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Value of podocalyxin immunohistochemical expression as a prognostic marker in colorectal carcinoma; a clinicopathologic study

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Abstract

Introduction: Podocalyxin-like 1 (PODXL) is an anti-adhesive transmembrane sialomucin whose expression on the membrane of tumor cells, indicates an integral role in tumor progression.
Objectives: To evaluate prognostic value of PODXL expression in colorectal carcinoma (CRC).
Materials and Methods: Fifty CRC cases were collected randomly from colectomy specimens. Sections were prepared from paraffin blocks and the slides immunohistochemically stained with PODXL. Correlations of PODXL expression with other clinicopathological variables and survival analysis were done.
Results: Fourteen percent of cases showed negative expression for PODXL and 86% showed positive expression. A statistically significant relationship was detected between high expression (+3, +4) and patients with stage IV CRC ($P=0.001$), positive circumferential margin ($P=0.006$), and patients with increased CEA after 6 cycles of chemotherapy ($P=0.002$). No statistically significant relationship was detected between PODXL expression and lymph node metastasis ($P=0.30$) or T stage ($P=0.60$). A higher median overall survival among cases with negative or +1 PODXL was obtained compared to lower median survival among cases with +3 and +4 and the difference is highly significant statistically ($P<0.0001$).
Conclusion: PODXL was a poor prognostic factor in CRC. PODXL can act as an attractive target for new strategies in treatment, and predicting prognosis in CRC.

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Introduction

Prediction of prognosis in colorectal cancer is important for the choice of therapeutic options. Histopathological factors are the most important in this respect. Factors such as histological type, degree of differentiation as well as the presence of lymphovascular invasion and lymph node involvement are well known factors that influence outcome (1). Prognosis is greatly dependent on disease stage at diagnosis; however, outcome can widely vary even within the same tumor stage. Thus, there is a great need for additional prognostic biomarkers to better identify patients at high risk of developing metastases (2).

Podocalyxin-like 1 (PODXL) is an anti-adhesive transmembrane sialomucin that is expressed by normal vascular endothelia, breast epithelial cells, hematopoietic progenitors, and renal podocytes. It is also a well-known stem cell marker, and is closely related to stem cell marker CD34 and to endoglycan (3). PODXL inhibits cell-cell interaction through charge-repulsive effects and it is thought to regulate cell morphology and adhesion through its connections to intracellular proteins and to extracellular ligands (4).

The role of PODXL in cancer was first described in testicular cancer. PODXL then has been found to be overexpressed in numerous cancer types and associated with a more aggressive tumor phenotype and poor outcome in breast, prostate, ovarian and bladder cancer. The poor prognosis seems

Key point

Podocalyxin-like 1 (PODXL) is a poor prognostic factor in colorectal carcinoma (CRC). PODXL can act as an attractive target for new strategies in treatment, and predicting prognosis in CRC.

to be conferred by PODXL expression on the membrane of tumor cells, and predominantly at the invasive tumor front, further indicating an integral role for this protein in the progression of some tumors (2). The role of PODXL as an independent prognostic marker in colorectal carcinoma (CRC) has been investigated in several studies (2,3,5).

Objectives

The aim of this work is to evaluate PODXL expression in CRC followed by correlation of the results with different clinic-pathologic parameters to evaluate its prognostic value.

Materials and Methods

This work included 50 cases of CRC obtained through collection of archival paraffin blocks of CRC during the period from 2009 to 2014, from the pathology lab.

Inclusion criteria were as follow: cases diagnosed as conventional adenocarcinoma, mucoid adenocarcinoma (cases diagnosed upon colonoscopy biopsy were not included in the study); cases of CRC presented by colectomy specimen; and cases with available data and files in Clinical Oncology department in Ain Shams University hospitals.

Methods

Positively charged slides were prepared from each paraffin block for immunohistochemical staining.

Histopathological re-evaluation

Re-examination of all H&E slides was done and the following histopathological features were evaluated;

1. CRCs were classified according to the World Health Organization (WHO) classification (2010) into conventional adenocarcinoma and mucinous adenocarcinoma (6).
2. Histological grading of colorectal adenocarcinomas was assessed according to WHO grading criteria (2010) and were divided into well, moderately and poorly differentiated. The percentage of the tumor showing formation of gland-like structures was used to define the grade. Well-formed glands are present in >95% of the tumors that are well differentiated, in 50% to 95% of moderately differentiated tumors, and in 0%-49% of poorly differentiated carcinomas (7).
3. The depth of tumor invasion, lymph node metastasis, distant metastasis and staging were assessed according to TNM staging (2010) (8).
4. Presence or absence of lymphovascular invasion, perineural invasion and circumferential margin involvement by the tumor.

Immunohistochemical staining for podocalyxin

The sections were deparaffinized in xylene, then were hydrated through a series of graded alcohols (95%-70%), distilled water and phosphate buffered saline (at pH 7.5). The slides were then immersed in 10mM citrate buffer (pH 6) and were twice pretreated by microwaving oven 800 w for 4 then 8 minutes. Between each period of heating, evaporated fluid was replenished. After a 25 minute cooling period, the endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide (H2O2) for 5 minutes. Antigen retrieval was done by immersing the slides in 10 mm citrate buffer, (pH 6), for 10-20 minutes at 100°C in a microwave followed by cooling at room temperature for 20 minutes. The tissues were blocked with protein blocking reagent for 30 minutes to reduce nonspecific staining. After washing with Tris-buffered saline, the sections were incubated with the primary antibody for 1 hour at room temperature. The primary antibody was rabbit polyclonal antibody, podocalyxin (NBP1-83348), concentrate antibody (Novus Biologicals), at a dilution of 1:100 from the stock. The dilution was based on dilution experiments. The sections were washed in Tris-buffer and incubated with avidin-biotin-peroxidase system (DAKO) for 30 minutes. The excess reagent was tapped off and the slides were washed with PBS and dried. Peroxidase reaction was detected by addition of diaminobenzidine tetrahydrochloride. Two or three drops of streptavidin enzyme label were placed on each slide for 30 minutes at room temperature. The excess reagent was tapped off and the slides were washed with PBS and dried. All slides were rinsed well in tap water for 5 minutes then slightly counterstained with Mayer's Hematoxylin for 1-2 minutes and dehydrated in ascending alcohol. The slides were cleared in xylene for 3 changes, and then Canada balsam and cover slips were applied.

Evaluation of immunohistochemical expression of PODXL

PODXL staining was evaluated (4,5) and was recorded as negative (0) (Figure 1A), weak cytoplasmic positivity in any proportion of cells (+1) (Figure 1B), moderate cytoplasmic positivity in any proportion (+2) (Figure 1C), distinct membranous positivity in ≤ 50% of cells (+3) (Figure 1D) and distinct membranous positivity in > 50% of cells (+4) (Figure 1E).

Overexpression of PODXL was considered if the tumor cells exhibited a distinct membranous staining in any proportion of the cells (+3 and +4). Normal colorectal mucosa adjacent to the cancers functioned as negative control (5). A sample of renal tissue and tumor-associated vasculature served as a positive control in each staining series (3). To avoid artificial effects, cells in areas with necrosis, poor morphology or at the margins of sections were not counted. The results of PODXL immunostaining in the tumors were correlated with collected data from patient files (age, sex, histopathological type, grade of differentiation, depth of invasion, lymph node metastasis, vascular invasion, staging and CEA levels after six cycles of chemotherapy). Correlations between the pattern of PODXL staining and histological type, histological grade, depth of invasion & stage were also done.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of faculty of medicine of Ain-Shams University.

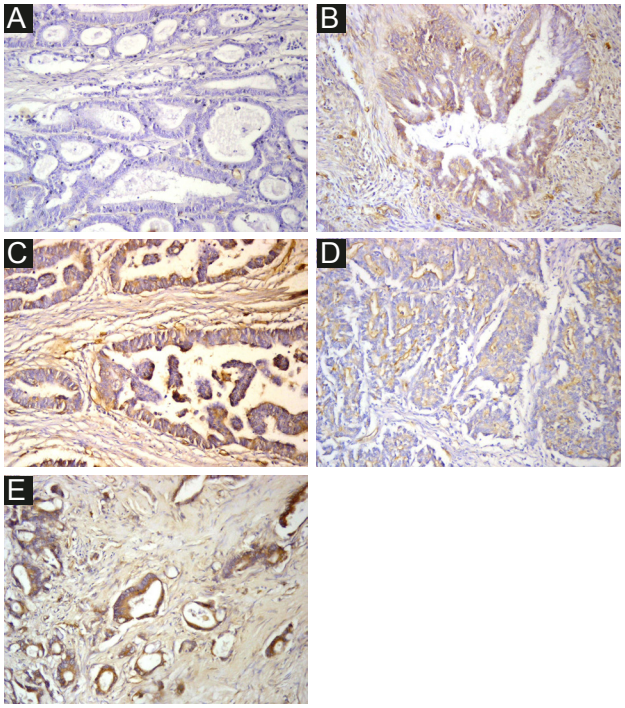

Figure 1. (A) A case of well differentiated colonic adenocarcinoma formed of malignant glands lined by hyperchromatic mild anaplastic epithelial cells with negative immunohistochemical expression of PODXL (x200). **(B)** A case of moderately differentiated adenocarcinoma with mild focal cytoplasmic staining +1 (x200). **(C)** A case of moderately differentiated colonic adenocarcinoma formed of irregularly sized and shaped glands lined by moderately anaplastic epithelial cells with tufting and intraluminal papillary projections, surrounded by desmoplastic stroma showing focal strong cytoplasmic immunostaining for PODXL without membranous staining 2+ (x200). **(D)** Moderately differentiated adenocarcinoma with moderate anaplasia with muscle layer infiltrated, showing focal strong positive cytoplasmic and membranous immunostaining for PODXL 3+ (x200). **(E)** Moderately differentiated adenocarcinoma infiltrating muscle fibers with diffuse strong immunostaining for PODXL mainly membranous 4+ (PODXLx200).

Statistical methods

Data was revised for its completeness and consistency. Double data entry on SPSS program version 16 was done. Quantitative data was summarized by mean, standard deviation (SD) while qualitative data was summarized by frequencies and percentages. Chi-square test, Spearman's correlation coefficient test, Student *t* test, Kaplan–Meier survival analysis were used in analysis of this paper. A "*P* value" of less than 0.05 was considered statistically significant.

Results

This study is a retrospective analytic cross section study, conducted on 50 CRC cases collected randomly from colectomy specimens sent to pathology lab of Ain-Shams University hospitals. Ages of the patients ranged from 21 to 75 years with mean age 49.1 ± 11.9 years. Twenty-four cases (28%) were below the age of 40. Thirty-two cases (64%) were females while 18 cases (36%) were males. Thirty-eight out of 50 cases (76%) were conventional adenocarcinomas, 13 cases (24%) were mucinous adenocarcinomas. The tumors located in left side were higher in incidence (representing 70% of cases) than tumors located in right side. Median follow up in all patients was 11 months, (range 3-40 months). As regards the degree of differentiation, the most common was moderately differentiated adenocarcinoma (66%), well differentiated adenocarcinoma (18%), while poorly differentiated type represented (16%) of cases.

CRC cases metastasized to lymph nodes representing (52%) of all cases. Most of the cases (78%) were infiltrating into sub-serosa or perirectal tissue (T3), 6% were T4, while the remainder were T2. No T1 was presented. Stage I was presented in 10% of cases, stage II 16%, stage III 26%, while stage IV was the highest presenting 48% of the cases. In the studied CRC cases, the percentage of carcinomas with negative CRM was presenting (88%) of the cases, while 12% were positive margins.

NB: Negative CRM include cases in which the tumor is located >1 mm away from the non peritonealized surface.

Regarding immunohistochemical expression of PODXL, +4 PODXL expression was the highest representing 28% of the studied cases (Table 1). The correlation of studied parameters with PODXL revealed a higher percentage of +3, +4 PODXL expression in patients with stage IV colon cancer, positive CRM, metastasis, mortality, and patients with increased CEA after 6 cycles of chemotherapy compared to other cases and the correlation is highly significant statistically with these parameters (Table 2).

There was a higher median overall survival among cases with negative or +1 PODXL compared to lower medial survival among cases with +3 and +4 and the difference is highly significant statistically (Table 3) (Figure 1A). Also there was a higher median survival time among cases with decreased CEA after 6 cycles of chemotherapy compared to cases with increased level and the difference is highly significant statistically (Table 4) (Figure 1B).

Discussion

The aim of the current study was to evaluate the expression of PODXL in 50 cases of CRC immunohistochemically followed by correlation with clinicopathological variables. As regard the immunohistochemical expression of PODXL in this study, 14% of the studied cases showed negative expression for PODXL while 86% showed positive expression (18% were +1, 16% were +2, 24% were +3 and 28% were +4). These results were different from those obtained from Larsson et al as they reported negative expression of PODXL in 75.3% of cases (2).

In this study no statistically significant association be-

Table 1. Distribution of PODXL expression in the studied cases

N = 50	Number	%
PODXL		
Negative	7	14.0
+1	9	18.0
+2	8	16.0
+3	12	24.0
+4	14	28.0

Table 2. Correlation between tumor characters and PODXL expression

	0 No. (%)	+1 No. (%)	+2 No. (%)	+3 No. (%)	+4 No. (%)	X2	P
Stage							0.001
I, N=5	1 (20.0)	2 (40.0)	2 (40.0)	0	0		
II, N=8	3 (37.5)	3 (37.5)	2 (25.0)	0	0	32.3	
III, N=13	3 (23.1)	4 (30.8)	1 (7.7)	3 (23.1)	2 (15.4)		
IV, N=24	0	0	3 (12.5)	9 (37.5)	12 (50.0)		
T stage							0.09
T2, N=8	1 (12.5)	3 (37.5)	2 (25.0)	1 (12.5)	1 (12.5)	13.5	
T3, N=39	6 (15.4)	6 (15.4)	6 (15.4)	8 (20.5)	13 (33.0)		
T4, N=3	0	0	0	3 (100)	0		
Metastasis							0.000
M0, N=26	7 (26.9)	9 (34.6)	5 (19.2)	3 11.5)	2 (7.7)	26.6	
M1, N=24	0	0	3 (12.5)	9 37.5)	12 (50.0)		
Type of tumor							0.5
Adenocacrcinoma, N=38	7 (18.4)	7 (18.4)	6 (15.8)	9 23.7)	9 (23.7)	3.2	
Others, N=12	0	2 (16.7)	2 (16.7)	3 (25.0)	5 (41.7)		
LN							0.01
N0, N=24	4 (16.7)	5 (20.8)	5 (20.8)	3 (12.5)	7 (29.2)	18.7	
N1, N=17	3 (17.6)	4 (23.5)	2 (11.8)	2 (11.8)	6 (35.3)		
N2, N=9	0	0	1 (11.1)	7 (77.8)	1 (11.1)		
Margin							0.006
Negative, N=44	7 (15.9)	9 (20.5)	8 (18.2)	11 (5.0)	9 (20.5)	10.8	
Positive, N=6	0	0	0	1 (16.7)	5 (83.3)		
Grade							0.000
1, N=9	3 (33.3)	3 (33.3)	1 (11.1)	2 (22.2)	0	29.1	
2, N=33	4 (12.1)	6 (18.2)	7 (21.2)	10 (30.3)	6 (18.2)		
3, N=8	0	0	0	0	8 (100.0)		
Survival							0.000
Living, N=24	7 (29.0)	9 (37.5)	5 (20.8)	3 (12.5)	0	45.1	
Dead, N=26	0	0	3 (11.5)	9 (34.6)	14 (53.8)		
CEA level							0.002
Decreased, N=22	6 (27.3)	6 (27.3)	6 (27.3)	1 4.5)	3 (13.6)	17.0	
Increased, N=25	1 (4.0)	3 (12.0)	2 (8.0)	11 (44)	8 (32.0)		

Chi square test: * $P<0.05$ Significant, ** $P<0.01$ highly significant.

Table 3. Correlation between PODXL results and the median overall survival of patients with CRC[a]

Kaplan-Meier product estimates[b]		
PODXL	**Median**	**SE**
0, N=7	36	7.4
+1, N=9	30	12.7
+2, N=8	20	4.2
+3, N=12	9	0.8
+4, N=14	6	0.9

[a] $P<0.01$ Highly significant
[b] Log rank test: 43.1; $P=0.000$

Table 4. Correlation between pattern of CEA after 6 cycles of chemotherapy and the median overall survival of patients with CRC[a]

Kaplan-Meier product estimates				
CEA pattern	**Median**	**SE**	**Log rank test**	**P**
Increased, N=25	8.0	0.6	18.6	0.000
Decreased, N=22	20.0	7.6		

[a] $P<0.01$ Highly significant

tween PODXL expression and LN ($P=0.3$) as well as the T-stage ($P=0.6$) of the tumor. This was in accordance with Larsson et al who detected the same findings (2). In contrast to the current study, the study of Larsson et al revealed a strong correlation between high PODXL expression and more advanced T-stage ($P<0.001$), N-stage ($P<0.001$) (5).

The variability of this data can be explained either by differences in patient selection or technical variability in the performance of IHC staining. It is widely accepted that IHC analysis is vulnerable to differences in tissue fixation, processing, in choice of primary antibody, detection system, epitope retrieval, interpretation and reporting (9).

A statistically significant positive correlation was detected between high PODXL expression (+3, +4) and advanced stage of tumor ($P=0.001$), metastasis ($P=0.001$) and mortality ($P=0.001$). This observation might be due to the fact that PODXL has been shown to interact with mediators of metastasis and to play an important role in epithelial-mesenchymal transition (EMT), a process involved in initiating the invasive and metastatic behavior of epitheli-

al cancer cells, by regulating and interacting with collagen type 1, E-cadherin and vimentin (4).

Analysis of the association between PODXL and CRM revealed a strong association ($P=0.003$). This finding could be explained by the fact that when overexpressed at high concentration, PODXL can drive epithelial cell morphogenesis and trigger intracellular cytoskeletal rearrangements, reduce adhesion to extracellular matrix and adjacent cells and facilitate cell shedding which in turn facilitate local spread. Moreover, a high expression of PODXL has been recorded in TGF-b induced EMT indicating that PODXL has an integral role in cancer progression (10,11).

A highly statistically significant correlation between high PODXL expression and CEA levels after 6 cycles of chemotherapy was attained ($P=0.002$). It was reported that CEA is elevated in cases with liver metastasis as it is known to be metabolized in the liver; that is why serum CEA is elevated in cases of impaired liver function (12). This association between elevated CEA and high PODXL expression can be explained by supposing that PODXL may be metabolized in the liver too; as in our cohort most of patients with stage IV who were presented by liver metastasis had high PODXL expression. But we did not find in literature a clear evidence about PODXL metabolism. Another explanation for this association is due to the novel finding that CEA is an E- and L-selectin ligand (13) and it was found that PODXL expressed by CRC cells can support E- and L-selectin-mediated adhesion and selectin-mediated interactions in the vasculature promote metastatic spread by facilitating circulating tumor cell binding to selectin-expressing host cells (14). It is obvious that both CEA and podocalyxin have same mechanism of action in cancer progression. Further studies on this issue as well as investigations on the molecular interaction between PODXL and CEA in progression of CRC are recommended.

Our study also showed a higher median survival time among cases with decreased CEA compared to cases with increased level after 6 cycles of chemotherapy and the difference is highly significant statistically ($P=0.001$). Similar results obtained from the study of Luo et al ($P=0.001$) (15). Several studies have shown that patients who exhibited a decrease in CEA while on chemotherapy had a better overall survival compared with those whose CEA concentrations failed to decrease and College of American Pathologists Expert Groups ranked preoperative serum CEA concentration as a category I prognostic marker for colorectal cancer (Category I factors include those — definitely proven to be of prognostic important based on evidence from multiple statistically robust published trials and generally used inpatient management) (12).

The current study showed a higher median overall survival among cases with negative or +1 PODXL (36 and 30 respectively) compared to lower medial survival among cases with +3 (9) and + 4 (6) and the difference is highly significant statistically ($P=0.001$). Larsson et al revealed that PODXL expression correlated with a significantly shorter OS (overall survival), with the worst outcome for tumors with high PODXL expression ($P=0.001$) (5). Since podocalyxin was supposed to have a functional role in tumor progression, it was suggested that can be a target for monoclonal antibody therapy (16).

There are several challenges to overcome in the development of PODXL-targeted therapies for the treatment of high-risk primary tumors or treatment of systemic cancers. First, we do not yet know if the high PODXL expression detected on primary tumors is maintained on metastatic tumor cells. Second, any PODXL-targeted therapies must carefully consider potential renal and vascular toxicity since PODXL is highly expressed on glomerular podocytes and on most vasculature. Fortunately, there are several examples of uniquely modified, tumor-specific forms of PODXL. These provide feasible targets for antibody-based drugs that are either directly oncolytic or block PODXL mediated functions without affecting the function of normal cells (11).

Conclusion

- Immunohistochemical expression of PODXL can act as a prognostic marker to predict poor outcome of patients with CRC.
- PODXL plays a role in both local and distant tumor spread.
- There is a link between PODXL and CEA through mechanism of action and metabolism which needs further investigations.
- PODXL acts as attractive target for the development of new strategies in the diagnosis and treatment of CRC in order to reduce the invasive and metastatic potential of CRC.

Conflicts of interest

None to be declared.

Authors' contribution

All authors contributed to the design of the research. RS, SS, MMS, GR and DS shared in analysis and interpretation of data. All authors drafted the first version. RS, SS, MMS, GR and DS edited the first draft. All authors reviewed and commented on final draft.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References

1. Marzouk O and Schofield J. Review of Histopathological and Molecular Prognostic Features in Colorectal Cancer. Cancers (Basel). 2011;3:2767-810.
2. Larsson AH, Nodin B, Syk I, Palmquist I, Uhlén M, Eberhard J, et al. Podocalyxin like protein expression in primary colorectal cancer and synchronous lymph node metastases. Diagn Pathol. 2013; 8:109.
3. Kaprio T, Fermér C, Hagström J, Mustonen H, Böckelman C, Nilsson O, et al. Podocalyxin is a marker of poor prognosis in colorectal cancer. BMC Cancer. 2014;14:493.
4. Larsson A, Fridberg M, Gaber A, Nodin B, Leveén P, Jönsson G, et al. Validation of podocalyxin-like protein as a biomarker of poor prognosis in colorectal cancer. BMC Cancer. 2012;12:282.
5. Larsson A, Johansson ME, Wangefjord S, Gaber A, Nodin B, Kucharzewska P, et al. Overexpression of podocalyxin-like

protein is an independent factor of poor prognosis in colorectal cancer. Br J Cancer. 2011; 105:666-72.
6. Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system. World Health Organization; 2010.
7. Hamilton SR, Bosman FT, Boffetta P, Ilyas M, Morreau H, Nakamura SI, et al. Carcinoma of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO Classification of Tumors of the Digestive System. Lyon: IARC; 2010. p. 134–46.
8. Grill S, Brown C, And Miller R. Colon Cancer. In: Blanke C, Rodel C, Talamonti M, eds. Gastrointestinal Oncology: A practical Guide. Berlin: Springer; 2011. p.337.
9. Seidal T, Balaton AJ, Battifora H. Interpretation and quantification of immunostains. Am J Surg Pathol. 2001;25:1204-7.
10. Meng X, Ezzati P, Wilkins JA. Requirement of podocalyxin in TGF-beta induced epithelial mesenchymal transition. PLoS One. 2011;6:e18715.
11. McNagny KM, Roskelley CD, DeBruin EJ, Cipollone J, Snyder K, Graves ML, et al. Podocalyxin in the diagnosis and treatment of cancer. INTECH Open Access Publisher; 2012.
12. Duffy MJ. Carcinoembryonic Antigen as a Marker for Colorectal Cancer: Is It Clinically Useful? Clin Chem. 2001;47:624-30.
13. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K. Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. J Biol Chem. 2008;283:15647-55.
14. Dallas MR, Chen SH, Streppel MM, Sharma S, Maitra A, Konstantopoulos K. Sialofucosylated podocalyxin is a functional E- and L-selectin ligand expressed by metastatic pancreatic cancer cells. Am J Physiol Cell Physiol. 2012;303:C616-24.
15. Luo J, Gao F, Zhang S, Chen L, Yang J. Analysis of prognosis of 122 colorectal cancer patients with concurrent liver metastasis. Clin Oncol Cancer Res. 2009;6:214-20.
16. Snyder KA, Hughes MR, Hedberg B, Brandon J, Hernaez DC, Bergqvist P, et al. Podocalyxin enhances breast tumor growth and metastasis and is a target for monoclonal antibody therapy. Breast Cancer Res. 2015;17:46.