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Correlation between cyclooxygenase-2 expression and body mass index on prognosis and survival of colorectal cancer of Egyptian patients

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SUMMARY

Introduction: The biologic behavior of colorectal cancer is complicated. In clinical studies, elevation ofcyclooxygenase-2 (COX-2) expression was detected in colorectal cancers when compared to the adjacent normal colonic mucosa. Moreover, obesity has been reported to cause 20% of all malignancy and contributed to almost 11% of colorectal carcinoma. Egypt Demographic Survey (2008) reported high rate of obesity among Egyptians. Both COX-2 overexpression and obesity support the different mechanisms of association between chronic inflammation and carcinogenesis.

The association between clincopathological characteristics and COX-2 overexpression and Body Mass Index (BMI) together with their effect on overall survival in Colorectal Carcinoma (CRC) will be evaluated in this study.

Methods: Seventy-one non-metastatic CRC patients were prospectively included in this study. Those patients diagnosed, treated and followed up at Tanta University Hospitals. Immunohistochemistry was used to assess COX-2 expression. Body mass index was calculated at time of presentation. COX-2 expression and BMI were reported and compared with other clinicopathological criteria. Survival was assessed and compared by Kaplan-Meier curves and log-rank test

Results: COX-2 overexpression was detected in 49.3% of patients and significantly correlated with tumor grade (p=0.003), lymph node metastasis (p<0.001), BMI>30kg/m^2 (p=0.001) and gender (p<0.001).

Univariate analysis revealed that COX-2 overexpression, higher grade of tumor, and high preoperative CEA status, were significantly associated with shorter overall survival. While in multivariate analysis, high COX-2 expression and tumor grade remained statistically significant with overall survival in [p =0.048; 95%CI (1.010: 12.757); HR=3.59) and p=0.034; 95%CI (1.086: 8.153); HR=2.976) respectively].

In this study, BMI>30kg/m2was reported in 53.5% of CRC cases and significantly correlated with older patients (p=0.015), femalegender (p =0.003) and lymph node involvement (p<0.001). However, no significant correlation was detected between high BMI and overall survival by univariate analysis.

Conclusion: Our study showed that COX-2 overexpression in addition to obesity, significantly associated with tumor characteristics and survival of CRC patients.

Key words: colorectal cancer, BMI, COX-2 over-expression

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INTRODUCTION

Colorectal cancer is one of the most common and lethal cancer in the world [1]. Colorectal cancer has a complicated biologic manner. The most effective determinants of CRC prognosis are stage, tumor grade, extramural extension, lymphvascular and perineural invasion, status of preoperative Carcinoembryonic Antigen (CEA), RAS and BRAF mutations and microsatellite instability [2].

Cyclooxygenase-2 (COX-2) is an enzyme, which is up-regulated in response to cytokines, tumors promoting factors and growth factors and reported to be over-expressed in inflammations and malignant tumors [3]. COX-2 suppresses apoptosis and stimulates cell proliferation, this carcinogenic effect has been evaluated in numerous cancers such as breast, lung, esophagus, and CRC [4-7].

Clinical Studies showed the rising of COX-2 expression incolon cancer when compared to the normal adjacent colonic mucosa [7-9]. Cyclooxygenase-2 over-expression provides a valuable prognostic impact for CRC and predicting the higher risk for recurrence [10].

Additionally, obesity has been reported to cause 20% of all malignancy and contributed to almost 11% of colorectal carcinoma [11]. Egypt Demographic Survey (2008), reported high rate of obesity among Egyptian people especially Egyptian women (65%-80%) [12].

Metabolic changes in sex hormone metabolism, adipokine, insulin, and insulin-like growth factor levelsmay predispose to cancer progression in obese persons [13].

The impact of COX-2 overexpression and Body Mass Index (BMI) on overall survival in CRC and their association with other clinic-pathological features will be evaluated in this study.

MATERIALS AND METHODS

This prospective study included 71 cases of CRC diagnosed, treated, followed up at the clinical oncology department, Tanta university hospitals, Egypt, from January 2015 to April 2019. Clinicopathological data were obtained and included gender, age, tumor type, size, site, grade, lymph vascular invasion,

lymph nodes status. Body mass index was calculated at time of presentation (BMI=kg/m^2).

The inclusion criteria were histologically confirmed invasive colorectal adenocarcinoma. While the exclusion criteria were in-situ lesions, recurrences and metastatic disease. The entire patient underwent a colorectal surgical approach regarding tumor criteria. Tumor stage, histologic diagnosis, and grading of each patient were evaluated. Postoperative adjuvant treatment and follow up were administered according to published guidelines.

The study was approved by the local ethical committee, and all the participants gave informed consent for use of their Formalin-fixed, paraffin-embedded specimens.

Therapeutic approach

Adjuvant chemotherapy: Patients with high-risk stage II and stage III colon cancer received 6 months FOLFOX4 (oxaliplatin 85mg/m^2, leucovorin 200mg/m^2,5 FU 400mg/m^2 bolus, 5 FU 600mg/m^2 22h infusion every 2 weeks) or XELOX (oxaliplatin130 mg/m^2, capecitabine 1000mg/m^2 twice daily every 3 weeks). For obese patients, doses of chemotherapy were calculated with capping the maximal Body Surface Area (BSA) to 2.0m^2 because of concern for excess toxicity.

Combined chemotherapy and external beam radiotherapy: chemoradiation with three or four-field technique using three-dimensional conformal RT (50 Gy-1.8 Gy/fx with capecitabine 850 mg/m^2 twice daily) was given as neoadjuvant or adjuvant therapy in stage II and III rectal cancer.

Immunohistochemistry and Staining

Sections were deparaffinized followed by rehydration, and after that heated in citrate buffer (0.01 M [pH 6.5]) for 9 minutes in an 800-watt microwave oven for antigen retrieval. 2% hydrogen peroxide was used for treating The sections for 10 minutes to inactivate endogenous peroxidase then blocked with 3% normal goat serum in 0.2 M Phosphate-Buffered Saline (PBS) (pH 7.4), For COX2 immunostaining: Two to three drops of COX2 rabbit polyclonal antibody (abcam 15191 1:100 dilution) were placed on each slide, with an overnight incubation at room temperature in the humidity, then Slides were washed for 5 min with PBS visualization obtained by streptavidin biotin ABC detection kit (Catalog#TA-015-HP, Lab-Vision CorporationFremont, USA) and counterstained with hematoxylin.

The immunostained sections were evaluated at a high power of the light microscope. Positive Immunoreactivity was estimated using an arbitrary semiquantitative four-step scoring system (0-3), based on the intensity of cytoplasmic staining:

- 0 (Negative): no cytoplasmic COX2 staining
- 1+: Weak cytoplasmic COX2 staining
- 2+: Moderate cytoplasmic COX2 staining
- 3+: Strong cytoplasmic COX2 staining

For statistical analysis, we combined the cases that scored as 0 and +1 (low score) and compared them to the cases that scored as 2+ and 3+ (high score) (Figure 1).

STATISTICAL ANALYSIS

IBM SPSS Statistics 20.0 software (IBM, lnc., Chicago, Illinois, USA) was used for statistical analysis. The associations of the clinicopathological data and COX-2 were performed using Chi-square. Survival was assessed and compared using KaplanMeier curves and log-rank test. Multivariate analysis using the Cox regression hazard model was used. p-values<0.05 were considered being significant.

RESULTS

Seventy-one patients, who diagnosed pathologically to have invasive colorectal adenocarcinoma, were included in this prospective study. The median age was 55 years (range 30-80). Female patients represented 52.1% of cases. Tumor size >5 cm was reported in 29 patients (40.8%). Thirty-one patients (43.7%) had lymph node metastases. Left colon tumors were reported in 39.4%. Clinicopathological features were gathered (Table1).

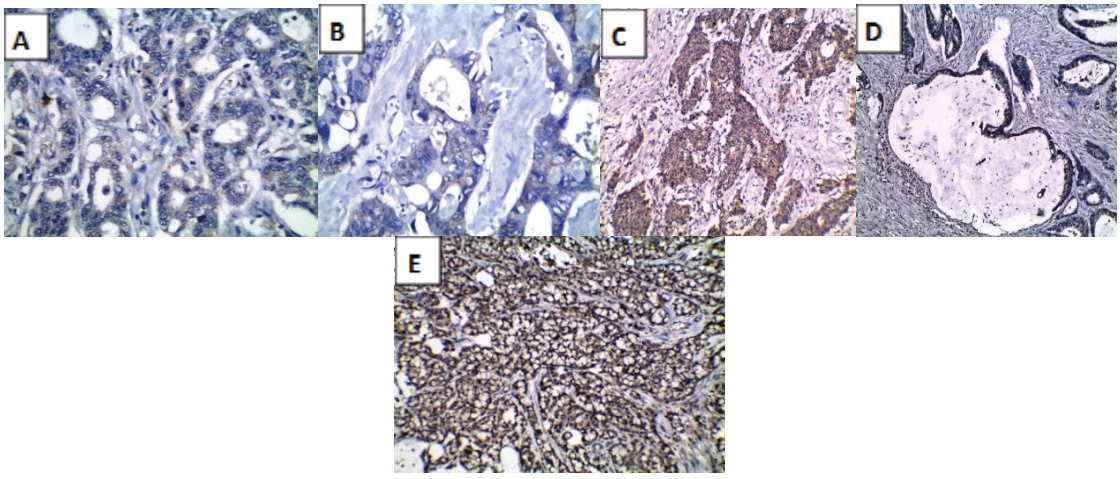

Fig. 1. (A): Well differentiated adenocarcinoma (grade I) showed mild positive expression of COX2 immunostaining (X400); **(B):** Moderately differentiated adenocarcinoma (grade II) showed moderate positive expression of COX2 immunostaining (X400); **(C):** Poorly differentiated adenocarcinoma (grade III) showed strong positive expression of COX2 immunostaining (X200); **(D):** Mucoid adenocarcinoma showed mild positive expression of COX2 immunostaining (200); **(E):** Signet ring adenocarcinoma showed strong positive expression of COX2 immunostaining (X200)

Tab. 1. Patient characteristics

Characteristics of all population	(n=71)	%
Age group		
≤ 55	28	39.4
>55	43	60.6
Gender		
Male	34	47.9
Female	37	52.1
Tumor size		
≤ 5 cm	42	59.2
>5 cm	29	40.8
Depth of invasion		
T1-T2	31	43.7
T3	29	40.8
T4	11	15.5
Lymph node involvement		
N0	40	56.3
N1-N2	31	43.7
Tumor location		
Right hemicolon	27	38
Left hemicolon	28	39.4
Rectum	16	22.5
Tumor grade		
G1-G2	52	73.2
G3	19	26.8
Lymph vascular invasion		
Absent	48	67.6
Present	23	32.4
CEA status		
Negative	53	74.6
Positive	18	25.4
COX-2 expression		
low	36	50.7
high	35	49.3
BMI		
>30 kg/m^2	38	53.5
≤ 30 kg/m^2	33	46.5

Tab. 2. Correlation of patient characteristics and COX-2 expression

Population Characteristics (N=70)	COX-2 expression				p
	Low (36)		High (35)		
	no	%	no	%	
Age group					
<55	16	44.4	12	34.3	0.381
≥ 55	20	55.6	23	65.7	
Gender					
Male	29	80.6	5	14.3	<0.001*
Female	7	19.4	30	85.7	
Tumor size					
≤ 5cm	24	66.7	18	51.4	0.192
>5cm	12	33.3	17	48.6	
Depth of invasion					
T1-T2	21	58.3	10	28.6	
T3	11	30.6	18	51.4	0.041*
T4	4	11.1	7	20	
Lymph node involvement					
N0	29	80.6	11	31.4	<0.001*
N1-N2	7	19.4	24	68.6	
Tumor location					
Right hemicolon	13	36.1	14	40.0	
Left hemicolon	16	41.7	14	40.0	0.940
Rectum	8	22.2	7	20.0	
Tumor grade					
G1-G2	32	88.9	20	57.1	0.003*
G3	4	11.1	15	42.9	
Lymph vascular invasion					
Absent	25	69.4	23	65.7	0.737
Present	11	30.6	12	34.3	
CEA status					
Negative	30	83.3	23	65.7	0.088
Positive	6	16.7	12	34.3	

BMI					
>30 kg/m²	12	33.3	26	74.3	0.001*
≤ 30 kg/m²	24	66.7	9	25.7	
*p value less than 0.05					

Tab. 3. Correlation of patient characteristics and BMI

Population Characteristics (N=70)	BMI				p
	>30 kg/m² (38)		≤ 30 kg/m² (33)		
	no	%	no	%	
Age group					
< 55	10	26.3	18	54.5	0.015*
≥ 55	28	73.7	15	45.5	
Gender					
Male	12	31.6	22	66.7	< 0.003*
Female	26	68.4	11	33.3	
Tumor size					
≤ 5cm	20	52.6	22	66.7	0.230
>5cm	18	47.4	11	33.3	
Depth of invasion					
T1-T2	10	26.3	21	63.6	0.007*
T3	20	52.6	9	27.3	
T4	8	21.1	3	9.1	
Lymph node involvement					
N0	14	36.8	26	78.8	<0.001*
N1-N2	24	63.2	7	21.2	
Tumor location					
Right hemicolon	17	44.7	10	30.3	0.446
Left hemicolon	13	34.2	15	45.5	
Rectum	8	21.1	8	24.2	
Tumor grade					
G1 -G2	25	65.8	27	81.8	0.128
G3	13	34.2	6	18.2	
Lymph vascular invasion					
Absent	27	71.1	21	63.6	0.505
Present	11	28.9	12	36.4	
CEA status					
Negative	25	65.8	28	84.8	0.066
Positive	13	34.2	5	15.2	
*p value less than 0.05					

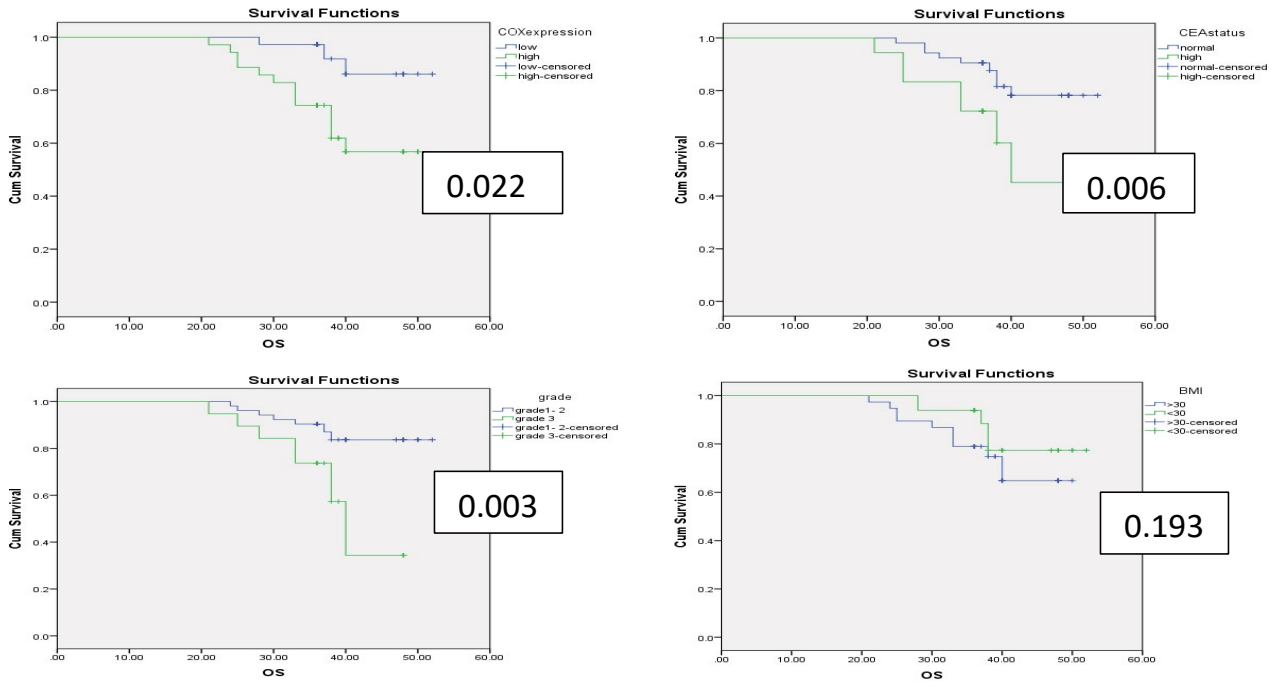

Fig. 2. overall survival in relation to prognostic features

Tab. 4. Clinicopathologic variables effects on overall survival rate by univariate and multivariate analysis

Population Characteristics (N=70)	Univariate			Multivariate		
	3-year OS (%)	95% CI	p	HR	95% CI	p
Age group						
< 55	71.6	0.71: 6.84	0.155			
≥ 55	69.2					
Gender						
Male	78.4.	0.74: 6.17	0.142			
Female	63.4					
Tumor size						
≤ 5cm	84.3	0.95: 7.21	0.050			
>5cm	50.6					
Depth of invasion						
T1-T2	78.5.					
T3	66	0.874:3.63	0.259			
T4	72					
Lymph node involvement						
N0	75	0.47: 3.47	0.128			
N1-N2	68					
COX-2expression						
Low	86.1	1.37: 16.92	0.006*	3.590	1.010: 12.757	0.048*
High	56.7					
Tumor location						
Right hemicolon	55.6					
Left hemicolon	89.3	0.30: 1.25	0.053			
Rectum	53.3					
Tumor grade						
G1-G2	74.8	1.46:10.64	0.003*	2.976	1.086: 8.153	0.034*
G3	34.4					
Lymph vascular invasion						
Absent	71.4	0.59: 5.79	0.271			
Present	72.9					
CEA status						
Negative	78.2	1.10: 8.10	<0.022*	2.343	0.866: 6.340	0.094
Positive	45.1					
BMI						
>30 kg/m^2	64.8	0.17:1.45	0.193			
≤ 30 kg/m^2	77.4					

*p value less than 0.05

COX-2 over-expression was detected in 49.3% of patients and significantly correlated with tumor grade (p=0.003), lymph node metastasis (p<0.001), BMI >30kg/m^2 (p=0.001), depth of invasion (p <0.041) and gender (p<0.001) (Table 2).

The BMI ≥ 30 kg/m^2 was reported in 53.5% of cases and significantly correlated with older patients (p=0.015), female gender (p=0.003), lymph node involvement (p<0.001) and depth of invasion (p=0.007) (Table 3).

The estimated mean overall survival was 46 months. The 3 year OS rate was 71%. The relation of overall survival to prognostic features was clarified in (Figure 2).

Univariate analysis revealed that COX-2 overexpression, higher grade of tumor and high preoperative CEA status, were significantly associated with shorter overall survival. High COX-2 expression and tumor grade remained statistically significant with overall survival in multivariate analysis [p =0.048; 95%CI (1.010: 12.757); HR=3.59) and p=0.034; 95%CI (1.086:8.153); HR=2.976) respectively (Table 4).

DISCUSSION

The cyclooxygenase-2 over-expression suppresses apoptosis and stimulates cell proliferation in numerous cancers including CRC [7].

Clinical studies showed that treatment with non-steroidal anti-inflammatory drugs, for example, acetyl salicylic acid, orcyclooxygenase inhibitors like celecoxib, participated in a reduction of CRC risk [14].

COX-2 level appears to have a significant prognostic value for CRC as well as predicting the higher risk for recurrence [15, 16].

The tumor development and progression by Cox-2 gene cause is still under study. Apoptosis inhibition is one of mechanisms that Cox2 can cause tumor progression [7, 17], in addition to angiogenesis [18] and local immunosuppression [19, 20]. So in our study we try to search the high body mass index as causative factor of COX 2 overexpression.

In our trial, COX-2 overexpression was significantly correlated with tumor grade (p =0.003), in agreement with Kazem et al., who reported statistically significant association of tumor grade and COX-2 positivity scores [10]. Our results revealed that Cox-2 significantly associated with depth of invasion and lymph node metastasis, matching with similar studies [21, 22].

Females represented 52.2% of all populations in our study and the expression of COX-2 was significantly higher in female patients. Other trials didn't show this correlation, maybe due to lower number of female than male in these studies [10, 22]

BMI ≥ 30 kg/m^2 was significantly correlated with older patients and female gender in keeping with Steele et al. [23].

The correlation of COX-2 expression and BMI wasn't discussed in previous clinical trials, but we reported a significant association between high COX-2 level and BMI ≥ 30 kg/m^2. This correlation can be explained by the role of Cyclooxygenase-2 which is up-regulated in response to cytokines and over-expressed in inflammations and malignant tumors. On the other hand, obesity is linked with rising of inflammation markers such as C-reactive protein and proinflammatory cytokines and oxidative stress [24-26].

Univariate and multivariate analysis in this study revealed that COX-2 overexpression is associated with shorter *survival. Similar results were reported in other trials [27, 28]. Interestingly, no significant correlation was detected between high BMI and overall survival.*

Several studies evaluated the BMI at the time of diagnosis in relation to outcome in CRC patients. Conflicting results were reported, including, no association [29, 30] or increased overall survival with obesity [31].

CONCLUSION

Our study showed that COX-2 overexpression and obesity significantly associated with tumor characteristics and survival in CRC patients that in turn could tailor therapy design, dose calculation and toxicity.

CONFLICT OF INTEREST

The authors clarify that they don´t have an interest in conflict.

REFERENCES

1. SEER Stat Fact Sheet; colon and rectum. National Cancer Institute. 2016.
2. Jessup JM, Goldberg RM, Asare EA. Colon and Rectum. In: AJCC cancer staging manual, 8th, Amin MB (Ed), AJCC, Chicago. 2017.
3. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. Role of cyclooxygenase-2 in cell proliferation and cell death inhuman malignancies. Int J Cell Biol. 2010;2010:1-21.
4. Denkert C, Winzer KJ, Hauptmann S. Prognostic impact of cyclooxygenase-2 in breast cancer. Clin Breast Cancer. 2004;4:428-433.
5. Mascaux C, Martin B, Paesmans M, Berghmans T, Dusart M, et al. Has COX-2 a prognostic role in non-small-cell lung cancer? A systematic review of the literature with metaanalysis of the survival results. Br J Cancer. 2006;95:139-145.
6. Li L, Zhao J, Wu Z, Wang G, Chen G. Meta-analysis: clinicopathological and prognostic significance of cyclooxygenase-2 expression on oesophageal squamous cell carcinoma. Aliment Pharmacol Ther. 2009;30:589-596.
7. Sheng H, Shao J, Kirkland SC, Isakson P, Coffey RJ, et al. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase 2. J Clin Invest. 1997;99:2254-2259.
8. DuBois RN, Radhika A, Reddy BS, Entingh AJ. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. Gastroenterol. 1996;110:1259-1262.
9. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterol. 1994;107:1183-1188.
10. Kazem A, El Sayed K, El Kerm Y. Prognostic significance of COX-2 and β-catenin in colorectal carcinoma. Alexandria J Med. 2014;50:211-220.
11. Wolin KY, Carson K, Colditz GA. Obesity and cancer. Oncol. 2010;15:556-565.
12. EL-Zanaty F, Way A. Egypt Demographic and Health Survey, 2008. Cairo, Egypt. Ministry of Health, El-Zanaty and Associates, and Macro International. 2009.
13. Gallagher EJ, LeRoith D. Obesity and Diabetes: The increased risk of cancer and cancer-related mortality. Physiol Rev. 2015;95:727-748.
14. Tougeron D, Sha D, Manthravadi S, Sinicrope FA. Aspirin and colorectal cancer: Back to the Future. Clin Cancer Res. 2014;20:1087-1094.
15. Li Y, Niu Y, Sun Y, Mei L, Zhang B, et al. An apple oligogalactan potentiates the growth inhibitory effect of celecoxib on colorectal cancer. Nutr Cancer. 2014;66:29-37.
16. Kraus S, Sion D, Arber N. Can we select patients for colorectal cancer prevention with aspirin? Curr Pharm Design. 2015;21:5127-5134.
17. Pang LY, Hurst EA, Argyle DJ. Cyclooxygenase-2: A role in cancer stem cell survival and repopulation of cancer cells during therapy. Stem Cells Int. 2016;2016:1-11.
18. Valverde A, Peñarando J, Cañas A, López-Sánchez LM, Conde F, et al. Simultaneous inhibition of EGFR/VEGFR and cyclooxygenase-2 targets stemness-related pathways in colorectal cancer cells. PLoS One. 2015;10:e0131363.
19. Liu B, Qu L, Yan S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. Cancer Cell Int. 2015;15:106.
20. Zelenay S, Reis e Sousa C. Reducing prostaglandin E2 production to raise cancer immunogenicity. Oncoimmunol. 2016;5:e1123370.
21. Soumaoro LT, Uetake H, Higuchi T, Takagi Y, Enomoto M, et al. Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. Clin Cancer Res. 2004;10:8465-8471.
22. Xie R, Yang Y, Zhang H, Hu Liu, Jing Guo, et al. c-Myb and its effector COX-2 as an indicator associated with prognosis and therapeutic outcome in colorectal cancer. J Cancer. 2019;10:1601-1610.
23. Steele CB, Thomas CC, Henley SJ, Massetti GM, Galuska DA, et al. Vital signs: trends in incidence of cancers associated with overweight and obesity- United States, 2005-2014. MMWR Morb Mortal Wkly Rep. 2017;66:1052-1058.
24. Romano M, Guagnano MT, Pacini G, Vigneri S, Falco A, et al. Association of inflammation markers with impaired insulin sensitivity and coagulative activation in obese healthy women. J Clin Endocrinol Metab. 2003;88:5321-5326.
25. Chen J, Wildman RP, Hamm LL, Muntner P, Reynolds K, et al. Association between inflammation and insulin resistance in U.S. nondiabetic adults: results from the Third National Health and Nutrition Examination Survey. Diabetes Care. 2004;27:2960-2965.
26. Doumatey AP, Lashley KS, Huang H, Zhou J, Chen G, et al. Relationships among obesity, inflammation, and insulin resistance in African Americans and West Africans. Obesity. 2010;18:598-603.
27. Jafarian AH, Kermani AT, Esmaeili J, Roshan NM, Seilanian-Toosi M. The role of COX-2 and Ki-67 over-expression in the prediction of pathologic response of rectal cancer to neoadjuvant chemoradiation therapy. Indian J Cancer. 2016;53:548-551.
28. Al-Maghrabi J. Cyclooxygenase-2 expression as a predictor of outcome in colorectal carcinoma. World J Gastroenterol. 2012;18:1793-1799.
29. Liu D, Li Q, Yang Z, Hu X, Qian W, et al. Association of body mass index and smoking on outcome of Chinese patients with colorectal cancer. World J Surg Oncol. 2013;11:271.
30. Yamamoto N, Fujii S, Sato T, Oshima T, Rino Y, et al. Impact of body mass index and visceral adiposity on outcomes in colorectal cancer: Obesity and outcome in colorectal cancer. Asia Pac J Clin Oncol. 2012;8:337-345.
31. Min YW, Kim SA, Lee JH, Kim JY, Chang DK, et al. Overweight is associated with a favorable survival in patients with colorectal cancer: a prospective cohort study in an asian population. Ann Surg Oncol. 2012;19:3460-3464.