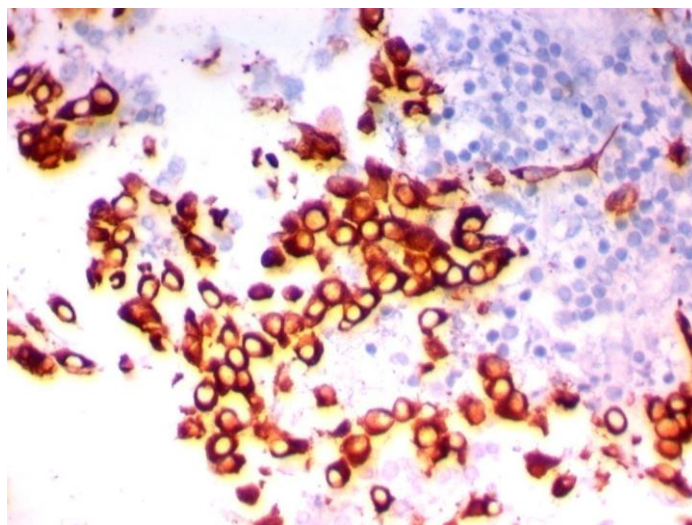


Figure 3 A case of poorly differentiated adenocarcinoma showing high cytoplasmic DDR2 expression (score 12) (IHC, original magnification X 400).

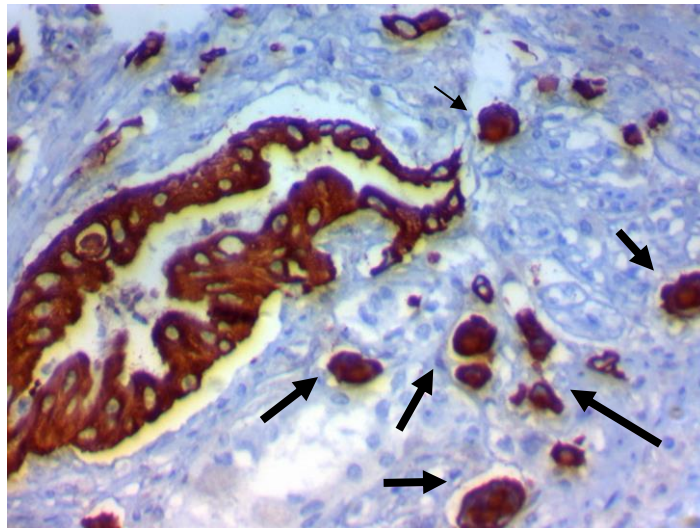


Figure 4 A case of adenocarcinoma with tumor budding highly reactive to DDR2, score (12) (arrows) (IHC, original magnification X 400).

DISCUSSION

CRC is a main public health problem, being a disease common in the developed world with an increasing incidence rates in developing countries [11], so, there is an obvious need for better prognostic marker to improve CRC outcome [4]. Discoidin domain receptor is a member of a family of receptor tyrosine kinases. Two known groups of DDRs, DDR1 and DDR2, are transcribed by chromosome 6 (6p21.3) and chromosome 1 (1q23.3), respectively. DDRs are activated by a various types of human collagen (12). Previous studies assessed the expression of DDR2 in various cancer types [13-15]. Up to our knowledge, Shin et al., (2017) was the only study that assessed its expression in CRC [8]. However, they only focused on peritoneal spread in relation to DDR2 expression with no other data regarding the well-established prognostic factors like grade, stage and other microscopic findings. As regards the tumor size, 20 cases of conventional adenocarcinoma and none of mucinous cases measured less than 5cm (41.6%), while 22 of conventional adenocarcinoma cases and 6 cases of mucinous carcinoma were measured ≥ 5 cm (58.33%). Our results revealed a significant association between high DDR2 expression and tumor size ≥ 5 cm ($P=0.002$) (Table 2). This may indicate the potential role of DDR2 in tumor progression through enhancement of proliferation and increasing tumor mass. In contrast to our study, Yi et al., (2016) and Mitra et al., (2015) found no significant association between DDR2 expression and tumor size in cancer ovary and cancer prostate ($P=0.352$; $P=214$, respectively) [10, 17]. This discrepancy can be explained by

the difference of the studied organs, cancer subtypes, and patients' groups regarding included numbers or geographic distribution

Lambert et al., (2017) explained the correlation between tumor budding and high DDR2 expression by its effect through epithelial mesenchymal transition (EMT), facilitating the dissemination process [18] (Figure 4) (Table 2). EMT is a biological process that permit a polarized epithelial cell to undergo various biochemical changes that convert it into a mesenchymal cell phenotype, those changes include increased migratory capacity, invasiveness, promotes resistance to apoptosis [19]. In support to our results, Callie et al., (2016) found a statistically significant relation between high DDR2 expression and tumor budding in cancer breast ($P=0.05$) [20]. Being encountered in metastasis, DDR2 promotes its action through EMT. So, lymph node metastasis and peritoneal spread are related to DDR2 expression and poor outcome [18]. This was confirmed by our results, which also has proven a high significant relation ($P=0.001$) (table 2). In support to our result, Velmurugan et al, (2018) found a significant relation between high DDR2 expression and lymph node metastasis in oral squamous cell carcinoma ($P=0.009$) [21]. Shin et al., (2017) and Yi et al., (2016) revealed a significant relation between high DDR2 expression and peritoneal spread in cancer colon and cancer ovary ($P=0.012$; 0.009 , respectively) [8, 10].

CONCLUSIONS

DDR2 overexpression could be considered as a marker in colorectal carcinoma defining cases with poor prognosis.

Conflict of interest: no conflicts of interest.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, CA Cancer J Clin, 2017; 67:7-30.
2. Mokhtar N, Asmaa S, Omnia B. Gastrointestinal tract tumor: Cancer pathology registry, 2011; 32-59
3. Chuanbing S, Xiaojun Y, Xiaodong B, Ning H, Pingsheng C. Alpha B-crystallin promotes the invasion and metastasis of colorectal cancer via epithelial mesenchymal transition, Biochemical and Biophysical Research Communications, 2017; 8:670-680.
4. Aoyagi T, Terracina KP, Raza A, Takabe K. Current treatment options for colon cancer peritoneal carcinomatosis. World J Gastroenterol, 2014; 20: 12493-12500.
5. Leitinger B. Discoidin domain receptor functions in physiological and pathological conditions. Int Rev Cell Mol Biol, 2014; 310: 39-87.
6. Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. J Clin Oncol, 2013; 31: 1097-1104.
7. Fu HL, Valiathan RR, Arkwright R, Sohail A, Mihai C, Kumarasiri M, et al. Discoidin domain receptors: unique receptor tyrosine kinases in collagen-mediated signaling. J Biol Chem. 2013; 288(11):7430-7437.
8. Shin S, Masami U, Tomohiro I, Manabu K, Hiroshi N, Toshiyuki W, et al. DDR2 Expression Is Associated with a High Frequency of Peritoneal Dissemination and Poor Prognosis in Colorectal Cancer. Anticancer research, 2017; 37: 2587-2591.
9. Zhang S, Zeng Y, Qu J, Luo Y, Wang X, Li W. Endogenous EGF maintains Sertoli germ cell anchoring junction integrity and is required for early recovery from acute testicular ischemia/reperfusion injury. Reproduction, 2013; 145(2): 89-177.
10. Yi F, Zhe X, Jin F, Liu H, Ming Y, Kun S, et al. Prognostic significance of discoidin domain receptor 2 (DDR2) expression in ovarian cancer. Am J Transl Res. 2016; 8 (6): 2845-2850.
11. Pasqualino F, Gabriele C, Marco G, Felice P, Raffaele E, Maria P, et al. Worldwide burden of colorectal cancer: a review. Updates in Surgery, 2016; 68(1):7-11.
12. Meng-Chen T, Wei-Ming L, Chun-Nung H, Hung-Lung K, Ching-Chia L, Hsin-Chih Y, et al. DDR2 overexpression in urothelial carcinoma indicates an unfavorable prognosis: a large cohort study. Oncotarget, 2016; 7(48): 78918-78931.
13. Bai Y, Kim JY, Watters JM, Fang B, Kinose F, Song L, et al. Adaptive responses to dasatinib-treated lung squamous cell cancer cells harboring DDR2 mutations. Cancer Res, 2014; 74: 7217-7228.
14. Tsai MC, Li WM, Huang CN, Hung-Lung K, Ching-Chia L, Hsin-Chih Y, et al. DDR2 overexpression in urothelial carcinoma indicates an unfavorable prognosis: a large cohort study. Oncotarget, 2016; 7: 78918-78931.
15. Fan Y, Xu Z, Fan J, Liu H, Ming Y, Kun S, et al. Prognostic significance of discoidin domain receptor 2 (DDR2) expression in ovarian cancer. Am J Transl Res, 2016; 8: 2845-2850.
16. Iker B, Elvira O, Olatz C, Scott L, Fernando V. Discoidin domain receptor 2 deficiency predisposes hepatic tissue to colon carcinoma metastasis BMJ journal, 2018; 61(10): 35-302.
17. Mitra A, Hamidreza A, Mahmoud A, Hosein R. Evaluation of discoidin domain receptor-2 (DDR2) expression level in normal, benign and malignant human prostate tissues. Res Pharm Sci, 2015; 10(4): 356-363.
18. Lambert AW, Pattabiraman DR, Weinberg RA. Emerging Biological Principles of Metastasis. Cell, 2017; 168:670-691.
19. Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT. Cell, 2016; 166:21-45.
20. Callie A, Audrey B, Whitney R, Samantha V, Andrew J, Kun Z, et al. The action of Discoidin Domain Receptor 2 in basal tumor cells and stromal Cancer Associated Fibroblasts is critical for breast cancer metastasis. Cell Rep. 2016 14; 15(11): 2510-2523.
21. Velmurugan BK, Chang WH, Chung CM, Yeh CM, Lee CH, Yeh KT, et al. DDR2 overexpression in oral squamous cell carcinoma is associated to lymph node metastasis. Cancer Biomark, 2018; 22 (4): 747-753.

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