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Progesterone Receptor Positive Colorectal Tumors Have Lower Thymidine Phosphorylase Expression: An Immunohistochemical Study

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Abstract: Present study aimed to find the clinicopathological significance of PgR and its association with TP expression in colorectal cancer. Immunohistochemical studies were performed on 83 colorectal adenocarcinoma patients using corresponding monoclonal antibodies of PgR and TP and LSAB detection kit. Mucin producing cells showed PgR expression and its expression was detected in 15.6% of normal and 59% of malignant tissues. Significant association were observed between PgR negative expression in malignant tissues and larger tumor size ($p = 0.006$), higher incidence of secondary organ metastasis ($p = 0.014$) and tumor pathological stage ($p = 0.041$). A close association was observed between PgR and lack of TP expression in malignant tissues that means out of 49 PgR (+) tumors, 43 cases (86%) were TP negative ($p = 0.046$). It seems that tumors with better prognosis were more likely to express PgR in their malignant tissues, which affects the expression of TP as one of the major therapeutic targets in CRC. Present study suggests progesterone therapy as a possible effective strategy to suppress colorectal cancers and as a novel anti-angiogenic therapy for tumor dormancy which needs complimentary studies for confirmation.

Key words: Progesterone receptor, thymidine phosphorylase, colorectal cancer, angiogenesis, immunohistochemistry

INTRODUCTION

Colorectal Cancer (CRC) is a common malignant disease, accounting for approximately 15% of all human cancers (Giatromanolaki *et al*., 2006). Sex hormone receptors are associated with carcinogenesis and tumor progression of malignant colonic mucosa (Korenaga *et al*., 1998) moreover existing data suggests a hormonal basis for colon cancer (Slattery *et al*., 2000). Although it is suggested that progesterone receptor (PgR) status could be considered as a critical factor for determining the proliferative activity of colorectal cancer tissue (Korenaga *et al*., 1997) but its alterations during tumor progression and tumor angiogenesis are remained unclear. Thymidine Phosphrylase (TP/PD-ECGF) is one of the most closely associated markers of tumor angiogenesis (Reinmuth *et al*., 2003) and chemotherapy response (Giatromanolaki *et al*., 2006). We have recently suggested the possible role of sex hormone receptors on TP expression in CRC (Arbabi Bidgoli *et al*., 2005). Although the effective role of sex steroid receptors on TP expression in uterine endometrium has strengthen our hypothesis (Fujimoto *et al*., 2005) but it is still not clear whether sex steroid receptors especially PgR promotes angiogenesis via expression of TP in CRC or not.

To understand CRC tumor biology and to predict the chemotherapy response to fluoropyrimidines and patient's survival in respect to PgR, we aimed in the present study to evaluate PgR as a prognostic marker and to identify its effect on TP expression.

MATERIALS AND METHODS

Patients: To prepare tissue samples, the questionnaires of all colorectal cancer patients who underwent surgery during the years 1999-2004 were completed in Imam Khomeini university hospital. By completed questionnaires, the clinicopathological features of patients were stated and shown in Table 1. Based on the questionnaires, patients with prior history of chemotherapy; radiotherapy and familial history of colorectal cancer were excluded from present study. During the next step, tissue samples of 83 patients with sporadic colorectal adenocarcinoma were selected for

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Table 1: Clinicopathological feature of CRC patients (n = 83)

Age	54.2±15.61 year
(Mean±SD)	(range: 18-89)
Sex	
Male	40 (48.2%)
Female	43 (51.8%)
Tumor size: (cm)	6.4±4.42 cm
(Mean±SD)	(range: 1-35)
Tumor location level	
Colon	33 (44.6%)
Rectum	46 (55.4%)
Histological grade	
G1[1]	19 (25.3%)
G2[2]	52 (69.3%)
G3[3]	4 (5.3%)
Lymphatic invasion	
Positive	47 (58.8%)
Negative	33 (41.3%)
Neural invasion	
Positive	49 (65.3%)
Negative	26 (31.3%)
Vascular invasion	
Positive	26 (33.8%)
Negative	51 (66.2%)
Secondary organ metastasis	
Positive	11 (14.1%)
Negative	67 (85.9%)
Pathological stage	
Stage A	1 (1.3%)
Stage B_1	9 (12.8%)
Stage B_2	23 (29.5%)
Stage C_1	11 (14.1%)
Stage C_2	23 (29.5%)
Stage D	11 (14.1%)

[1]: Well differentiated, [2]: Moderately differentiated, [3]: Poorly differentiated

present study. Case selection was in accord with the ethical committee on human experimentation of Tehran University of Medical Sciences.

Histological evaluation: According to the Ackerman's guidelines (Rosai, 2004) and by studying the H and E stained tissue specimens, histopathological data containing tumor anatomical location, tumor pathological type, tumor size, histological differentiation (malignancy grade), stage, lymphatic invasion, neural invasion and vascular invasion were collected and presented in Table 1.

IHC method: Tissues were deparaffinized and rehydrated before the blocking steps. Following the blocking steps with H202 0.3% and Bovine Serum Albumin (BSA) 1%, the slides were incubated with primary antibodies at 1:50 optimized dilution for PgR (M3569 dakocytomation) and 1:100 for TP (MS-499 lab Vision Corporation) for 30 min. at room temperature. 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. As a positive control, an adenocarcinoma sample known to positively stain for PgR and TP antibodies were included in each staining run.

Scoring method: For each tumor sample normal, malignant and Lymphoid and stromal cells were assessed and scored separately by two expert pathologists who blinded to the clinical outcomes. They performed scoring of the samples using quantization of the intensity and number of positive tumor cells. Each cell type of stained sections was classified into four categories (negative, 1+, 2+, 3+) based on nuclear or cytoplasmic expression of PgR and TP. Staining was considered negative only after careful examination of the entire tissue section. By our scoring criteria, tumors with <5% positive cells considered as negative, 5-25% as 1+ (mild staining), 25-50% as 2+ (moderate staining) and >50% as 3+ (strong staining).

Statistical analyses: Values were expressed as frequency rates or as the mean±standard deviation (SD). To compare means student t test, to assess the association between expressions of markers, nonparametric spearman's rho test, to compare two nonparametric variable non-Mann-Whitney U test and to compare more that two variables Kruskall Wallis H test were used (Rober and Clarke, 2002). Probability values of $0<0.05$ were considered significant. Statistical analysis was performed with SPSS 10 statistical software.

RESULTS

Patient's characteristics: There were no significant difference between the mean age of the male and female patients (56.4 vs. 52, $p = 0.204$). The mean size of tumors were also not significantly different between male and female patients (6.4 vs 6.3, $p = 0.930$) other parameters were also not significantly differed between males and females groups (Table 1).

PgR staining: PgR was localized in the mucinous parts of normal and malignant tissues and its expression decreases when the normal tissues were compared with their corresponding normal malignant tissues (Fig. 1a). Out of 83 colorectal tumor samples, 13 (15.6%) of normal tissues expressed the receptor. This receptor was also expressed in the cytoplasm of 49 (59%) of malignant tissues (Fig. 1b). Figure 2 shows the differential expression of PgR in malignant and normal tissues of CRC cases. No association were observed between the expression pattern of PgR in malignant tissues in respect to

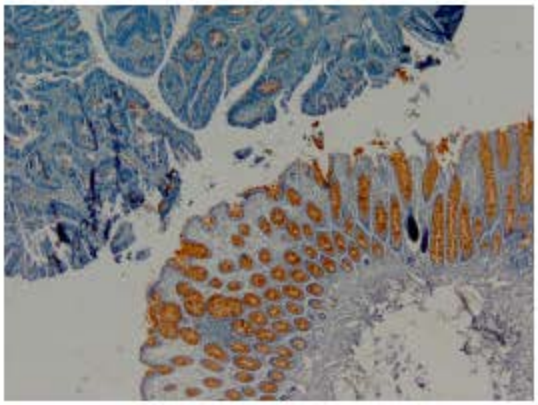

Fig. 1a: Immunohistochemical expression of PgR in normal mucin producing cells of colorectal cancer. PgR expression decreases when the normal tissues compare with their corresponding malignant tissues (Chromogen DAB, x100)

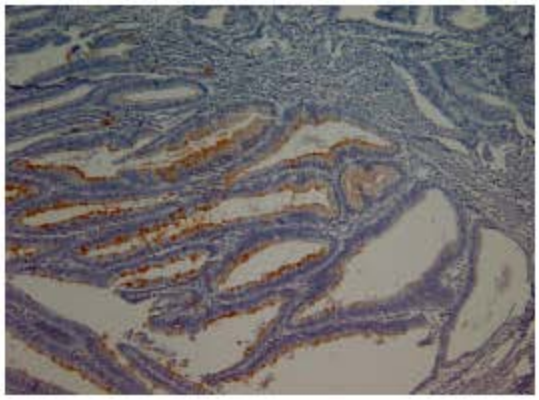

Fig. 1b: Immunohistochemical expression of PgR in malignant mucin producing cells of colorectal cancer (Chromogen DAB, x100)

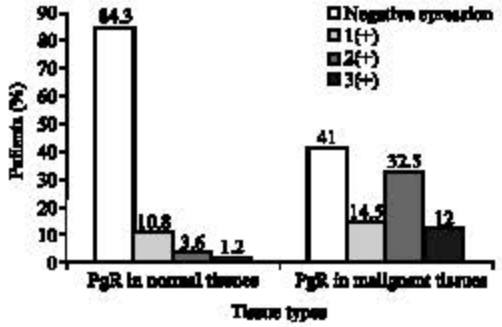

Fig 2: Differential expression of PgR in normal and malignant tissues of colorectal cancer

their corresponding normal tissues (Kruskal Wallis Test p = 0.446). The same results were observed when the expression pattern of PgR in normal and malignant tissues of females (p = 0.346) and males were compared (p = 0.422).

Clinicopathological significance of PgR in malignant tissue: Kruskal Wallis test showed a highly significant associations between PgR expression pattern in malignant tissue and tumor size (p = 0.006). This result was confirmed when we compared the mean tumor size of PgR positive cases (5.5± 3.3 cm) to the mean tumor size of PgR negative cases (7±2.80 cm) (p = 0.05, student t test). Significant associations between PgR expression and Duke stage and secondary organ metastasis was also confirmed by spearman's association test (p = 0.041 and p = 0.014, respectively). That means, PgR losses its expression when the tumor progresses to higher stages. There was also a significant association between PgR expression and secondary organ metastasis that means out of 11 metastatic cases, 9 cases lacked their PgR expression and only 2 metastatic cases showed PgR positivity. Additionally out of 77 non metastatic cases only 22 cases was PgR negative.

When we repeated the same tests in the female group, a significant association was observed between PgR expression and vascular invasion (p = 0.03) but the same association was not observed in the male group (p = 0.387). Despite of male patients significant association was found between PgR expression and neural invasion in female cases (p = 0.047).

TP staining and its clinicop athological significance: TP expression was detected in four different cell types in colorectal cancer cases. The malignant cells loss their TP expression when compared to normal tissues. A highly significant difference were recorded regarding to the expression of TP in malignant and stromal tissues (79.2 vs. 18.8% p = 0.004). Significant association was detected between TP expression in malignant tissues and survival (p = 0.031) and vascular invasion (p = 0.054). Significant association was also recorded between Stromal TP expression and secondary organ metastasis (p = 0.014) and relatively to patient's survival (p = 0.082). No clinicopatholigical significance was detected in respect to TP expression in normal and lymphoid tissues (Table 2).

Associations between TP and PgR expression: The associations between expression of PgR and TP were evaluated as well as their significant immunophenotypes

Table 2: Expression pattern of TP (%) in four different cell types of CRC

	Scores of expression			
TP expression	Negative	1 (+)	2 (+)	3 (+)
Malignant cells	79.2	16.7	3.10	1.0
Normal cells	63.5	34.9	1.60	0.0
Stroma cells	18.8	74.0	7.30	0.0
Lymphatic cells	13.5	70.8	12.50	3.1

in all categories (data is not showed). Significant associations were recorded between TP and PgR expression in malignant tissues ($p = 0.026$) that means the significant immunophenotype was PgR (+)/TP (-) in this respect and also out of 49 PgR (+) tumors 43 cases (86%) were TP negative. This immunophenotype was associated with smaller tumor size (7.47±5.42 vs 5.49±3.24, $p = 0.012$) and lower incidence of secondary organ metastasis ($p = 0.02$). Moreover a significant association was observed between lack of TP expression in malignant tissues and PgR expression in normal tissues ($p = 0.006$).

DISCUSSION

Present study showed obviously that PgR has a tendency to be expressed in mucinous parts of colorectal tissues regardless to the presence of malignancy. Our results also showed lower incidence of PgR expression in tumors with larger tumor sizes when compared by tumors with smaller sizes (7±2.80 vs 5.5±3.3 cm, $p = 0.006$). Moreover significant association between PgR expression and Duke's stage as well as the lack of PgR expression in metastatic cases suggested its protective role on colorectal tumor progression. According to present results significant association between PgR and TP expression (PgR+/TP-) and its association with smaller tumor size (5.49±3.24 vs 7.47±5.42, $p = 0.012$) and lower incidence of secondary organ metastasis ($p = 0.02$) suggested again the protective role of PgR against TP expression.

Although we didn't aim to assess the association between PgR and mucin profile and this relationship was found unexpectedly. Mucins are large glycoproteins which undergo changes in structure and expression in neoplastic transformation. New roles in metastatic disease including a paradoxical protection from immune response and increase in metastatic potential have been recently proposed in colorectal cancer (Nguyen *et al*., 2006). Although negative PgR expression has been recorded among metastatic mucinous carcinomas of gastrointestinal tract with colorectal origin (Russell Vang, 2006) and this study recorded negative PgR expression in most primary tumors with secondary organ metastasis but lack of study on the exact interaction between mucin and PgR during the tumor progression and metastasis is one of the limitations of present study which needs to be clarified by further studies. It seems that this preliminary observation may help to understand the controversial role of PgR in colorectal cancer progression in respect to mucin producing cells.

Earlier studies showed sensitive regulation of TP expression in uterine by ovarian steroids (Ichigo *et al*., 1998; Fujimoto *et al*., 2005) but to date this study is the first study which suggests PgR inhibitory role on TP expression in colorectal cancer like uterine.

Some controversial data exists which considered PgR expression as a feature of the tissue characteristics rather than a consequence of a malignant process (Slattery *et al*., 2000; Oshima *et al*., 1999) moreover one other researches suggests the lack of clinicopathological significance of PgR in colorectal cancer (Raigoso *et al*., 2001), but we are recording for the first time several clinicopathological significance of PgR as well as higher clinicopathological significance of this receptor in females than males. The inhibitory role of PgR on tumor progression is considered as one of the major findings of present study which is in agreement with one previous work (Korenaga *et al*., 1997).

TP is identical to platelet-derived endothelial cell growth factor and high TP expression has been suggested as a hallmark of tumor invasiveness with less response to chemotherapy (Longley *et al*., 2006). According to the present results, the prognostic value of TP was confirmed again and its significant association with PgR expression in malignant tissue (PgR+/TP-) suggested the protective role of PgR against TP expression in tumoral cells.

The valuable role of long term hormone therapy in better prognosis of colorectal cancer is recently reported (Nazeri *et al*., 2006; Schurmann *et al*., 2004). Present data shows a conserve hormone dependency for colorectal cancer, which decreased the angiogenic potential and tumor advancement of colorectal cancer. We suggest progesterone therapy as a possible effective strategy to suppress colorectal cancers and as a novel anti-angiogenic therapy for tumor dormancy which needs complimentary studies for confirmation. This study brought new insight about the treatment of colorectal cancer but further studies are required to show the PgR effects on other angiogenic factors and further clinical studies are also necessary to the find the potential significance of progestin in the treatment of colorectal carcinoma specifically in combination with chemotherapeutic agents which recognize TP as their therapeutic targets.

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