Pathological Study of Renal Cell Carcinoma by Expression of Vascular Endothelial Growth Factor (VEGF) and P53 (Immunohistochemical Approach)

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Abstract

Background: Renal cell carcinomas (RCC) represent about 1-3% of all visceral cancers. There are 30,000 new cases per year and 12,000 deaths from the disease in USA [1].

Aim: To estimate the rate of VEGF & P53 immunohistochemical overexpression in RCC in relation to histological types of tumor.

Methods: Fomalin fixed, paraffin-embedded blocks from 48 patients (28 male and 20 female) with RCC included in this study. A group of 12 patients with non specific inflammation –pyelonephritis were included as a control group. LSAB+ (Labeled Strept-Avidin Biotin) method was employed for immunohistochemical detection of VEGF & P53.

Results: In assessment of the correlation of VEGF overexpression in RCC with histological types, there is correlation between the intensity of VEGF expression & classical type as (R= 0.618) while in other histological types no correlation & no significant difference (p>0.05) between them. In assessment of the correlation of P53 overexpression in RCC there is a negative correlation (R=-0.843) in classical, in papillary (R=-0.674) & no significant difference between them (p>0.05).

Conclusion: these finding provide further evidence for the role of VEGF & P53 in the carcinogenesis of RCC. However both markers not well correlated with histological types of the tumor.

Keyword: VEGF: vascular endothelial growth factor, P53, RCC: renal cell carcinoma.

الخلاصة

سرطان الكلية يمثل 1-3%من الامراض السرطانية للاحشاء هناك 30,000 حالات جديدة في كل سنة و 12,000حالة وفاة بهذا المرض في الولايات المتحدة.

الهدف: تقييم درجة التعبير المناعي الكيميائي النسيجي لعامل نمو ألبطانة ألوعائية VEGF عامل (p53) في سرطان الكلية. تقييم علاقة كلا العاملين مع الانواع النسيجية.

الطريقة: طبقت هذه الدراسة على ثمانية وأربعون حالة مرضية لسرطان الكلية واثنى عشر حالة التهاب الجهاز البولي, استخدمت طريقة +LSAB لتحديد التعبير المناعي النسيجي ل (VEGF وp53).

النتيجة:أثبتت الدراسة أن يوجد فرق ذا مغزى للتعبير المناعي لعامل النمو البطانة الوعائية (VEGF) مع بعض الانواع (القياسي) ولا يوجد فرق ذا مغزى مع بقية الأنواع النسيجية. ولا يوجد فرق ذا مغزى للتعبير المناعي لعامل(p53).

الاستنتاج: النتائج نستنتج الدور المهم لعامل نمو البطانة الوعائية (VEGF) و عاملp53 في تولد نشأة خبيث

Introduction

Renal cell carcinomas (RCC) represent about 1-3% of all visceral cancers. There are 30,000 new cases per year and 12,000 deaths from the disease in USA [1]. RCC accounts 90% of renal cancers in adults [1]. The tumor usually occurs in the 6th-7th decades of life, male to female ratio is 2 to 3:1[2]. Several factors have prognostic abilities included grade, stage, tumor

size, nuclear morphology, and tumor vascularity [3]. Several pathologic subtypes have been described, included the typical (convential; clear &/or granular), papillary, renal medullary carcinoma, chromophobe cell, and collecting (Bellini) duct subtypes [4]. *Clear cell carcinoma* accounts to 70-80% of renal cancers [5].

VEGF is an important signaling protein, involved in both vasculo-genesis and angiogenesis [6]. **VEGF** gene is located on chromosome 6p12. **VEGF** is related to the platelet derived family (PDF) [7]. **P53** is a tumor suppressor gene acting as a key role in the regulation of cell cycle at G1/S regulatory point and the prevention of cancer development [8].

P53 is located in chromosome 17 p13.1. **P53** over expression is present in 10% to 35% of RCC [9]. RCC in relation to histological types of the tumor among a group of patients who referred to Kufa School of medicine teaching hospital for histopathological evaluation.

Material and Method

This study was carried out in the Department of Pathology and Forensic Medicine, Faculty of Medicine, University of Kufa, during the period from September 2009 through June 2010, 48 cases of patients with RCC (28 male & 20 female) & 12 cases of non-specific inflammation pyelone-phritis were subjected to the present investigation.

All cases, whether malignant or benign lesions, were examined by two histopathologists independently and then subjected to the immunohisto-chemical method using the LSAB$^+$ (Labelled Strept-Avidin Biotin) technique. The malignant renal cases were staged according to the TNM (tumor size, lymph node involvement, distant metastasis) staging system [10]. The mean age of the patients was 53.08 years. Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor, 0.2 ml, Clone VG1, Code M7273, LOT 00028659 was used as primary antibody for the detection of VEGF protein & Monoclonal Mouse Anti-Human P53 Protein, 11 ml, Ready-To-Use, DAKO, Clone DO-7, Code N1581, LOT 00005848. The staining kit used was the Ready-To-Use staining kit, Code K0673. The criterion for positive reaction confirming the presence of VEGF protein is dark, brown, intracellular cytoplasmic precipitate, while the criterion for P53 protein is dark, brown, intracellular nuclear precipitate. The intensity of the staining was assessed by using a semiquantative scoring method by counting the percentage of positive cells in 100 malignant cells, which is performed at the objective 40 total magnification. The scoring system according to Sophia KA et al.[11] for P53 (score 0: Negative, none of the cells revealed positivity for the marker; score 1: Weak or mild staining, (5-10%) positive of tumor cells; score 2: Moderate staining, less than 25% of tumor cells are stained positive; score 3: Strong staining, (25-50%) of tumor cells are stained positive; & score 4: Highly strong staining, over 50% of tumor cells are stained positive.), and Raica M et al.[12] for VEGF (Score 0: Negative, none of the cells revealed positivity for the marker; score 1: weak positive, weak reaction in less than 10% of tumor cells; score 2: moderate positive, weak-moderate reaction in (10–50%) of tumor cells; & score 3: intense positive, strong or moderate intensity in more than 50% of tumor cells) . Statistical analyses of all results were performed by the help of SPSS software statistical package

(version 15) using Chi Square test, P value at level of significance less than 0.05, and correlation regression test (R at a significant level of 0.3).

Result

The assessment of intensity of P53 overexpression in malignant renal cells revealed that; 33(68.75 %) cases out of 48 were negative (score 0), 10(20.83 %) cases with score +1, 2 (4.16%) cases with score +2, 2 (4.16%) cases with score +3 and 1 (2.08%) with score +4. The classical type 26(68.43 %) of 38 score 0, 8(21.05%) of 38 score + 1, 2(5.26%) of 38 score + 2, 1(2.63 %) of 38 score + 3, 1(2.63%) out of 38 with score +4, the papillary type 6 (66.67 %) of 9 score 0 , 2(22.22 %) of 9 score +1, 1 (11.11%) out of 9 score + 3,0 in score + 2& +4 and the collecting duct type 1 (100 %) is score 0. There is a negative correlation (R=-0.843) in classical type, in papillary type (R=-0.674) & no significant difference between them (p>0.05) (Table 1) and (Figure 1). The assessment of intensity of VEGF overexpression in malignant renal cells revealed that 25 (52.1 %) cases out of 48 were negative (score 0); 7 (14.58 %) cases with score +1; 4 (8.34%) cases with score +2 and 12 (25%) cases with score +3. The classical type 18 (47.37 %) of 38 score 0; 6 (15.79%) of 38 score 1; 3 (7.89%) of 38 score 2; and 11 (79.17 %) of 38 score 3. While the papillary type 6 (66.67 %) of 9 score 0; score 1, 2, 3 are 1 (11.11 %) of 9 and the collecting duct type 1 (100

%) is score 0. There is correlation between the intensity of VEGF expression & classical type as (R= 0.618) while in other histological types no correlation & no significant difference (p>0.05) between them (Table2) and (Figure 2).

Discussion

Angiogenesis is crucial for tumor development. In assessment of the correlation of VEGF and P53 overexpression in RCC with histological types, there is correlation between the intensity of VEGF expression & classical type as (R= 0.618) while in other histological types no correlation & no significant difference (p>0.05) between them (Table 2). This means that histological types does not play a role in increasing the expression of VEGF, as has been reported elsewhere who found that no significant difference in VEGF expression was noticed in relation to histological types [13]. However further investigation has shown that there were no difference in VEGF expression with histological types [14].

Though the study that had quantitatively assessed vessel area (VA) and expression of VEGF/VEGF-Rs in tumor suggested that no clear differences in expression patterns were seen based on histological subtypes [15], other investigation had suggested that the conventional RCC has significantly lower VEGF and COX-2 levels than those with papillary RCC [16].

Table 1: The correlation between intensity of P53 expression & histological type

Histological types	Immunostaining of p53					Total	R
	Score 0 no stain	Score +1	Score +2	Score +3	Score +4		
Classical	26 (68.43%)	8 (21.05)	2(5.26%)	1(2.63%)	1(2.63%)	38(79.17%)	R=-0.843
Papillary	6(66.67%)	2(22.22%)	0	1(11.11%)	0	9(18.75%)	R=-0.674
Collecting duct	1(100%)	0	0	0	0	1(2.08%)	
Total	33(68.75%)	10(20.83)	2(4.16%)	2(4.16%)	1(2.08%)	48(100%)	

Table 2: The correlation between intensity of VEGF expression & histological type

Types of carcinoma	Score 0 no stain	Score +1	Score +2	Score +3	Total	R
Classical	18 (47.37 %)	6 (15.79 %)	3 (7.89%)	11 (28.95%)	38 (79.17%)	R=0.618
Papillary	6 (66.67%)	1 (11.11%)	1 (11.11%)	1 (11.11%)	9 (18.75%)	
Collecting duct	1 (100%)	0	0	0	1 (2.08%)	
Total	25 (52.08%)	7 (14.58%)	4 (8.34%)	12 (25%)	48 (100%)	
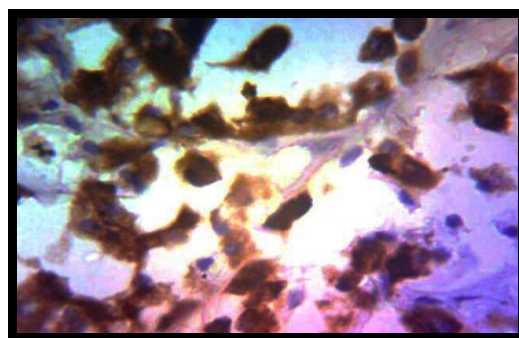
Figure.1: Renal cell carcinoma positive P53 score +4 classical type 40X
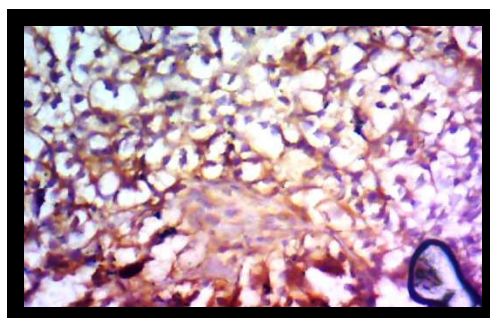
Figure 2: Renal cell carcinoma classical type positive VEGF score +3 10X.

Furthermore**,** the VEGF staining intensity not correlated with any histological types [14]. There was a negative correlation (R=-0.843) in classical type, in papillary (R=-0.674) & no significant difference between them (p>0.05).

This means that histological types have no role in the expression of P53 which is in consistent with previous investigation which found that P53 expression was not associated with cell type or histological pattern [17, 18]. However a group of workers shown that a significant difference in p53 immunoreactivity was found among the histological subtypes [19] & the papillary RCCs showed nuclear p53 overexpression in 70% compared with 27.3% of chromophobe and 11.9% of conventional RCC [19]**.** Indeed it has been suggested that significant difference was found in papillary and chromophobe tumor types but not in conventional RCC [20].

Conclusion

These finding provide further evidence for the role of VEGF & P53 in the carcinogenesis of RCC. However both markers not well correlated with histological types of tumor. Larger study including all types of renal carcinoma for further investigation of VEGF &P53 overexpression is needed.

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