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The Role of Angiogenesis Factor in Bladder Tumors

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Abstract

The study designed to detect Ang-1 expression as angiogenesis factor in bladder tumor tissues, and to clarify the relationship between Ang-1 and clinicopathological grade of bladder cancer. In this study, the expression of Ang-1 in tumor cells was investigated in (30) patients with transitional cell carcinoma of bladder (TCC) and (20) patients with inflammatory lesions of the bladder (BIL) as acontrol group from the histopathology laboratories of Al-Hussain Teaching Hospital and private laboratories in Thi-Qar Province,Iraq . The tissue sections were analyzed immunohistochemically (IHC) to detection of Ang-1. The results showed a high positive immunohistochemical Ang-1 expression of TCC tissues than in BT tissues (76.7 % versus 40%; $p \leq 0.01$). Furthermore, the significantly highest level of Ang-1 positive cells was detected in the groups with the highest clinical grade ($p \leq 0.05$). Ang-1 antigen expression could be indicator of aggressive behavior of bladder cancer.

دور العامل المتعلق بتكوين الأوعية الدموية في أورام المثانة

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الخلاصة

هدفت الدراسة تحديد التعبير البروتيني لـ Ang-1كمعلم حيوي لتكوين الأوعية الدموية الجديدة في أنسجة المثانة الورمية ,وتوضيح العلاقة بينه وبين الدرجات السريرية الامراضية لسرطان المثانة .تم تشخيص التعبير لمعلم Ang-1في خلايا الورم لـ 30مريض يعانون من سرطان المثانة البولية الانتقالي الحرشفي 20 ,مريض يعانون من قرح المثانة البولية الالتهابية (أورام حميدة كمجموعة سيطرة) في مختبر الأنسجة المرضية في مستشفى الحسين التعليمي والمختبرات الخاصة في محافظة ذي قار .تم استخدام تقنية التعبير المناعي الكيمونسيجي لتحديد التعبير البروتيني لـ . Ang-1وقد وجدت الدراسة أن مستوى التعبير المناعي الكيمونسجي الموجب الـ +Ang-1كان مرتفع نسبيا مع فرق معنوي إحصائي في مرضى سرطان المثانة البولية مقارنة مع مرضى أورام المثانة البولية الحميدة .(76.7 % versus 40% ; $p \leq 0.01$)وبينت النتائج أن هناك علاقة معنوية بين الدرجات السريرية الامراضية لمرضى سرطان المثانة البولية والتعبير المناعي الكيمونسيجي للـ . +Ang-1إن التعبير المناعي الكيمونسيجي الموجب لانتيجين +Ang-1في مرضى سرطان المثانة يمكن إن يكون كدليل في التطور أو التصرف الحاد لسرطان المثانة .

Introduction

Bladder cancer (BC) is one of the most prevalent diseases in economically advanced countries. Practically, all bladder cancers initiate in the urothelium, which is a 3- to 7-cell mucosal layer in the interior of the muscular bladder (Lopez-Beltran and Cheng, 2006) .The spectrum of bladder cancer contains muscle-invasive low-and high-risk non-muscle-invasive and metastatic disease (Von Bernstorff *et al*., 2001). Angiogenesis is recognized as important processes that play a key role in the cancer evolution and development of solid tumors further than 1-2 mm in diameter, that is defined the creation of new blood vessels starting from the current vasculature, and neovascularization is an essential for the growth (Folkman,1990). So, through the tumorigenesis, the tumor growth and development reaches a growth-limiting stage when nutrients and oxygen ranks are inadequate to remain the proliferation and propagation. Tumors obtain blood vessels by co-option of bordering vessels. This process comes from vasculogenesis from endothelial precursor cells and in many cases from emergent or intussuscepted microvascular growth (Carmeliet and Jain, 2000). Basically, in most kind of solid tumors, the recently formed vessels are plagued by special functional and structural aberrations due to the sustained and extravagant exposure to angiogenic elements created by the tumor (Jain,2003). The new tumor-associated vasculature is anomalous and inefficient however; it is necessary for the tumor development as well as the metastasis. Even though being abnormal, these new vessels permit tumor growth at primary stages of carcinogenesis and development from in situ lesions to locally invasive, and finally to metastatic tumors. As a result, tumors tend to become hypoxic. The ordinary ways for the cellular response to hypoxia is to create growth factors for instance vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor alpha (TGF-α), and platelet derived growth factor (PDGF). These mechanisms were done by neoplastic, stromal cells or inflammatory cells (Witzenbichler *et al.*,1998), and might prompt an angiogenic switch to permit the tumor to induce the creation of microvessels from the nearby host vasculature (Hanahan and Folkman,1996), that stimulate neoangiogenesis (Kaelin,2005). The function of the Angiopoietins (Ang1 and Ang2) which are established a family of endothelial growth factors is to ligand the tyrosine kinase receptor Tie2 (Hansen *et al.*, 2010). Tie2 is critical factor for vascular progress and maintenance furthermore; it is stated in endothelial cells (ECs) and up controlled in microvessels of tumor (Jones *et al.*, 2001). In vitro, Ang1 elevate EC growth, survival, and migration, while Ang2 prevents the activation of Tie2 that induced by Ang1 (Witzenbichler *et al.*, 1998). Therefore, the recently tumor-associated vasculature is abnormal and passive, but it is vital for tumor development and metastasis. The cancer is a disease described by unbalanced growth and extent of abnormal cell. This process can happen by both external factors and internal factors (American Cancer Society, 2007). In relation to bladder cancer, there is no publication dealing with Ang-1 in Iraq. So the aim of this study was to evaluate the role of Ang1 protein in bladder tumor prognosis.

Materials and Methods

In this study, we were selected 30 patients with Transitional Cell Carcinoma (TCC) and 20 cases of inflammatory lesions of the bladder as control (BIL). The patient samples were collected from the histopathology laboratories of Al-Hussain Teaching Hospital and private laboratories in Thi-Qar province, Iraq during the period from October 2014 to March 2015. Basically, the diagnosis and analysis of studied tissue blocks were mainly based on the achieved histopathological records of bladder biopsy sections in hospital laboratory. Then all samples checked by specialist pathologist to made confirmatory histopathological reevaluation for each studied tissue blocks of each. For each case, the histopathological diagnosis was revised by using Hematoxylin and Eosin and for each representative section. While other sections were put on positive charged slides and stained immunohistochemically for Ang-1. Immunohistochemical staining was carried out using the Novocastra TM Polymer Detection Systems (Envision technique) by using commercial kit from Novocastra, Newcastle, UK, RE7150-K, the slides were deparaffinized, rehydrated then blocked. All of the slides were treated with anti-Ang1 monoclonal antibody, dilution 1:50 (Dako, Denemark), then incubated with a post primary block solution for 30 minutes. In the next step , the slides were rinsed gently in PBS 2× 5 and tissue sections incubated with a secondary antibody Novolink TMpolymer mouse and rabbit immunoglobulins) for 30 minutes, washed in PBS 2× 5 with gentle rocking . After washing, the samples were stained with diluted liquid DAB, and then counter stained with hematoxylin. Slides washed,

dehydrated, mounting, and examining under light microscope at 10X, 20X, 40X magnification (Shi *et al.*, 1998).

Results and Discussion

The results was observed that out of the 30 total cases 11, 16 and 3 had grade I (low) , grade II and grade III (high) carcinoma respectively . Our results showed that there were many places to expression of Ang-1 in TCC and BIL, such as stromal cells, the smooth muscle cells of large blood vessels in addition to endothelial cells and tumor cells (Figure-1) with significant different between TCC and BIL (Table-1) . These results agreed with Szarvas *et al*. (2009) who studied the serum Angiopoietin (Ang-1) levels in patients of bladder cancer and they found there was significant difference. This difference was higher ($p \leq 0.001$) than those in control (Szarvas *et al.,* 2009). The results of other studies found high expression of Ang1 correlated with tumor angiogenesisthe. Interestingly, our study showed a significant relation between the expression of Ang1 and clinical grades ($P \leq 0.01$, Table-2). These results agreed with Szarvas *et al.* (2008) who found in muscle invasive stages of bladder cancer, a part of tumor stromal cells was strongly positive for Ang-1 while Ang-1 expression was significantly lower ($P < 0.001$) in tissues of noninvasive bladder tumors (Szarvas et al., 2008). Furthermore, our study agreed with Audero *et al.* (2001) who demonstrated that there was increasing through the progression from low- to high-grade astrocytomas for Ang. Consequently, there was density of Ang1 staining and the amount of positive vessels was increased (Audero *et al.*, 2001). Cancer cells are cells which lost their capacity to division in a controlled fashion. A malignant tumor contains populations of quickly, rapidly dividing and developing cancer cells that gradually become mutations. Interesting, tumors can grow beyond a certain size (generally 1–2 mm) and this can happened by supplying the tumor with dedicated blood. This blood is necessary to provide the oxygen and other essential nutrients for tumor (McDougall *et al.*, 2006). ANG-1 is critically important in the formation of vascular networks during developmental angiogenesis (Suri *et al*., 1996). The function of Ang1 that secreted by tumor cells in neoplastic angiogenesis, have been recognized in amount of Ang1-expressing glioblastoma cell lines have set up in vitro coculture system with ECs. ECs seemed distinctly flattened and migrated throughout the Matrigel surface, creating an efficient network of anastomosing, not split, cordlike structures when Ang1-expressing glioblastoma cells were existence. Indeed the creation of such tubular structures is a very difficult process that includes the joint effects on cell-cell and cell-matrix adhesion, proteolytic remodeling of the matrix and migration. It is well known that the using of a blocking anti-Ang1 antibody mainly influenced cell-cell adhesion and migration. In fact, the most clear feature for ECs remained grouped were unsuccessful to align, elongate, giving origin to less extended cordlike structure and nests created by an increased number of cells (Von Bernstorff *et al*., 2001) . Neovascularization has been shown to be related with offensive behavior in various adult malignancies (Hyeok Seok *et al*., 2013). Ang1 has the ability to converted macrophage differentiation toward a pro-inflammatory phenotype, even in the existence of an anti-inflammatory mediator. All these findings propose that Ang1 shows an essential role in stimulating pro-inflammatory responses. This fact might afford a new strategy by which to achieve inflammatory responses (Suri *et al*., 1996). When the Ang1 knockout in mice, the strategy will change, reduced association between ECs and periendothelial cells in addition to a lack of EC spreading and flattening are the main reasons for immature vessels . It has been demonstrated that Ang1 has critical role in ECs. It was responsible on promotes sprouting, cell survival, and migration (Sato *et al.*,1995). Consequently, it can be guessed that Ang1 not only shows a role in the retaining of interactions between ECs and support cells but it appears to have obvious and direct effects on EC behavior through vascular remodeling , Vainstein *et al.* (2004) demonstrated that the modulation of ANG1 production seemed to have a complex effect: In speedily growing tumors a specific ANG1 creation level prompted highest tumor growth, whereas both the greater and lesser levels affected relative tumor growth repression (Vainstein *et al*., 2004). Interestingly, in slow growing tumors, the tumor growth might accelerate by inhibition of ANG1 production even though its motivation had little effect. Therefore, the stimulation in addition to the inhibition of ANG1 creation by tumor cells might have different and reverse effects on tumor growth and developing, relying on the patho-physiological conditions.

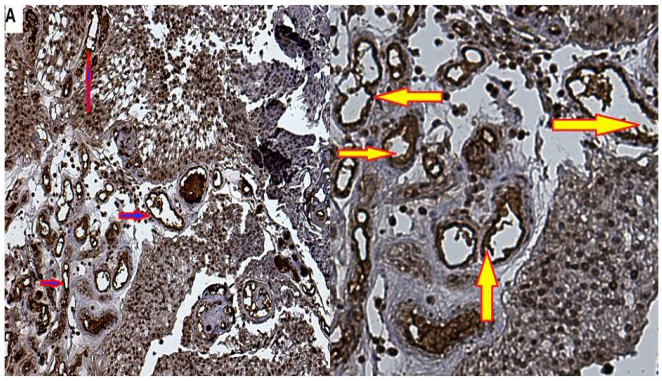

Figure (1): A: Invasive transitional cell carcinoma, poorly differential (Grade II) showing strong positive Ang1 staining (Score +++, brown) (10, 20X,arrow)

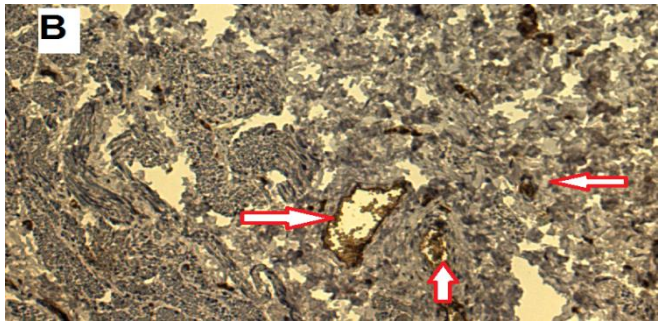

Figure (1): B: Moderated positive Ang1 staining in Bladder tumor (Score +, brown) (20X,arrow)

Table (1): IHC Ang1 expression in bladder patients group

Groups	Ang-1 expression	
	Ang-1^{+}	ANg-1 $^{-}$
TCC	23/30 (76.7 %)	7/30 (23.3 %)
BIL	8/20 (40 %)	12/20 (60%)
Total	31/50(62%)	19/50 (38%)
Chi-square value P value		6.8478 0.008875 *

* (P≤ 0.01) high significant

Table (2) Association between Ang1 IHC expression and tumor grade

Grades	Ang-1 expression	
	Ang-1^{+}	ANg-1 $^{-}$
High	18/19(94.7 %)	1/19 (5.3 %)
low	5/11 (45.5 %)	6/11 (55.5%)
Total	23/30(76.7%)	7/30 (23.3%)
Chi-square value P value		9.4585 0.002102*

* (P≤ 0.05) significant

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