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Correlation Between Thymidine Phosphorylase Expression and Sex of Patients in Colorectal Carcinoma

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Abstract: The present study was carried out to determine the TP status in colorectal cancer and to correlate molecular alterations with sex and other clinicopathological findings. Out of 129 surgically resected colorectal cancer patients, 51 tumor samples (23 males, 28 females) were randomly analyzed by immunohistochemical techniques using primary antibody for TP and LSAB2 detection kit. Despite the lack of significant correlation between patients' sex and most other clinicopathological parameters, the mean tumor size in males (5.7174±1.8453 cm) was significantly (p=0.016) more than females (4.4643±1.8453 cm) in this study. Out of 51 postoperative colorectal tumor samples, 24 (47.1%) showed positive TP expression. Unlike other clinicopathological parameters, TP immunostaining was significantly correlated with tumor size (p=0.007), lymphatic invasion (p= 0.036) and sex of patients (p=0.002). The prevalence of TP positive immunostaining was significantly higher in males than females (66.6% versus 33.3%, respectively). Due to the importance of high TP expression in predicting the tumor responses to fluoropyrimidines, the results of the present study possibly show the role of sex hormones in TP expression and angiogenesis. This finding might be important in being considered as a valuable prognostic or predictive marker in clinical settings.

Key words: Colorectal cancer, Thymidine Phosphorylase (TP), sex, angiogenesis

INTRODUCTION

Colorectal cancer is a commonly diagnosed cancer in both men and women. It is estimated that in 2004, 146,940 new cases would be diagnosed and 56,730 deaths from colorectal cancer would occur[1]. It is accepted that the standard regimen in colorectal cancer is the fluoropyrimidine-based chemotherapy and this group of drugs is the most commonly used class of drugs in the treatment of patients with colorectal malignancies[2,3]. Recent studies have shown that the angiogenic factor Thymidine Phosphorylase (TP), also known as platelet-derived endothelial cell growth factor, influences the survival of patients with colorectal tumors[4]. Compared with *in vitro* studies showing that an increased intracellular level of TP in tumor cells treated with 5-FU-based protocols potentiates the activity of 5-FU by converting it to the more cytotoxic nucleoside form, it was found that sometimes patients who had tumors with the higher basal TP expression levels did not respond to 5-FU-based chemotherapy[2,3]. Therefore, the therapeutic interest in TP falls into two categories: Firstly, there are drugs, which are the substrates for TP and for these compounds, enhancers of TP levels and gene delivery of TP, promotes their cytotoxic activities[5]. Since the expression of TP, is strongly associated with clinical outcome for colorectal cancer, the newer colorectal cancer chemotherapy should be targeted to TP[6]. Secondly, TP is identified as a major angiogenic factor which promotes angiogenesis and metastasis of tumors if it is linked to their angiogenic properties[7]. Therefore, the realization that the growth and spread of tumors are dependent on angiogenesis has created new avenues of research designed to help us to better understand cancer biology and to facilitate the development of new therapeutic strategies[8]. Of the identified angiogenic factors,

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PD-ECGF/Thymidine Phosphorylase (TP) has been recognized as one of the most frequently associated proteins with tumor progression and metastasis[9,10].

On the other hand, microvascular endothelial cells express the estrogen receptor and estrogen stimulates endothelial cell proliferation[11]. Estrogen may also promote new blood vessel development by acting directly on microvascular endothelial cells. It is proposed in endometrium that estrogen, by regulating expression and secretion of angiogenic factors by glandular epithelial cells, regulates endometrial angiogenesis[12] .

Nevertheless, it is not clear in the colorectal cancer whether estrogen promotes angiogenesis directly and/or indirectly via expression of angiogenic factors by particular colorectal glandular epithelial cells. As the identification of high TP expression may allow us to understand colorectal tumour biology and to predict the chemotherapy response to fluoropyrimidines with respect to sex of patients and sexual hormones and to use alternative therapeutic strategies, we aimed in the present study to evaluate the importance of this angiogenic factor as prognostic marker and its correlation with sex of patients in a clinical setting.

MATERIALS AND METHODS

Patients' characteristics: The questionnaires of 129 colorectal cancer patients were completed in three different university hospitals of Tehran (Imam Hossein, Shohadaye Tajrish and Imam Khomeini) during the years 2000-2003. Based on questionnaire, patients with prior chemotherapy or radiotherapy before surgery, familial history of colorectal cancer, history of background diseases and/or history of addiction to alcohol and abuse-substances were excluded from immunohistochemical study. Then, tissue samples of 51 patients with colorectal adenocarcinoma were randomly selected for the study. All of included patients were Iranian from different geographical locations within Iran. Based on the designed questionnaire, data were collected for their age at surgery, sex and pathological diagnosis. Histopathological data contained tumor anatomical location, tumor pathological type, tumor size, histological differentiation (malignancy grade), stage and lymphatic invasion.

TP immunohistochemical analyses: As previously described[13], dewaxed and rehydrated tissue sections were subjected to antigen retrieval using microwave oven and boiling citrate buffer (pH = 6.0). Endogenous peroxidase activity and nonspecific binding sites were blocked by incubating sections in 3% hydrogen peroxide in methanol for 30 min and 5% BSA for 60 min, respectively. Sections were then incubated overnight at 4°C with TP mouse monoclonal antibody (P-GF.44C, LabVision Corporation) that recognizes nuclear and cytoplasmic expression of the human TP protein. The primary antibody was used at dilution of 1:100. The results were visualized using the Streptavidine-biotin immunoperoxidase detection kit (LSAB2; Dakocytomation-Denmark) and DAB chromogen (Dakocytomation-Denmark) based on the manufacturer's instruction with necessary modifications. Sections were also counterstained with Meyer's haematoxyline. In each series, a section in which incubation with the primary antibody was omitted used as negative control. The ideal staining conditions were established in our preliminary experiments. Staining was considered negative only after careful examination of the entire tissue section. Quantitation of the intensity and number of positive tumor cells was performed by two independent pathologists (B. M. and M. D.) blinded to the clinical outcome. In cases in which the observers disagreed, the immunohistochemical scoring was repeated to agree on same scoring by both observers. Tumor samples were then classified into four categories based on the cytoplasmic expression of TP. Tumor cells were scored as 3+ if they had strong cytoplasmic staining (>50%), 2+ if they had moderate cytoplasmic staining (25-50%), 1+ if they had mild cytoplasmic staining (5-25%) and 0 if staining was <5% or no staining. According to the guidelines for pathological studies on colorectal carcinoma histological grade and pathological stage of tumors were also determined[14].

Statistical analyses: For the statistical analyses, descriptive data were expressed as the mean±SD. To compare means of continuous variables, independent sample T test, to compare discontinuous variables Man Whitney U test and to determine the correlation between molecular features and clinicopathological parameters the spearman's correlation test were performed using SPSS12 software[15]. The correlation between scores of TP expression and tumor anatomical location, size, histological differentiation, lymphatic and secondary organ metastasis, stage, as well as patients' sex and age at surgery were statistically evaluated.

RESULTS

Patients' characteristics: This study included 129 postoperative colorectal carcinoma, 69 (53.5%) males and 60 (46.5%) females. The tumor anatomical location was 60 (46.5%) in colon and 69(53.5%) in rectum. The mean

Table 1: Clinicopathological features of colorectal cancer patients

Age	54.4 year±15.28	Tumor size	5±1.84 cm
(mean±SD)	(range: 15-85)	(mean±SD)	(range: 0.5-9)
Sex		Lymphatic invasion	
Male	23 (45.1%)	Positive	24 (47.1%)
Female	28 (54.9%)	Negative	27 (52.9%)
Tumor anatomical level		Duke stages	
Colon	32 (62.7%)	Stage A	3 (5.9%)
Rectum	19(37.3%)	Stage B1	2(3.9%)
Histological Grade			
I	23 (45.1%)	Stage B2	22(43.1%)
II	22 (43.1%)	Stage C1	2(3.9%)
III	3 (5.9%)	Stage C2	10(19.6%)
IV	3 (5.9%)	Stage D	12(23.5%)
Secondary organ metastasis			
Positive	16 (31.4%)		
Negative	35 (68.6%)		

Table 2: Correlation between sex and clinicopathological features of patients

Variables	Males (n=23)	Females (n=28)	p-value
Mean of age	58.2±12.6	52.1±16.2	0.137
Tumor anatomical level			
Colon	12	20	0.161
Rectum	11	8	
Histological grade			
I	9	14	0.655
II	12	10	
III	0	3	
IV	2	1	
Tumor pathological stage			
A	0	3	0.117
B1	1	1	
B2	9	13	
C1	1	1	
C2	5	5	
D	7	5	
Lymphatic invasion			
Positive	13	11	0.228
Negative	10	17	
Mean of tumor size	5.7±1.81	4.47±1.7	0.014*
Secondary organ metastasis			
Positive	10	6	0.095
Negative	13	22	

* = Significant

age of these patients was 55.6 year±13.31 (range: 15-85). No significant difference were observed between the mean age of females 54.91±14.3 when compared to the mean age of males 56.42±12.5 (p=0.525).

Clinicopathological features: As described in materials and methods, 51 patients with lack of prior chemotherapy or radiotherapy before surgery, familial history of colorectal cancer, history of background diseases and/or history of addiction to alcohol and abuse-substances were selected for immunohistochemical studies. In randomly selected patients the distribution of tumor anatomical level was 32 (62.7%) in colon and 19 (37.3%) in the rectum. The mean macroscopic size of the resected tumors was 5 cm (range: 0.5-9). The carcinomas were 23 (45.1%) well differentiated, 22 (43.1%) moderately differentiated, 3 (5.95%) poorly differentiated and 3 (5.9%) undifferentiated. More than half of the samples 27 (52.9%) were also lymph node negative and 24 (47.1%) of these samples had positive lymphatic invasion (Table 1). Among these cases, 16 patients (31.4%) had also secondary organ metastasis.

Relationships between sex and clinicopathological features of patients: Despite the lack of significant difference between patients' sex and age (p=0.137), tumor anatomical level (p=0.161), histological grade (p=0.655), tumor pathological stage (p=0.117), lymphatic invasion (p=0.228) and secondary organ metastasis (p=0.095) (Table 2) we observed a highly significant difference between males and females with respect to tumor size (p=0.014). The mean tumor size of colorectal tumors in males (5.72±1.84 cm) were significantly higher than females (4.46±1.85 cm).

Results of immunostaining with TP antibody: Out of 51 postoperative colorectal cancer patients, 24 (47.1%) showed TP expression. The distribution of positive

Table 3: Clinicopathological significance of TP (+/-) cases of colorectal cancer

Variables	Negative	1(+)	2(+)	3(+)	p-value
Age					
>54	15	6	4	0	0.366
<54	12	9	4	1	
Sex					
Male	7	9	6	1	0.002**
Female	20	6	2	0	
Tumor location					
Colon	18	10	3	1	0.402
Rectum	9	5	5	0	
Histological grade					
I	14	7	3	0	0.405
II	11	6	4	1	
III	1	1	1	0	
IV	1	0	0	0	
Tumor size					0.007**
>5 cm	11	10	7	1	
<5 cm	16	5	1	0	
Lymphatic invasion					
Positive	11	10	7	1	0.036*
Negative	16	5	3	0	
Secondary organ metastasis					
Positive	8	6	2	0	0.982
Negative	19	9	6	1	
Pathological stage					
A	3	0	0	0	0.276
B1	0	1	1	0	
B2	14	6	2	0	
C1	1	0	1	0	
C2	3	3	3	1	
D	6	5	1	0	

*, ** Represent significant and highly significant, respectively

staining for TP was 1 (2%) with strong staining (3+), 8 (15.7%) with moderate staining (2+) and 15 (29.4%) with mild staining (1+).

Relationship between TP expression and clinicopathological findings: Based on the results of IHC staining, the percent of patients did not differ significantly between negative and positive TP with respect to age (p=0.336), tumor location (p=0.402), histological differentiation (p=0.405), secondary organ metastasis (p=0.982) and pathological stage (p=0.276). Unlike mentioned parameters, TP immunostaining was significantly correlated with tumor size (p=0.007). The prevalence of TP negative staining was significantly higher in tumors with <5 cm of size when compared with tumor size >5 cm (59% versus 41%, respectively). The prevalence of TP positive staining was significantly higher in tumors with size >5 cm when compared with <5 cm (75% versus 25%, respectively) (Fig. 1).

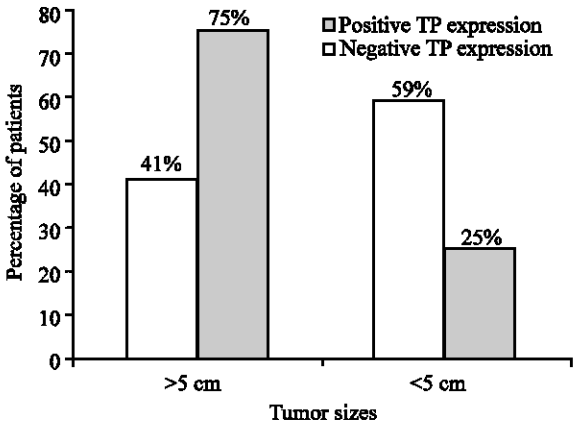

Fig. 1: Status of TP expression with respect to tumor size. The prevalence of TP negative staining was significantly higher in tumors with <5 cm of size when compared with >5 cm of size. The prevalence of TP positive staining was significantly higher in tumors with size >5 cm when compared with <5 cm

TP immunostainings were also correlated with sex of patients (p=0.002) (Table 3). The prevalence of TP negative immunostaining was significantly higher in females than males (74% versus 26%, respectively) and the prevalence of TP positive staining was significantly

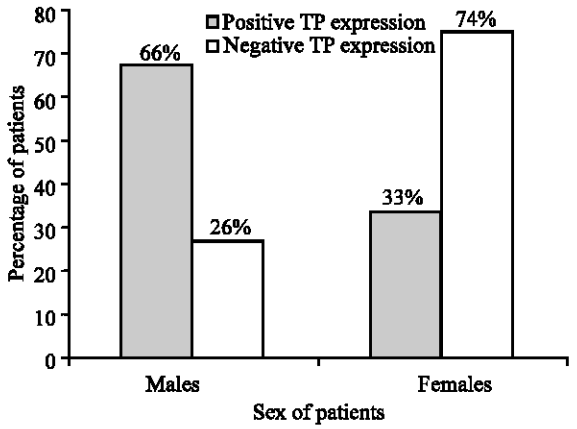

Fig. 2: Status of TP expression with respect to sex. The prevalence of TP negative immunostaining was significantly higher in females than males. The prevalence of TP positive staining was significantly higher in males than females

higher in males than females (66.6% versus 33.3%, respectively) (Fig. 2). A significant correlation were observed between TP expression and lymphatic invasion (p=0.036) (Table 3). Out of 29 lymph node positive patients, 18 (62%) had positive TP staining as opposed to 8 patients (38%) in 24 lymph node negative patients.

DISCUSSION

Colorectal cancer represents one of the best-studied models of tumor angiogenesis. A large number of angiogenic factors have been identified that regulate angiogenesis in colon cancer, including VEGF, b-FGF, TP/PD-ECGF, IL-8, Ang-1, Ang-2, TGF-β, TNF-α and angiogenin[8,16,17]. Among these factors, VEGF and TP/PD-ECGF are the most closely associated with tumor angiogenesis and progression in colon cancer[9]. Analysis of primary human malignancies, including colon, esophageal, gastric, breast, bladder, ovarian and lung cancers showed that TP expression was elevated up to 10 fold in the tumors when compared with the corresponding non-neoplastic regions of the same organs[18]. In the majority of these studies, higher TP expression was linked to increases in angiogenesis, invasion, metastasis and to shorter patient survival[18]. In the present study, close association between TP expression and lymphatic invasion was observed (p=0.036). In studies of GI cancers in which multiple angiogenic factors were examined, TP was found to be an independent prognostic marker of tumor aggressiveness[19,20]. Takahashi *et al.* [21] hypothesized that TP/PD-ECGF drives angiogenesis in colon tumors. Present results showed the correlation between size of colorectal tumors and positive expression of TP/PD-ECGF (p=0.007). It is clear that the growth and spread of tumors are dependent on angiogenesis which is a new avenue of research and potential therapeutic opportunities[8]. A highly significant difference between males and females with respect to tumor size (p=0.016) were observed in our study. The mean tumor size of colorectal tumors in males was significantly more than females. This result indicates the possible different angiogenic inducers in males and females, which could be affected by the sex hormones. However, there is no data about the role of sex hormones on angiogenic process in colorectal cancer. Aoki *et al.*[22] have recently reported that estradiol could induce the expression of TP/PD-ECGF and its mRNA and tamoxifen greatly could diminish the estrogen-induced TP/PD-ECGF in uterine endometrial cancer cells. They reported that the estrogen-induced TP/PD-ECGF might be driven via estrogen receptor cascade. The suggested estrogen-induced TP/PD-ECGF expression was increased approximately two-fold by progesterone and by its metabolite 17 alpha hydroxyprogesterone in well-differentiated endometrial cancer cells, but not with other various steroid hormones[22]. Although we observed a possible negative role of female sex hormones on TP/PD-ECGF expression and tumor size in colorectal tumors but the mean age of female cases were showed their menopause phase of life. Furthermore, the role of male sex hormones is not even understood in relation with angiogenesis. It seems that male hormones can induce TP expression in colorectal carcinoma with larger size.

There is no specific study on the role of sex on angiogenesis and TP expression and optimization of chemotherapy regimen with respect to the sex of patients as well as other factors. Present data suggest the possible role of sex hormones on colorectal tumor growth. Due to significance of high TP expression in predicting the chemotherapy response to fluoropyrimidines[23] and present findings on correlation of TP and sex, it seems to be important to consider the role of sex in choosing chemotherapy regimen for patients.

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