Virulence 1:2, 84-87; March/April 2010; © 2010 Landes Bioscience

Schistosoma haematobium and bladder cancer

What lies beneath?

Monica Catarina Botelho,[1,2,*] Jose Carlos Machado[2,3] and Jose Manuel Correia da Costa[1]

[1]CIBP—Centre for Parasite Immunology and Biology; National Institute of Health; Porto, Portugal; [2]IPATIMUP—Institute of Pathology and Molecular Immunology of Porto University; Porto, Portugal; [3]FMUP—Faculty of Medicine of Porto University; Porto, Portugal

©2010 Landes Bioscience
Do not distribute.

***Schistosoma haematobium* is a parasitic flatworm that infects millions of people, mostly in the developing world, and is associated with high incidence of bladder cancer although why is not clear. But our group was able to define the mechanistic relationship for the first time between infection of *S. haematobium* and cancer. We used in vitro models to demonstrate the presence of informative carcinogenesis-associated phenotypes in CHO cells exposed to Sh total antigen, in which we showed increased cell proliferation, decreased apoptosis, upregulation of the anti-apoptotic molecule Bcl-2, downregulation of the tumor suppressor protein p27, and increased cell migration and invasion.**

We further discuss the molecular and cellular events that might be responsible for schistosomiasis-related bladder cancer.

Key words: *Schistosoma haematobium*, squamous cell carcinoma of the bladder, CHO cells, carcinogenesis-associated phenotypes, animal models, kras mutations, estrogenic DNA adducts

Submitted: 10/24/09

Accepted: 10/30/09

Previously published online: www.landesbioscience.com/journals/virulence/article/10487

*Correspondence to: Mónica C. Botelho; Email: monicabotelho@hotmail.com; monica.botelho@insa.min-saude.pt

Addenda to: Botelho M, Ferreira AC, Oliveira MJ, Domingues A, Machado JC, da Costa JMC. *Schistosoma haematobium* total antigen induces increased proliferation, migration and invasion, and decreases apoptosis of normal epithelial cells. Int J Parasitol 2009; 39:1083–91; PMID: 19285502; DOI: 10.1016/j.ijpara.2009.02.016.

Schistosomiasis, also known as snail fever—since part of the parasite life cycle occurs in these animals—is a potential fatal disease that, according to the World Health Organization, infects 200 million people and is endemic in as much as 76 tropical developing countries. The disease, spread through contaminated waters, is only second to malaria in rates of infection and public health impact throughout the developing world, but has been one of many neglected tropical diseases until very recently, when globalization and its associated intense migration flux has brought it into the light. And infection by *S. haematobium*—a member of this family—is particularly relevant due to its association to bladder cancer. In fact, in some of the regions where *S. haematobium* is endemic, bladder cancer is the most common cancer in men and the second in women, just behind breast cancer, accounting for as much as 30% of all cancer cases.[1]

In an attempt to understand this worrying link between infection and cancer our group exposed cells growing in laboratory to extracts of *S. haematobium* looking for changes, particularly in those traits associated with cancerous processes. In fact, cancer cells can be defined by rapid uncontrolled division, high resistance to death and—in the late stages of the disease—an abnormal capability to migrate through tissues (normal cells, except for a few exceptions, are not capable of move out of their "home" tissue/organ). We found that, in fact, cells exposed to *S. haematobium* cells divided faster and more than those not exposed to the parasite, and also died much less. When searching for molecular clues to explain such alterations it was found that the altered cells presented increased levels of bcl2—a protein involved in cellular death and linked to cancer—while the tumor suppressor protein p27 was reduced. These cells were also more mobile than controls, a crucial characteristic for metastasis formation. These results revealed *S. haematobium* is able to induce the formation of cancer-like cells.[2]

Next, in a second study we injected *S. haematobium*—exposed cells into mice with no immune system and, remarkably, this led to tumors very similar to those found in bladder cancer. Crucially, animals injected with non-exposed cells showed no growths (**Fig. 1**).[3]

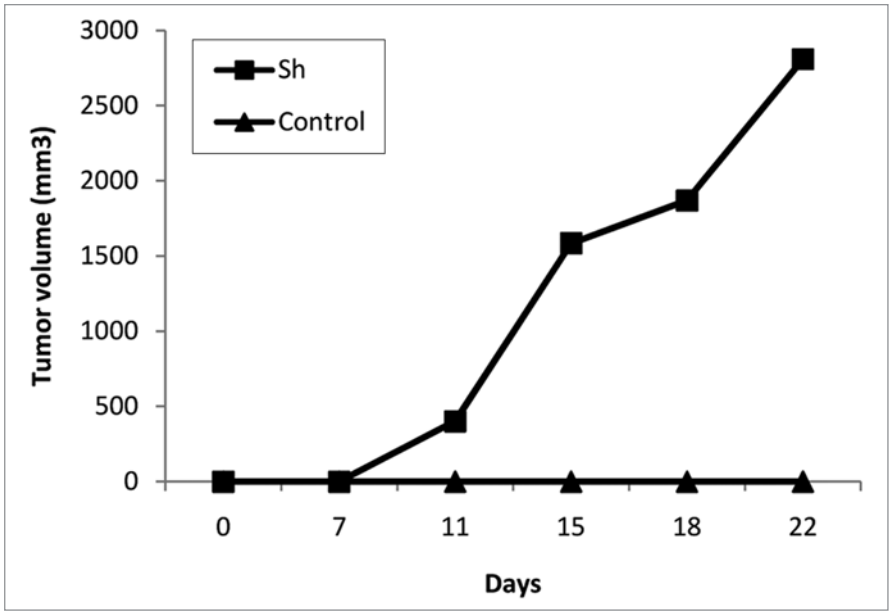

Figure 1. Analysis of the tumour volume induced by control CHO and Sh treated CHO in nude mice. Tumour volumes were measured at the indicated days after injection. Each point represents the mean of five individual determinations.

©2010 Landes Bioscience. Do not distribute.

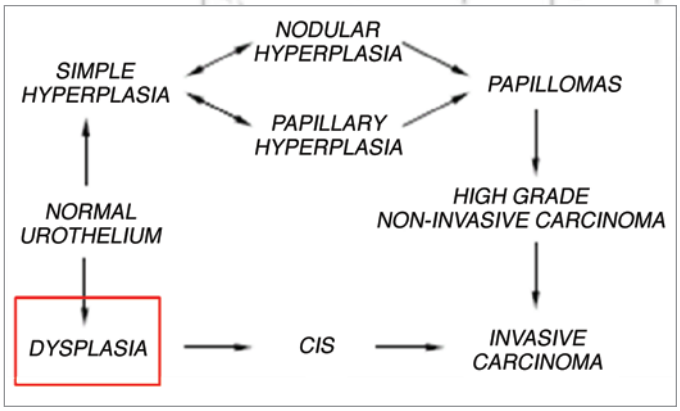

Figure 2. Pathogenic pathways of rat and mice urinary bladder carcinogenesis (reviewed in ref. 3).

We have further used a CD-1 mice model to show that *Schistosoma haematobium* total antigen (Sh) has a carcinogenic ability. Intravesically administration of Sh gave rise to a high incidence of urothelial dysplasia (**Fig. 2**).[4]

The urothelium of the animals exposed to Sh described in this paper presented similar phenotypes. The urothelium of these animals presented dysplasia (LGIUN—Low Grade Intra-Urothelial Neoplasia), a non-invasive malignant flat lesion, in 70% of the tested subjects after 40 weeks of treatment (**Table 1, Fig. 3**). Our results suggest that Sh induce the malignization of the urothelium.[5]

Although squamous cell carcinoma of the urinary bladder has been associated with *Schistosoma haematobium* infection in many parts of Africa, the cellular and molecular mechanisms linking *S. haematobium* infection with cancer formation are not yet defined. Carcinoma of the bladder frequently harbours gene mutations that constitutively activate the receptor tyrosine kinase-Ras pathway.[6] The ras-signaling pathway has attracted considerable attention as a target for anticancer therapy because of its important role in carcinogenesis. The Ras gene product is a monomeric membrane-localized G protein of 21 kd that functions as a molecular switch linking receptor and nonreceptor tyrosine kinase activation to downstream cytoplasmic or nuclear events. Each mammalian cell contains at least three distinct ras proto-oncogenes encoding closely related, but distinct proteins, Kras, Hras and Nras Activating mutations in these Ras proteins result in constitutive signaling, thereby stimulating cell proliferation and inhibiting apoptosis. Oncogenic mutations in the ras gene are present in approximately 30% of all human cancers.[7] In our latest study we used the dysplastic bladders induced by Sh and screened them by sequencing

Table 1. Histopathological findings in the Sh instilled bladders and controls

Group	n	W	N	U	I	L	M	D
Group 1 (Sh treated)	10	20	7/10 (70%)	5/10 (50%)	9/10 (90%)	9/10 (90%)	0/10 (0%)	3/10 (30%)
Group 2 (Control)	10	20	10/10 (100%)	9/10 (90%)	2/10 (20%)	2/10 (20%)	0/10 (0%)	0/10 (0%)
Group 3 (Sh treated)	10	40	0/10 (0%)	0/10 (0%)	10/10 (100%)	9/10 (90%)	4/10 (40%)	7/10[a] (70%)
Group 4 (Control)	10	40	10/10 (100%)	10/10 (100%)	3/10 (30%)	3/10 (30%)	0/10 (0%)	0/10 (0%)

n, number of animals; W, weeks between treatment and sacrifice; N, normal urothelium; U, presence of umbrella cells; I, inflammatory infiltrate; L, infiltration of lymphocyte; M, infiltration of mastocytes; D, dysplasia; [a](p = 0.001, Group 3 Vs. Group 4).

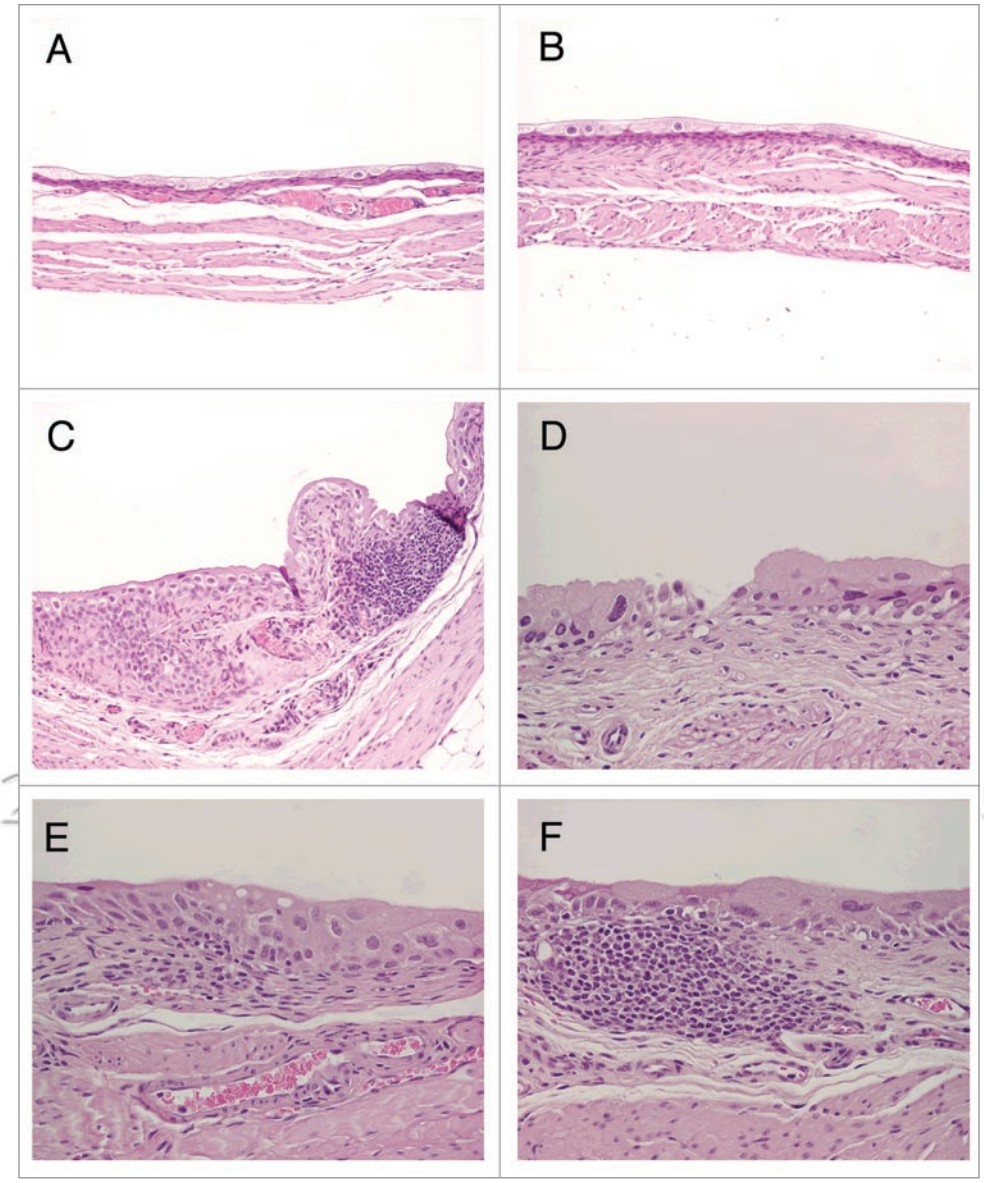

Figure 3. (A and B) control, (C) urothelium displaying an inflammatory nodule and a lesion with characteristics of nodular hyperplasia, (D) denudation and dysplasia, (E) inflammatory infiltrate and dysplasia, (F) inflammatory nodule and dysplasia.

for mutations in the Kras codon hotspots (12 and 13) gene. Two (out of 10) of the bladders with dysplasia presented a KRAS mutation in codon 12 of exon 2 (**Fig. 4**). We concluded from these results that the parasite extract of *S. haematobium* has carcinogenic ability possibly through oncogenic mutation of KRAS gene.

Further investigations using *Schistosoma haematobium* antigens and the identification of the carcinogenic component(s) may be useful to understand oncogenesis associated with this parasite. We have attempted to identify such carcinogenic compounds and they are probably quinone forms generated by metabolic activation of catechol estrogens. In a first report, high levels of oestradiol but not LH, and Testosterone were detected in sera from urinary schistosomiasis patients.[8] This evidence was unexpected. Estradiol is a steroid hormone secreted principally by the ovarian follicles in vertebrate animals. How could this be possible? The hormone levels in sera patients neither could nor be attributed to hypothalamic-pituitary-gonadal axis. How to explain this non sense biological evidence? The production of an estradiol-related molecule by Schistosoma was hypothesized. Finally, we show that this molecule related to estradiol is present in schistosome worm extracts. In a first attempt, the detection method ELISA specific for estradiol, revealed the expression of this estradiol-related molecule in schistosome worm extracts, but not in *Fasciola hepatica* worm extracts. We were able to characterize four estrogenic molecules. These molecules probably mediated an interaction between the parasites and their hosts, because their presence was detected in both organisms.

The quinone forms generated by metabolic activation of catechol estrogens are highly electrophilic species. Some authors

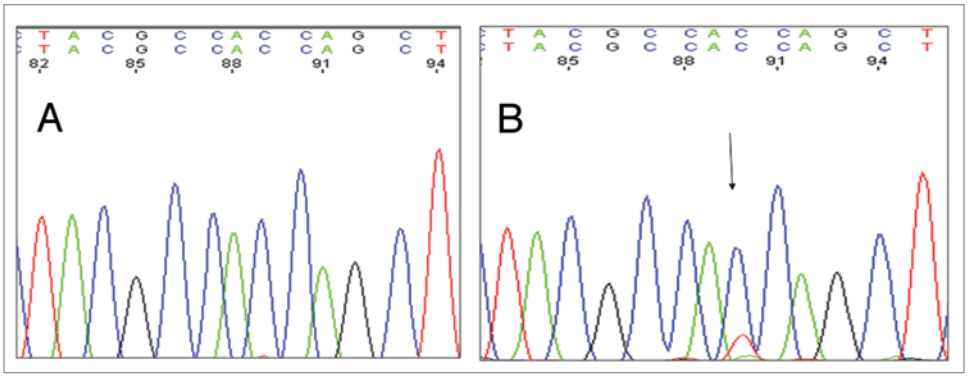

Figure 4. *KRAS* exon 2 sequencing results. (A) control, (B) dysplastic bladder with a mutation at codon 12 (resulting in a Gly12Asp mutation).

reported that these species are able to covalently bind DNA bases, forming stable adducts.[9-12] These adducts can be considered as estrogen-bound metabolites generated in vivo that are produced by nucleophilic attack of the DNA base on different positions of the steroid ring.[10-13] Catechol estrogen-3,4-quinone adducts of DNA have been proposed as endogenous initiators of breast, prostate and other human cancers.[14]

The carcinogenic effect of this estrogen adduct-mediated pathway of the estrogenic molecules present in *S. haematobium* extracts could explain the link between this parasite and squamous cell carcinoma of the bladder. More studies are ongoing aiming a better comprehension of these biological interactions and their carcinogenic effect.

©2010 Landes Bioscience.
Do not distribute.

References

1. Mostafa MH, Sheweita SA, O'Connor PJ. Relationship between schistosomiasis and bladder cancer. Clin Microbiol Rev 1999; 12:97-111.
2. Botelho M, Ferreira AC, Oliveira MJ, Domingues A, Machado JC, da Costa JM. *Schistosoma haematobium* total antigen induces increased proliferation, migration and invasion, and decreases apoptosis of normal epithelial cells. Int J Parasitol 2009; 39:1083-91.
3. Botelho M, Oliveira P, Gomes J, Gartner F, Lopes C, Correia da Costa JM, et al. Tumourigenic effect of *Schistosoma haematobium* total antigen in mammalian cells. Int J Exp Path 2009; 90:448-53.
4. Oliveira PA, Colaco A, De la Cruz PLE, Lopes C. Experimental bladder carcinogenesis-rodent models. Exp Oncol 2006; 28:2-11.
5. Botelho MC, Oliveira PA, Lopes C, Correia da Costa JM, Machado JC. Urothelial dysplasia and inflammation induced by *Schistosoma haematobium* total antigen instillation in mice normal urothelium. Urol Oncol 2009; In press.
6. Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. Nat Rev Cancer 2005; 5:713-25.
7. Adjei AA. Blocking oncogenic Ras signaling for cancer therapy. J Natl Cancer Inst 2001; 93:1062-74.
8. Botelho MC, Crespo M, Almeida A, Vieira P, Delgado ML, Araujo L, et al. *Schistosoma haematobium* and *Schistosomiasis mansoni*: production of an estradiol-related compound detected by ELISA. Exp Parasitol 2009; 122:250-3.
9. Stack DE, Byun J, Gross ML, Rogan EG, Cavalieri EL. Molecular characteristics of catechol estrogen quinones in reactions with deoxyribonucleosides. Chem Res Toxicol 1996; 9:851-9.
10. Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, et al. Molecular origin of cancer: catechol estrogen-3-4-quinones as endogenous tumor initiators. Proc Natl Acad Sci USA 1997; 94:10937-42.
11. Akanni A, Tabakovic K, Abul-Hajj YJ. Estrogen-nucleic acid adducts: reaction of 3,4-estrone-o-quinone with nucleic acid bases. Chem Res Toxicol 1997; 10:477-81.
12. Akanni A, Abul-Hajj YJ. Estrogen-nucleic acid adducts: reaction of 3,4-estrone-o-quinoneradical anion with beoxyribonucleosides. Chem Res Toxicol 1997; 10:760-6.
13. Farmer PB, Sweetman GMA. Special feature: perspective. Mass spectrometry of carcinogen adducts. J Mass Spectrom 1995; 30:1369-79.
14. Zahid M, Kohli E, Saeed M, Rogan E, Cavalieri E. The greater reactivity of estradiol vs. estradiol-2,3-quinone with DNA in the formation of depurinating adducts: implications for tumor-initiating activity. Chem Res Toxicol 2006; 19:164-72.