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Dysregulated *KDR* and *FLT1* Gene Expression in Colorectal Cancer Patients

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Abstract

Background: Colorectal cancer (CRC) is one of the most commonly-diagnosed malignancies throughout the world and the fourth-leading cause of cancer deaths globally. Angiogenesis and the resultant tumor neovascularization is a well-known cancer hallmark. Here we investigated the expression of *FLT1* and *KDR*, the influential genes in angiogenesis regulation, in CRC patients.

Methods: We assessed *FLT1* and *KDR* mRNA expression in 47 CRC samples and matched adjacent non-cancerous tissues (ANCT) by quantitative real-time PCR. The Spearmen correlation coefficient and receiver operating characteristic (ROC) curves were also examined.

Results: Both genes were expressed at significantly greater levels in CRC tissues than in ANCT ($p < 0.05$). A significant association was found between *KDR* expression and disease stage and lymph status in CRC patients. Furthermore, the Spearman correlation demonstrated a moderate correlation between *FLT1* and *KDR* expression in CRC samples. Finally, ROC curve analysis demonstrated that *FLT1* had the greatest sensitivity (85.1%), while the greatest specificity was achieved by a combination of the two genes.

Conclusions: The dysregulated *FLT1* and *KDR* expression, in addition to the observed correlation and ROC curve results, indicate the critical importance of angiogenesis among the cancer pathways in CRC. These data can broaden our current knowledge of angiogenesis in CRC to improve disease diagnosis and patient treatment.

Keywords: Colorectal cancer, *FLT1*, *KDR*, Gene expression.

Introduction

Colorectal cancer (CRC) is one of the most commonly-diagnosed malignancies throughout the world and the fourth-leading cause of cancer deaths globally (1). This major public health problem is expected to increased worldwide by 60% to over 2.2 million new cases and 1.1 million deaths by 2030 (2). Although non-genetic factors, such as diet and lifestyle, are thought to have considerable impact on CRC risk, controversial evidence exists regarding predisposing or protective effects of several dietary components (3), allocating a large portion of disease-causing

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factors for genetic involvement in the pathophysiology of the disease (4).

Induction of angiogenesis, combined with the resultant tumor neovascularization, is a well-known hallmark of cancer, and the balance between proangiogenic and inhibitory elements controls angiogenesis (5, 6). Angiogenesis is well known as a principal factor in the development and dissemination of CRC, displaying significant implications in the clinical management of the disease. Previous studies have shown angiogenesis inhibition enhances the effectiveness of CRC treatment (7). Angiogenesis is regulated mainly via various growth factors and their associated receptor tyrosine kinases (8). At the heart of this signaling network, vascular endothelial growth factors (VEGFs) and their receptors have key roles in pathological angiogenesis in various cancers. The VEGF receptors (VEGFRs) *FLT1* (VEGFR-1) and *KDR* (VEGFR-2) have been demonstrated to be critical in controlling tumor angiogenesis (9).

FLT1 is expressed primarily on precursors and mature endothelial cells and has been shown to play direct roles in angiogenesis related to human disease. It is the common receptor for the three members of pro-angiogenic family, namely VEGF-A, VEGF-B, and placental growth factor (PGF), and binding of FLT1 to each of these three ligands activates the receptor and initiates the consequent signaling transduction cascade, which leads to the regulation of both biological and pathological events associated with cellular proliferation, transformation, migration, apoptosis, and vascularization (10, 11).

The kinase insert domain receptor (KDR) is the crucial VEGF receptor mediating signaling transduction triggered by VEGF ligands. VEGF signaling through KDR is the major pathway that promotes angiogenesis via endothelial cell proliferation, survival, migration, and permeability. These cumulative effects, exerted by the VEGF/KDR signaling cascade, can facilitate tumor growth, invasion, and therapeutic resistance (12). The interaction between VEGF and KDR results in the activation of downstream signaling pathways including PLCɣ-PKC-MEK-MAPK and PI3K/AKT pathways, which promote endothelial cell survival (12, 13).

Here we investigated *FLT1* and *KDR* expression, in CRC tissues and healthy adjacent samples. Moreover, the correlation between expression of these two genes and disease state, as well as receiver operating characteristics (ROCs) were also assessed.

Materials and methods

Ethics approval and consent to Participant

All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Patients and tissue samples

This study was conducted on 47 CRC samples and 47 matched adjacent non-cancerous tissues (ANCT) from patients admitted to the Imam Reza Hospital of Tabriz city of Iran. The mean age of the 27 male and 20 female CRC patients was 55.4 ± 13.64 (mean ± standard deviation (SD)) years. No patient had any previous or secondary malignancy or had received preoperative chemotherapy, radiotherapy, or immunotherapy. Samples were selected based on clinicopathological characteristics determined by expert pathologists. The study design was approved by the local Ethical Committee of the Tabriz University of Medical Sciences (TUMS).

Sample preparation and RNA isolation Phage One-mm tissue cores from each specimen were obtained and immediately transferred into RNAlater (Qiagen, Germany) microtubes. Then, the RNA was extracted from all samples using RNeasy™ Mini Kit (Qiagen) based on the manufacturer's protocols. The quality and quantity of the RNAs were determined via agarose gel electrophoresis and NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA), respectively. We incubated all the RNA samples in a solution containing DNase, RNase inhibitor, DNase buffer, and DEPC-treated water at 37°C for 30 min to remove any contaminating DNA. Then, ethylenediaminetetraacetic acid (EDTA) was added and samples were incubated at 65 °C for 10 min to remove the DNase (14).

cDNA synthesis and quantitative RT-PCR

The cDNA library was synthesized by RT-PCR with a combination of random hexamers, oligo-dT primers, and reverse transcriptase in in 20 µl reaction volume. In brief, 500 ng of total RNA from each sample was reverse transcribed. cDNA was then synthesized using Prime Script II reverse transcriptase (TaKaRa, Japan) at 37 °C for 15 min followed by 85 °C for 10 second to inactivate the reverse transcriptase (15).

The specific primers for *FLT1*, *KDR*, and *GAPDH* were designed using Allele ID software version 7.5 (Premier Biosoft International, Palo Alto, CA, USA) and blasted with NCBI Primer-BLAST (Table 1). Real-time PCR was performed in a Rotor-Gene 6000 machine (Corbet Life Science). SYBR Premix Ex Taq II (TaKaRa, Japan) was used to detect gene expressions All amplifications were performed in duplicate and the synthesis of single PCR products was confirmed by determining melting curves. Negative controls with no cDNA template were included in all runs to identify possible contamination. Gene expression changes were calculated after normalizing for *GAPDH* expression. Results were reported as fold increase or decrease relative to controls.

Table 1. PCR Primers

Genes	Forward primers	Reverse primers
GAPDH	CATGGCCTCCAAGGAGTAAG	GCTTGAGCACAGGGTACTTTA
FLT1	GCCGTGTCATCGTTTCCAGA	GGTTACAGGGGTGCCAGAA
KDR	CTACTGATTTTTGCCCTTGTTC	TAGTCATTGTTCCCAGCATTTC

Statistical analysis

We applied SPSS statistical software version 20 (SPSS Inc., Chicago IL, USA) for all statistical measurements in this study. The mean normalized expression ± standard deviation was calculated for each gene. Relative expression levels were evaluated in CRC and ANCT samples. Spearman's correlation coefficient was used to compare correlations between different variables.

Likewise, the ROC curve was determined to ascertain the sensitivity and specificity of gene expression levels as diagnostic biomarkers. We applied the Youden index (j) to establish the most discrepancies between sensitivity (true-positive) and 1 – specificity (false-positive). ROC curves can be paramount in characterizing the potential of KDR and FLT1 to discriminate between malignant and non-malignant samples. The significance level was set as p value less than 0.05 in all experiments.

Results

Clinicopathological data

The clinicopathological features of our CRC patients are presented in Table 2. Table 3 demonstrates the associations between up- and down-regulated levels of *FLT1* and *KDR* and clinicopathological characteristics. A significant association was found between *KDR* expression and disease stage, lymph status, and CRC ($p < 0.05$).

Table 2. Clinicopathological characteristics of colorectal cancer (CRC) patients in our study.

		Case
Age (years old)		55.4 ± 13.64 (23-76)
Gender	**Male**	57.4 %
	Female	42.6 %
Histology	**Well**	57.5 %
	Mod	32.5 %
	Poor	10 %
Stage	**I**	2.5 %
	II	32.5 %
	III	52.5 %
	VI	12.5 %
Metastasis	**Absent**	90 %
	Present	10 %
Depth	**T2**	7.5 %
	T3	22.5 %
	T4	70 %
Lymph	**Absent**	40 %
	Present	60 %
Venous	**Absent**	27.5 %
	Present	72.5 %
Liver	**Absent**	87.5 %
	Present	12.5 %

Table 3. The association of *FLT1* and *KDR* with colorectal cancer (CRC) patients' clinicopathological characteristics.

	***FLT1* up-regulation**	***FLT1* down-regulation**	**P value**	***KDR* up-regulation**	***KDR* down-regulation**	**P value**
Age			**0.6**			**0.87**
60 >	14 (63.6%)	8 (36.4%)		14 (63.6%)	8 (36.4%)	
60 ≤	10 (55.6%)	8 (44.4%)		11 (61.1%)	7 (38.9%)	
Gender			**0.58**			**0.88**
Female	17 (63%)	10 (37%)		17 (63%)	10 (37%)	
Male	11 (55%)	9 (45%)		13 (65%)	7 (35%)	
Histology			**0.15**			**0.8**
Well	13 (56.5%)	10 (43.5%)		14 (60.9%)	9 (39.1%)	
Mod	10 (76.9%)	3 (23.1%)		9 (69.2%)	4 (30.8%)	
Poor	1 (25%)	3 (75%)		2 (50%)	2 (50%)	
Stage			**0.19**			**0.02**
I	1 (100%)	0 (0%)		1 (100%)	0 (0%)	
II	5 (38.5%)	8 (61.5%)		4 (30.8%)	9 (69.2%)	
III	15 (71.4%)	6 (28.6%)		16 (76.2%)	5 (23.8%)	
IV	3 (60%)	2 (40%)		4 (80%)	1 (20%)	
Metastasis			**0.63**			**1**
Absent	21 (58.3%)	15 (41.7%)		22 (20.8%)	14 (79.2%)	
Present	3 (75%)	1 (25%)		3 (75%)	1 (25%)	
Depth			**0.74**			**1**
T2	1 (33.3%)	2 (66.7%)		2 (66.7%)	1 (33.3%)	
T3	6 (66.7%)	3 (33.3%)		6 (66.7%)	3 (33.3%)	
T4	17 (60.7%)	11 (39.3%)		17 (60.7%)	11 (39.3%)	
Lymph			**0.08**			**0.04**
Absent	7 (43.8%)	9 (56.3%)		7 (73.8%)	9 (56.3%)	
Present	17 (70.8%)	7 (29.2%)		18 (75%)	6 (25%)	
Venous			**1**			**0.52**
Absent	7 (63.6%)	4 (36.4%)		6 (54.5%)	5 (45.5%)	
Present	17 (58.6%)	12 (41.4%)		19 (65.5%)	10 (34.5%)	
Liver			**1**			**0.63**
Absent	21 (60%)	14 (40%)		21 (60%)	14 (40%)	
Present	3 (60%)	2 (40%)		4 (80%)	1 (20%)	

Relative mRNA expression levels of *FLT1* and *KDR* genes

FLT1 and *KDR* expression were significantly greater in tumor tissues than in ANCT (p values 0.014 and 0.009, respectively, Table 4). A similar result was seen for both genes in the male subgroup (*FLT1*: $p < 0.01$; *KDR*: $p < 0.03$), but the results were not significant in the female subgroup ($p > 0.05$). The expression ratios for both genes in tumor and ANCT tissues are shown in Figure 1 (Fig. 1).

Table 4. Relative expression of *FLT1* and *KDR* in tumors and adjacent non-cancerous tissues (ANCTs) from colorectal cancer (CRC) patients.

		Tumor tissue vs ANCT (Total)	**Tumor tissue vs ANCT (Male)**	**Tumor tissue vs ANCT (Female)**
FLT	Expression ratio	1.48	1.65	1.26
	P value	**0.014**	**0.01**	0.36
KDR	Expression ratio	1.59	1.66	1.5
	P value	**0.009**	**0.03**	0.12

Correlation and ROC curve analysis

The correlations between *FLT1* and *KDR* expression in CRC tumor tissues are shown in Figure 2. An intermediate correlation exists between these two genes ($R^2 = 0.353$) (Fig. 2).

In this study, we found that *FLT1* and *KDR* expression was significantly greater in tumor tissues than in ANCT. We then attempted to determine the predictive value of these altered expression levels separately and combined to discriminate between malignant and non-malignant status using ROC curves (Figs. 3 and 4). Critical cut-off values of significantly different *FLT1* and *KDR* expression levels were investigated. The area under the curve (AUC), sensitivity, and specificity for *FLT1* and *KDR* expression, and their combination, are presented in Table 5. Of these results, *FLT1* had the greatest sensitivity at 85.1%, while a combination of the two genes had the greatest specificity at 57.4%. These significant values suggest the altered expression of *FLT1* and *KDR*, as well as their combination, may be diagnostic for CRC.

Table 5. Receiver operating characteristic (ROC) curve analysis of *FLT1* and *KDR* in colorectal cancer (CRC) tissues.

	Estimate criterion	AUC	J[a]	Sensitivity	Specificity	P value[b]
FLT	≤ 5.18	0.63	0.25	85.1	40.4	**0.016**
KDR	≤ 4.61	0.61	0.23	70.2	53.2	**0.05**
Combination of two genes	> 0.47	0.64	0.25	68.1	57.4	**0.014**

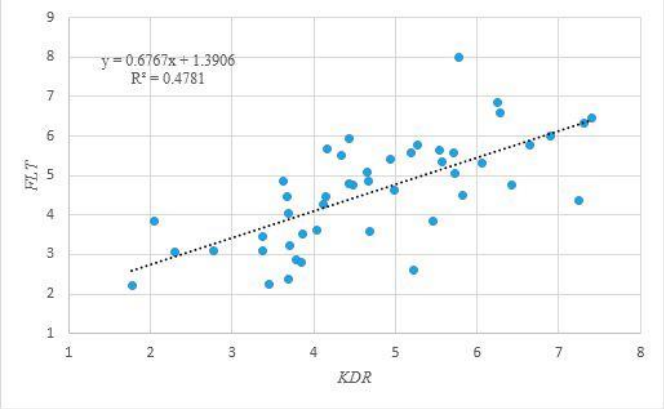

Fig. 1. *FLT1* and *KDR* expression in colorectal cancer (CRC) tissues vs. adjacent non-cancerous tissues (ANCT).

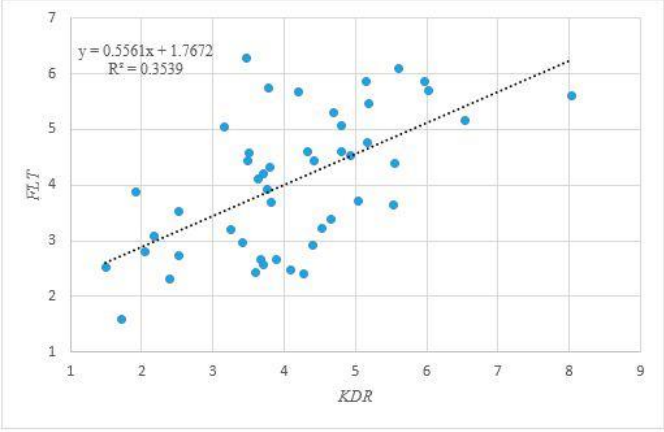

Fig. 2. The Spearmen correlation between *FLT1* and *KDR* expression in colorectal cancer (CRC) tissues.

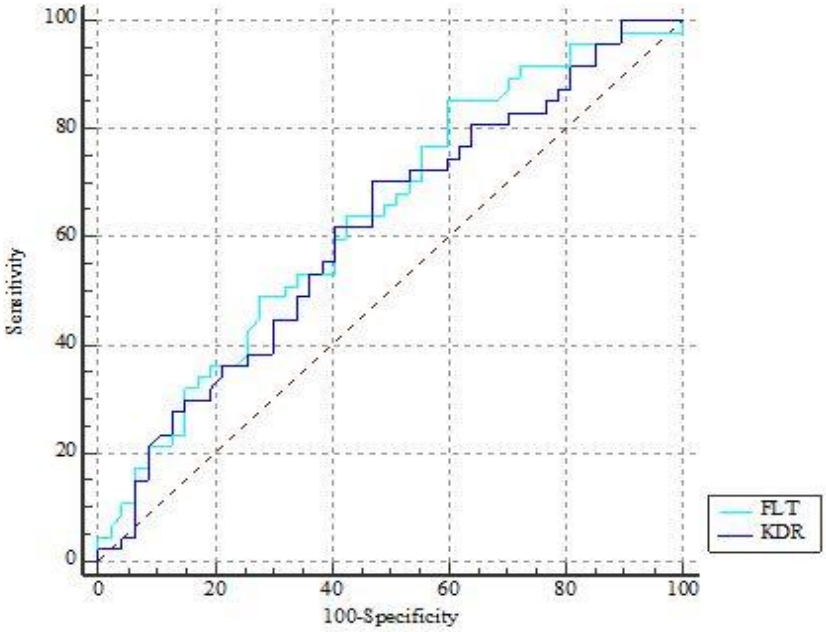

Fig. 3. Receiver operating characteristic (ROC) curve depicting the areas under the curves (AUCs) for discriminating between malignant and non-malignant colorectal tissues by *FLT1* and *KDR*, separately.

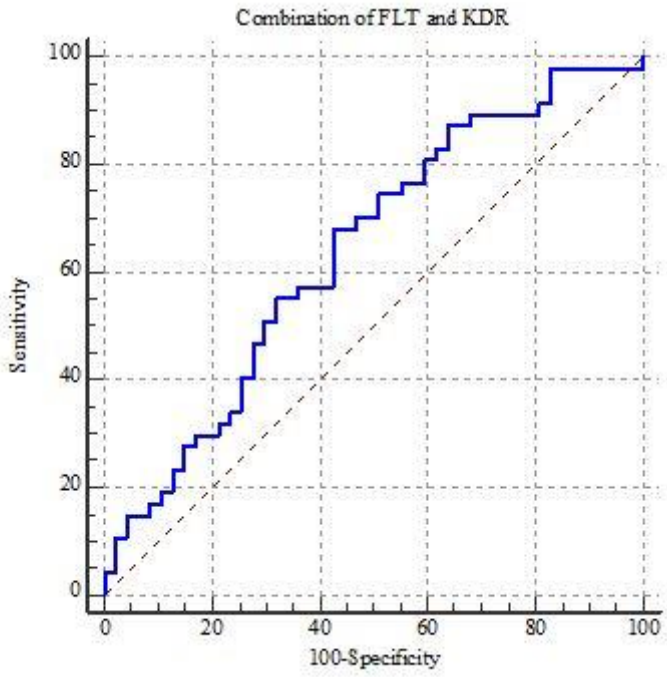

Fig. 4. Receiver operating characteristic (ROC) curve depicting the area under the curves (AUC) for discriminating between malignant and non-malignant colorectal tissues by combination of *FLT1* and *KDR*.

Discussion

In the present study, we examined the expression of *FLT1* and *KDR*, as the genes among the most important factors in cancer angiogenesis, in patients with CRC. We found significantly greater expression of both genes in tumor tissues than in ANCT in male patients. Neovascularization is a critical step in the tumor growth and dissemination of CRC. Angiogenesis, the formation of de novo vessels via developing endothelial cells, and vasculogenesis, formation of new vascular components from precursor endothelial cells in bone marrow, are recognized as the essential mechanisms by which this phenomenon occurs. This up-regulated process makes a synergistic

crosstalk with the epidermal growth factor receptor (EGFR) pathway and further reinforces the MAPK kinase cascade and PI3K/ATK pathway. The final outcome would be increased angiogenesis, cellular proliferation, and loss of apoptosis (16). In accordance to our findings, it was shown that inhibition of EGFR and VEGF by tyrosine kinase inhibitors (TKIs) augments the anti-tumoral and anti-angiogenic efficacy in CRC cell lines via diminished VEGF production by cancer cells, and inhibits cellular proliferation, G1 cell cycle arrest, and apoptosis induction (17).

Studies have found that a correlation exists between vessel density and VEGF expression in human renal cell carcinoma, breast tumors, and colon cancer (18). Here, we reported up-regulated *FLT1* expression in tumor tissues. FLT1 protein is a receptor tyrosine kinase with high binding affinity for its ligand, VEGF-A, and PGF. Mouse models have revealed that not only is it functionally required for embryonic vasculogenesis and macrophage function, but also $FLT1^{+}$ progenitor-derived monocytes in bone marrow promote the premetastatic niche (19, 20). This kinase receptor is expressed in both endothelial and epithelial cells and its activation contributes to the epithelial-mesenchymal transition (EMT) along with an aggressive phenotype in particular cancer cells such as those found in CRC (21). The regulatory mechanism of *FLT1* expression in cancer cells is, however, largely uncertain (22).

Similar to FLT1, KDR (VEGFR-2), is another receptor tyrosine kinase involved in angiogenesis. This receptor binds multiple VEGFs and is an influential component in angiogenesis through its downstream signaling pathways. Furthermore, KDR cooperates in pathologic angiogenesis and has been shown to have altered mRNA levels in several malignancies (23, 24). In the present study, we observed up-regulated *KDR* expression in CRC tissues, which is in line with previous findings showing the association of VEGFR-2 with increased vascularity and metastatic potential in CRC (25). Likewise, our results are consistent with the observation in which mutations in KDR were categorized as a novel predictive biomarker regarding exceptional response to regorafenib in metastatic CRC patients (26). For instance, it was established that KDR inhibition abrogates the VEGF-mediated activation of hypoxia-inducible factor 1-alpha (HIF1A), which in turn enhances the survival of HCT116 CRC cells that are sensitive to bevacizumab, the most commonly utilized anti-angiogenesis agent in CRC treatment (12). Taken together, such experimental investigations emphasize the critical functions of FLT1 and KDR in both etiology and clinical outcomes of CRC patients, through adjusting VEGF-induced signaling transduction. Interestingly, no statistically significant differences were observed for both studied genes in female subgroup. This might be due to the fact that the number of females was less than that of males (20 vs. 27), and that these genes, simply, are not highly expressed in female CRC patients as in male patients.

In addition, a moderate correlation was seen between *FLT1* and *KDR* expression in CRC samples. The significant association between *KDR* expression and clinicopathological characteristics such as disease stage and lymph status again highlight the importance of their network in angiogenesis and relevant pathways in CRC development (27). Such findings agree with earlier assessments displaying notable relationships between VEGFR-3 expression and clinical stage, lymph node metastasis, and T stage (28). Given the ROC curve results, we concluded that the combination of *FLT1* and *KDR* is a good predictive biomarker to discriminate malignant from non-malignant tissues in CRC, due to the highest level of AUC and specificity. Meanwhile, the 85.1% sensitivity of *FLT1* expression offers promise in clinical applications.

As a next step, we recommend the assessment of more extended gene networks from related pathways to improve our current knowledge of CRC. Studying a larger sample size as well as investigating these expression alterations at the protein level may also be beneficial.

In conclusion, we demonstrated up-regulated *FLT1* and *KDR* expression in CRC tissues compared with healthy adjacent samples. Understanding such dysregulated expression in critical cancer pathways, such as angiogenesis, can broaden our current knowledge of precise mechanisms in CRC to improve disease diagnosis and patient treatment.

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References

1. Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi REM, Corcione FJUis. Worldwide burden of colorectal cancer: a review. 2016;68(1):7-11.
2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray FJG. Global patterns and trends in colorectal cancer incidence and mortality. 2017;66(4):683-91.
3. De la Chapelle AJNRC. Genetic predisposition to colorectal cancer. 2004;4(10):769.
4. Jeon J, Du M, Schoen RE, Hoffmeister M, Newcomb PA, Berndt SI, et al. Determining risk of colorectal cancer and starting age of screening based on lifestyle, environmental, and genetic factors. 2018;154(8):2152-64. e19.
5. Zhang S-D, McCrudden CM, Meng C, Lin Y, Kwok HFJO, therapy. The significance of combining VEGFA, FLT1, and KDR expressions in colon cancer patient prognosis and predicting response to bevacizumab. 2015;8:835.
6. Song EK, Tai W, Messersmith WA, Bagby S, Purkey A, Quackenbush KS, et al. Potent antitumor activity of cabozantinib, ac-MET and VEGFR 2 inhibitor, in a colorectal cancer patient-derived tumor explant model. 2015;136(8):1967-75.
7. Goh V, Padhani AR, Rasheed SJTLO. Functional imaging of colorectal cancer angiogenesis. 2007;8(3):245-55.
8. Mohamed AH, Said NMJJogc. Immunohistochemical Expression of Fatty Acid Synthase and Vascular Endothelial Growth Factor in Primary Colorectal Cancer: a Clinicopathological Study. 2018:1-8.
9. Stacker S, Achen MJB. Emerging roles for VEGF-D in human disease. 2018;8(1):1.
10. Huang Y, Huang Y, Liu D, Wang T, Bai GJO. Flt-1-positive cells are cancer-stem like cells in colorectal carcinoma. 2017;8(44):76375.
11. Cicatiello V, Apicella I, Tudisco L, Tarallo V, Formisano L, Sandomenico A, et al. Powerful anti-tumor and anti-angiogenic activity of a new anti-vascular endothelial growth factor receptor 1 peptide in colorectal cancer models. 2015;6(12):10563.
12. Dong G, Guo X, Fu X, Wan S, Zhou F, Myers RE, et al. Potentially functional genetic variants in KDR gene as prognostic markers in patients with resected colorectal cancer. 2012;103(3):561-8.
13. Shibuya M, Claesson-Welsh LJEcr. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. 2006;312(5):549-60.
14. Ghafouri-Fard S, Oskooei VK, Omrani MD, Taheri MJJoad. Dysregulation of cytokine coding genes in peripheral blood of bipolar patients. 2019;256:578-83.
15. Mazdeh M, Zamani M, Eftekharian MM, Komaki A, Arsang-Jang S, Taheri M, et al. Expression analysis of vitamin D receptor-associated lncRNAs in epileptic patients. 2019:1-9.
16. Wang Q, Zhao X, Yan H, Kang F, Li Z, Qiao Y, et al. A cross-talk EGFR/VEGFR-targeted bispecific nanoprobe for magnetic resonance/near-infrared fluorescence imaging of colorectal cancer. 2018;8(3):1008-17.
17. Valverde A, Peñarando J, Cañas A, López-Sánchez LM, Conde F, Hernández V, et al. Simultaneous inhibition of EGFR/VEGFR and cyclooxygenase-2 targets stemness-related pathways in colorectal cancer cells. 2015;10(6):e0131363.
18. Fong TAT, Shawver LK, Sun L, Tang C, App H, Powell TJ, et al. SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. 1999;59(1):99-106.
19. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. 2005;438(7069):820.
20. Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, et al. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. 2002;2(4):289-300.
21. Marcucci F, Stassi G, De Maria RJNrDd. Epithelial–mesenchymal transition: a new target in anticancer drug discovery. 2016;15(5):311.

22. Ye X, Fan F, Bhattacharya R, Bellister S, Boulbes DR, Wang R, et al. VEGFR-1 pseudogene expression and regulatory function in human colorectal cancer cells. 2015.
23. Fallah A, Sadeghinia A, Kahroba H, Samadi A, Heidari HR, Bradaran B, et al. Therapeutic targeting of angiogenesis molecular pathways in angiogenesis-dependent diseases. 2019;110:775-85.
24. Khan K, Cunningham D, Chau IJCdt. Targeting angiogenic pathways in colorectal cancer: complexities, challenges and future directions. 2017;18(1):56-71.
25. Mashreghi M, Azarpara H, Bazaz MR, Jafari A, Masoudifar A, Mirzaei H, et al. Angiogenesis biomarkers and their targeting ligands as potential targets for tumor angiogenesis. 2018;233(4):2949-65.
26. Loaiza-Bonilla A, Jensen CE, Shroff S, Furth E, Bonilla-Reyes PA, Deik AF, et al. KDR mutation as a novel predictive biomarker of exceptional response to regorafenib in metastatic colorectal cancer. 2016;8(2).
27. Cârţână E-T, Gheonea DI, Cherciu IF, Streaţă I, Uscatu C-D, Nicoli E-R, et al. Assessing tumor angiogenesis in colorectal cancer by quantitative contrast-enhanced endoscopic ultrasound and molecular and immunohistochemical analysis. 2018;7(3):175.
28. Zhu G, Huang Q, Zheng W, Huang Y, Hua J, Yang S, et al. LPS upregulated VEGFR-3 expression promote migration and invasion in colorectal cancer via a mechanism of increased NF-κB binding to the promoter of VEGFR-3. 2016;39(5):1665-78.