Angiogenesis in *Schistosoma haematobium*-associated urinary bladder cancer

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Schistosoma haematobium, a parasitic flatworm that infects more than 100 million people, mostly in the developing world, is the causative agent of urogenital schistosomiasis, and is associated with a high incidence of squamous cell carcinoma (SCC) of the bladder. During infection, eggs are deposited in the bladder causing an intense inflammatory reaction. Angiogenesis is defined as the formation of new blood vessels from preexisting ones and is recognized as a key event in cell proliferation and carcinogenesis and spread of malignant lesions. A growing amount of evidence points to angiogenesis playing a key role in schistosomiasis-associated bladder cancer. Thus, identifying biomarkers of this process plays an important role in the study of cancer. Here, we review recent findings on the role of angiogenesis in bladder cancer and the growth factors that induce and assist in their development, particularly SCC of the bladder associated to urogenital schistosomiasis.

Key words: Schistosomiasis; urothelial carcinoma; blood vessels; urogenital schistosomiasis; angiogenic markers.

SCHISTOSOMIASIS

Schistosomiasis is a neglected tropical disease transmitted to humans from freshwater snails. It is caused by a blood fluke of the genus *Schistosoma*. Schistosomiasis is considered the most important of the helminthiases and the second most important parasitosis, after malaria, causing high rates of morbidity and mortality. As of 1989, schistosomiasis was endemic in 76 countries (1). Recent WHO report declared schistosomiasis endemic in 78 countries (2, 3). *S. haematobium* is endemic in 53 countries in the Middle East and most of the African continent, including the islands of Madagascar and Mauritius. Due to successful eradication programs, the infection is no more of significant public health significance in Egypt, Lebanon, Oman, Syria, Tunisia and Turkey because transmission is low or nonexistent (4). A disputed and ill-defined focus exists in India and requires further confirmation (5). After more than 50 years in which no more autochthonous cases of schistosomiasis were recorded in Europe, *S. haematobium* infection has recently emerged in Corsica (6). This disease affects 200 million people worldwide. From these, 20 million have severe disease and 120 million are considered symptomatic. Risk of infection affects 600 million; others including travelers from developed countries (7).

This review focuses on the morphological variables and prognosis in carcinoma of the urinary bladder associated with schistosomiasis, and the

fact that the appearance and formation of angiogenesis alters the course of cancer development, in the context of *S. haematobium* infection. The present work attempts to integrate a variety of studies and experimental approaches with *S. haematobium* models, while giving particular emphasis to the *in vitro* studies that have contributed to expanding our understanding of the mechanisms of action of growth factors and formation of new vessels in urinary bladder cancer. In particular, we suggest that the presence of eggs of *S. haematobium* plays a key role in angiogenesis and contributes to the development of urinary bladder cancer (Fig. 1).

UROGENITAL SCHISTOSOMIASIS

Three major species of schistosomes are the agents of human schistosomiasis – *Schistosoma japonicum* and *Schistosoma mansoni* cause intestinal schistosomiasis in East Asia, Africa, South America and the Caribbean, while *S. haematobium*, occurring widely throughout Africa and the Middle East, causes urogenital schistosomiasis. Recent recalibration of health burdens revealed that in the range of 4.5–70 million disability adjusted life years (DALYs) are lost to schistosomiasis. More people are infected with *S. haematobium* than with the other schistosomes combined. Of 112 million cases of *S. haematobium* infection in sub-Saharan Africa, 70 million are associated with hematuria, 18 million with major urinary bladder wall pathology, and 10 million with hydronephrosis leading to kidney damage (8–10). In many patients, deposition of *S. haematobium* parasite ova eventually leads to squamous cell carcinoma (SCC) of the urinary bladder (11, 12). Accordingly, *S. haematobium* has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) (13, 14).

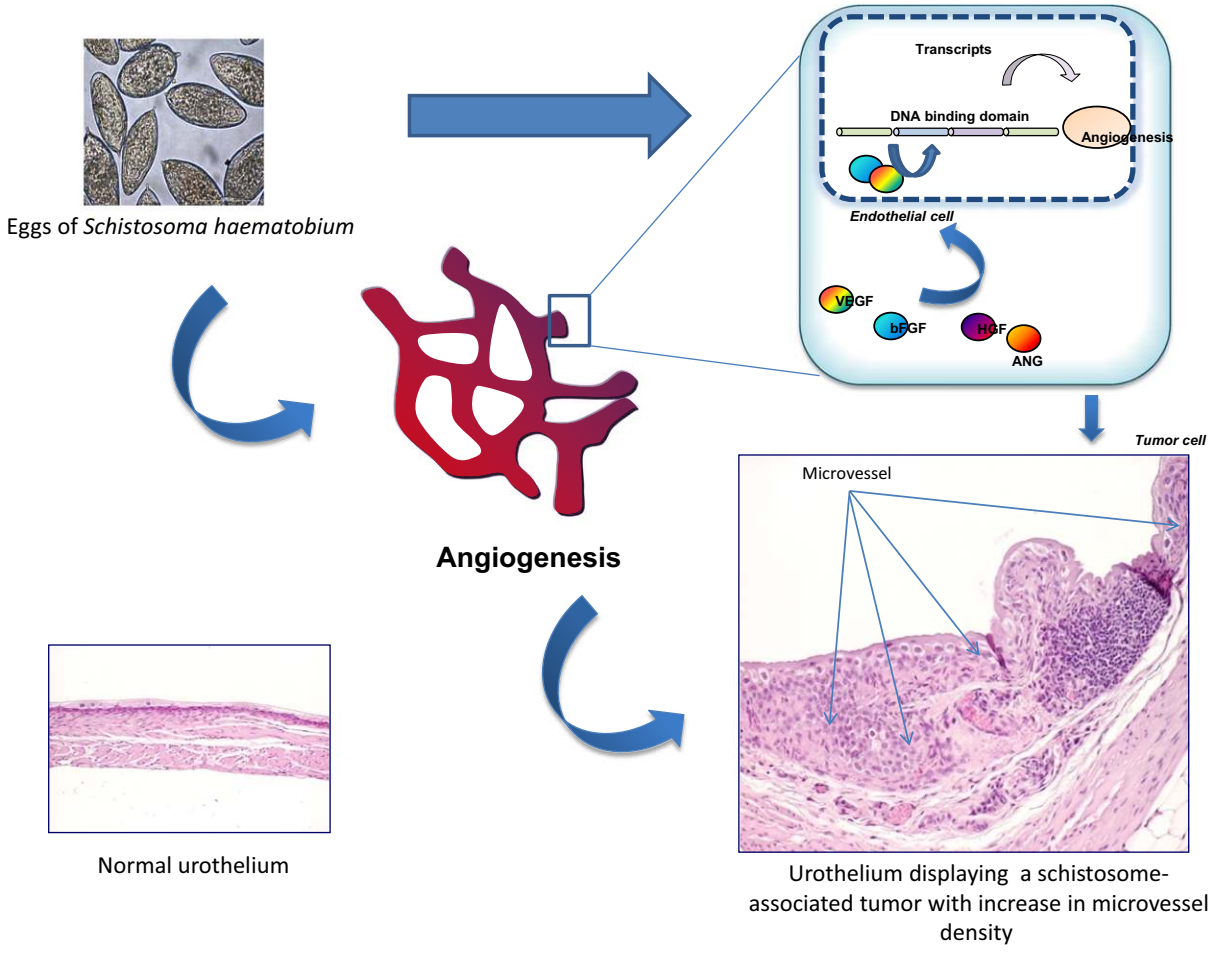

Fig. 1. Schematic representation of angiogenesis associated with urogenital schistosomiasis. Microphotographs from urinary bladder sections stained with Hematoxylin and Eosin (400×).

SCHISTOSOMA HAEMATOBIUM-ASSOCIATED URINARY BLADDER CANCER

Squamous cell carcinoma is a malignant, poorly differentiated neoplasm. SCC is the common form of urinary bladder cancer in rural Africa where *S. haematobium* is prevalent (15, 16). By contrast, the majority of urinary bladder cancer in developing countries and regions not endemic for urogenital schistosomiasis is transitional cell carcinoma (TCC), which arises from the transitional epithelium lining of the urinary bladder. The parasite eggs trapped in the urinary bladder wall release antigens and other metabolites (presumably evolved to expedite egress to the urine, and hence to the external environment). The phenomenon leads to hematuria and to chronic inflammation, in turn increasing the risk of SCC of the urinary bladder. The epidemiological association between SCC of the urinary bladder with schistosomiasis hematobia is based both on case–control studies and on the correlation of urinary bladder cancer incidence with prevalence of *S. haematobium* infection within diverse geographic locations. The incidence of urogenital schistosomiasis-associated SCC is estimated in 3–4 cases per 100 000 (17). Schistosomiasis hematobia is a chronic infection. The adult, egg-producing schistosomes live for many years, re-infections frequently occur, and schistosomiasis-associated urinary bladder SCC appears relatively early, often by the mid-decades of life (TCC usually presents in the later decades of life). In its most recent monograph, IARC confirmed that chronic infection with *S. haematobium* causes cancer of the urinary bladder (14).

The cellular and molecular mechanism linking infection with *S. haematobium* and cancer is usually related to adult parasite invasion in the venous plexus around the urinary bladder, the eggs released by worms, cause chronic granulomatous inflammation of the mucosa and submucosa of the urinary bladder. Chronic granulomatous inflammation and irritation subsequently leads to the development of squamous metaplasia of the transitional epithelium. Chronic granulomatous inflammation also leads to fibrosis in the urinary bladder that causes urinary stasis and super-infection by bacteria. The bacteria convert the nitrates and nitrites in dietary nitrosamines, which are then excreted in urine. These nitrosamines are carcinogenic and acting on metaplastic epithelium, promote subsequent progression of squamous cell carcinoma. The infection can spread and involve the ureters and kidneys, causing chronic obstructive disorders and kidney failure (18). Several models have been proposed to explain the genesis of urinary bladder cancer induced by Urogenital Schistosomiasis (UGS). Some attribute initiation of carcinogenesis to low doses of nitrosamines and/or other environmental carcinogens associated with the infection. In other models, it is suggested that UGS-induced carcinogenesis is due to exposure to tobacco smoke, industrial and agricultural dyes and vitamin A deficiency (19). However, the mechanism by which infection contributes to carcinogenesis is still unresolved. Recent contributions suggest a crucial role of *S. haematobium*: Chinese Hamster Ovary cells (CHO) cells experimentally treated with parasite antigens show increased proliferation, cell migration and invasion, decreased apoptosis, increased Bcl-2 expression and reduced p27 expression. Altogether, these biological processes are characteristic of tumorigenesis and tumor cell survival (7). Further, intravesical administration in a murine model of *S. haematobium* extract induces urothelial dysplasia (20), implying that infection by *S. haematobium* induces malignant transformation of the urothelium, even in the absence of nitrosamines. Previous reports of our group revealed that schistosomes produce estrogen metabolites called catechols and that these molecules can be used as biomarkers for the detection of schistosomiasis-associated urinary bladder cancer (21–23). Based on this scientific evidence, and the discovery of parasitic origin of estrogen metabolites, becomes fundamental to understanding the role of this parasite as initiator of carcinogenesis (24).

ANGIOGENESIS AND LYMPHANGIOGENESIS IN URINARY BLADDER CANCER

Angiogenesis, or the formation of new endothelial sprouts from preexisting postcapillary venules, is a well-known characteristic of inflammatory diseases, wound repair and cancer (25, 26). Accordingly, angiogenesis is a process in which endothelial cells migrate and divide to form new capillaries, providing support for tumor progression. As such, much attention has been focused on pathological significance and detailed mechanism of the vascular system and angiogenesis in cancer. Moreover, the spread of tumor cells through the bloodstream and lymphatic system plays an important role in metastases development (26). A large number of pro-angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and angiogenin (ANG) are often overexpressed in tumors (27). Several studies have indicated that angiogenic activators play an important part in the

growth and spread of tumors. On immunohistochemical examination, the VEGF family and their receptors were found to be expressed in about half of the human cancers investigated (28). These factors are known to affect the prognosis of adenocarcinomas that have developed in the uterine cervix, (29), endometrium, (30), ovary (31) and stomach (32). In addition, a significant correlation between the expression of VEGF and prognosis has been described in colorectal cancer (33), breast cancer (34), lung cancer (35), head and neck squamous cell carcinoma (36), Kaposi sarcoma (37) and malignant mesothelioma (38). These studies also indicated that the levels of angiogenic factors in tissue reflect the aggressiveness with which tumor cells spread, and thus have predictive value in the identification of the high-risk patients with poor prognosis.

Metastatic spread to regional lymph nodes is an early step in systemic dissemination of tumors, being usually associated with poor survival (39). Moreover, exacerbated angiogenesis together with presence of lymph node metastasis are poor prognostic factors for transitional cell carcinoma and urinary bladder carcinoma (40).

While both the blood and lymphatic vascular systems have been implicated, preclinical experimental systems supported by clinical evidence suggest the most common pathway of initial metastasis is through the lymphatic system (41). In recent years, several works discuss the importance of pathological lymphangiogenesis in urinary bladder cancer and in its importance in the invasiveness toward adjacent muscle tissue (42–44). Similarly, recent reports evidence the activation of VEGF signaling that controls and promotes lymphangiogenesis by several parasites such as filariasis and leishmaniasis agents (45, 46). Although lymphangiogenesis is shown to be increased in both urinary bladder cancer and in infection caused by parasites, it is a question needed to be answered whether lymphangiogensis would be involved in urinary bladder cancer associated with schistosomiasis.

SCHISTOSOMIASIS AND ANGIOGENESIS

Angiogenesis plays a complex and extraordinary role in schistosomiasis. This statement may seem a paradox, since schistosomes are intravascular parasites that cause damage by destroying the blood vessels (47). Angiogenesis plays an important role during the formation of perioval granulomas as well as in the genesis of schistosomiasis fibrosis. From the point of view of general pathology, schistosomal perioval granulomas are dynamically similar to the healing of wounds, with the production of granulation tissue, which becomes increasingly less vascularized with time (48).

It has been demonstrated that intact live eggs, excretory/secretory products of eggs and the extracts of homogenized eggs stimulate the proliferation and migration of endothelial cells. Formation of endothelial capillary-like outgrowths, was stimulated by egg extracts (49). The effects mediated by eggs of schistosomes revealed that the soluble egg antigen induces endothelial cell proliferation and upregulates vascular endothelial growth factor (VEGF) (47). Loeffler et al. (50) investigated the effects of *Schistosoma mansoni* soluble egg antigen (SEA) on angiogenic processes: proliferation, tube formation and apoptosis of human umbilical vein endothelial cells (HUVECs). In this study, SEA increased HUVEC tube formation and decreased HUVEC apoptosis after serum and growth factor deprivation. These authors showed that messenger RNA for vascular endothelial growth factor (VEGF) increased 2-fold in SEA-treated HUVECs. Their findings suggest that products secreted by schistosome eggs may promote angiogenesis by upregulating endothelial cell VEGF (50). Other authors analyzed VEGF levels in sera from people diagnosed with schistosomiasis. These patients had significantly high VEGF levels compared with healthy people (51, 52). Therefore, this angiogenic capacity has been suggested as an early marker of preneoplastic and neoplastic lesions in schistosomiasis associated SCC (53). Several growth factors and other molecules produced by the schistosome itself have been reported to be associated with tumor growth, progression and survival of urinary bladder cancer. Moreover, tumor microvessel density (MVD) is thought to be the most useful prognostic marker for cancer development, the relapse-free survival and overall survival (54). El-Sobky and collaborators (55) found a significant relationship between angiogenesis and tumor grade. These findings suggest that assessing angiogenesis using the MVD provides an independent predictor of survival in patients with schistosome-associated carcinoma of the urinary bladder.

Studies to quantify the concentration of angiogenic factors in cases of SCC of the urinary bladder associated to schistosomiasis may be of great clinical importance for urinary bladder cancer detection, assessing their stage and level of development. The methodologies that can be performed to evaluate angiogenesis associated with *S. haematobium* infection are by means of microscopy/immunochemical, ELISA, PCR-based techniques and other molecular biology techniques that can be used to evaluate vascularization markers in both samples of infected patients and in animal models. Such markers may

include CD31, CD34, vonWillebrand factor (vWF) and VGFRs. Angiogenic markers showed significant association with clinical stage (27). It was reported that Basic fibroblast growth factor (bFGF) increased significantly in urinary bladder squamous cell carcinoma cases. These authors found that bFGF and hepatocyte growth factor (HGF) significantly correlated with tumor grade (27). Understanding the growth factors that influence the progression of angiogenesis and lymphangiogenesis during infection by schistosomes becomes feasible from the point of view of a possible intervention in the spread of tumor cells. Moreover, cancers are genetically diversified using different exposures, DNA repair effects and cellular origin, which may suggest that a particular exposure (parasite antigen) can lead to a cascade of events that promote cancer in susceptible hosts, and that angiogenic factors, as reported in *S. haematobium*, could be used as diagnostic and prognostic markers of urinary bladder cancer in UGS. An in the case of Symmers' fibrosis associated to schistosomiasis, angiogenesis inhibitors are indicated as an effective tool for the treatment of this liver fibrosis (55).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In the last years, urinary bladder cancer-associated schistosomiasis has been a field under extensive investigation. Increasing knowledge in the field has opened up important new perspectives with respect to how this type of cancer is perceived. Among them, there are crucial findings on the hallmarks of cancer and the contribution of these to the carcinogenic process, specially angiogenesis and lymphangiogenesis. On the basis of these results, a new mechanistic approach to the development of schistosomiasis-associated urinary bladder cancer arose, enabling us to further comprehend their underlying molecular mechanisms and contribution to the development of this type of cancer. Studies are needed to identify and characterize angiogenic and lymphangiogenic markers in carcinoma of the urinary bladder associated to UGS. Tumor induction, proliferation, invasion and metastasis represent a complex and incompletely understood series of events (56). Thus, the development of additional biological markers of prognosis for tumor angiogenesis and lymphangiogenesis, may add information to the initial risk assessment. It is to be hoped that the rapidly increasing volume of information in these field can be used in the future to develop specific and more effective anti-cancer therapies, not only toward schistosomiasis-associated urinary bladder cancer but also other types of cancer.

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