Review

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Controversies and challenges in research on urogenital schistosomiasis-associated bladder cancer

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Urogenital schistosomiasis, infection with *Schistosoma haematobium*, is linked to increased risk for the development of bladder cancer, but the importance of various mechanisms responsible for this association remains unclear, in part, owing to lack of sufficient and appropriate animal models. New advances in the study of this parasite, bladder regenerative processes, and human schistosomal bladder cancers may shed new light on the complex biological processes that connect *S. haematobium* infection to bladder carcinogenesis.

The link between *Schistosoma haematobium* and bladder cancer

The association between urogenital schistosomiasis and bladder cancer was documented in the early 1900s [1] and has since been corroborated by many retrospective studies of human bladder cancer in diverse regions endemic for this infection [2–4]. The World Health Organization's International Agency for Research on Cancer (IARC) thus regards infection with *Schistosoma haematobium* – the causative agent for urogenital schistosomiasis – as carcinogenic to humans (Group 1, the classification reserved for suspected carcinogens with the strongest evidence) [5,6]. However, there is minimal evidence of any cancer association with infection by the related schistosomes *Schistosoma mansoni* and *Schistosoma japonicum*; these species predominantly cause hepatoenteric disease and rarely affect pelvic organs [4,5]. Analogous to *S. haematobium* and bladder cancer, other macroparasites such as the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis* show strong associations with the onset of cholangiocarcinoma, a form of bile duct cancer [6–8]. Indirect and direct mechanisms may be responsible for the association of these parasites with their particular cancers [9]. Helminths may directly induce cancer through the activity of parasite molecules on host cells. Indirectly, they may permit co-infection by other potentially oncogenic biological species – such as viruses or bacteria [10–16] – and also elicit genetic lesions from spillover effects of host inflammatory processes directed against the parasite, such as production of reactive nitrogen and oxygen intermediates [9,17]. Additionally, the risk of bladder cancer owing to infection by *S. haematobium* seems to be significantly promoted by concurrent risk factors commonly associated with bladder cancer in areas of nonendemicity, including nitrosamines and other chemical exposures from industrial and agricultural sources, as well as from tobacco smoking (reviewed in [18,19]). Thus, multiple factors may intersect to confer increased risk for bladder cancer associated with *S. haematobium* infection in humans.

Although the association between *S. haematobium* infection and bladder cancer is strong, identification of the underlying mechanisms has progressed slowly and probably hampers the diagnosis and successful treatment of urogenital schistosomiasis-associated bladder cancer. The interrelated reasons for slow progress in this field include: (i) lack of a tractable animal model to study the progression of urogenital schistosomiasis; (ii) until recently, a paucity of genomic information about *S. haematobium*; (iii) few genetic tools to manipulate life stages of *S. haematobium*; and (iv) an incomplete catalog of mutations that may be unique to human schistosomal bladder cancer compared with other bladder cancers. Over the past few years, new research efforts have directly targeted some of these key roadblocks [20–22], opening new avenues to investigate *S. haematobium* infection and its association with bladder cancer.

This review attempts to integrate recent insights into the regenerative pathways at work in bladder homeostasis and injury repair with the growing literature on the roles host inflammatory mechanisms may play in promoting initial neoplastic transformation and cancer progression. These findings may suggest ways to frame future experimentation on the oncogenic effects of particular *S. haematobium* pathogenesis mechanisms and host inflammatory pathways, as well as roles for potential co-infections in precipitating neoplastic transformation. Ultimately, such work may reveal new diagnostic and treatment modalities,

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with potential to test broader questions regarding the role of inflammation in epithelial cancers.

The inflammatory environment during acute and chronic *Schistosoma haematobium* infection of the bladder

The pathology of urogenital schistosomiasis is primarily caused by the eggs laid by *S. haematobium* adult worm pairs residing in the venous plexus of the bladder and other pelvic organs. On their way to exiting the body through the urinary stream, eggs transit through the bladder mucosal tissue, causing substantial tissue damage and initiating granulomatous inflammation that can progress over many years to complications including fibrosis and bladder cancer [23,24]. Temporally cross-sectional, histological observations of *S. haematobium*-infected human bladder tissues, especially autopsy series, combined with extrapolations from rodent hepatoenteric pathology caused by experimental *S. mansoni* and *S. japonicum* infections, outline the likely timeline of changes occurring in bladder tissue in the setting of urogenital schistosomiasis [25–27]. However, the potentially unique properties of inflammatory responses in the bladder mucosa [28] imply that animal models of urogenital schistosomiasis could greatly improve our grasp of the important immune factors involved in the acute and chronic phases of this infection.

There are limitations to the available experimental models for the study of the full course of *S. haematobium* infection in mammalian hosts [29]. Unfortunately, transdermal infection of mice with *S. haematobium* cercariae, the route of natural infection of humans, results in very low rates of worm maturation and egg deposition in the pelvic organs, the key sites of human pathology [30]. Infection of hamsters and non-human primates with *S. haematobium* cercariae leads to worm maturation and oviposition [31]. Hamsters, however, exhibit low rates of pelvic organ infection and instead develop predominantly hepatoenteric schistosomiasis. By contrast, *C. sinensis*- and *O. viverrini*-infected hamsters make an excellent model and even acquire cholangiocarcinoma when fed a diet that is high in nitrosamines, mimicking the human scenario [32–34]. Although non-human primates develop pathology similar to human disease, ethical considerations and financial cost preclude their regular use, and a relative lack of genetic and scientific tools in general complicate testing of relevant host pathways [35].

Several years ago we sought to help overcome this impasse in animal models of urogenital schistosomiasis. By microinjecting a bolus of *S. haematobium* eggs into the bladder wall of mice [36], we were able to replicate several important changes observed in human urogenital schistosomiasis, such as hematuria, increased urinary frequency, persistent and fibrotic granulomata, and systemic and regional type 2 immune activation [20]. Importantly, egg exposure also triggered persistent urothelial hyperplasia and squamous metaplasia, two potentially preneoplastic lesions of the bladder (Figure 1). Analysis of the bladder transcriptome in this model demonstrated that dramatic decreases occur in the expression of genes important for urothelial differentiation and function [37]; these processes of dedifferentiation may be relevant to bladder carcinogenesis and cancer progression [38]. Thus, it is possible that helminths such as *S. haematobium* may have co-evolved so closely with their human hosts that they specifically modulate host epithelial (de)differentiation to benefit their own survival and reproduction (reviewed in [39]). Regardless, our animal model provides a foothold for experimental investigation of the acute (and possibly some chronic) inflammatory changes induced in the bladder by *S. haematobium* infection. However, our model has important limitations, given that it features administration of a single egg bolus to mice, whereas naturally infected humans experience continuous bladder oviposition by worms. Although it is unknown how this central difference in oviposition affects the pathology observed in our model, in all likelihood it results in less chronic inflammation than natural infection. Therefore, room for improvement exists; a mouse model of worm-based oviposition in pelvic tissues would be better. Ultimately, the short life span of *Mus musculus* (~2 years) may constrain our ability to model schistosomal bladder cancer using mice, given that these neoplasms do not arise in *S. haematobium*-infected humans for decades [3,40–42].

Regardless, the capacity to model the inflammatory aspects of *S. haematobium* infection may reveal mechanisms by which this infection facilitates bladder oncogenesis. Inflammation participates in both the initiation and progression of many cancers [43]. When taking up residence within particular host tissues, microbes and macroorganisms may initiate inflammation that can lead to eventual cancer formation [9,44,45]. Although appropriate levels and forms of inflammation confer protection from pathogen dissemination during acute infection, persistent and dysregulated inflammation can create opportunities for malignant transformation of host cells. Continual tissue turnover increases risk of loss of genomic integrity in progenitor cells, and the ongoing wound healing response can construct a persistent microenvironment conducive to and immunologically tolerant of survival of cancerous cells [46–50]. The microenvironment and neoplastic cells can become fixed in positive feedback loops that perpetuate cancer and allow it to progress, invade adjacent tissue, and metastasize to distant sites.

Comparison of inflammation at different mucosal sites: the intestine and bladder

Our understanding of mechanisms involved in inflammation-associated bladder cancer significantly lags behind our understanding of inflammation-associated cancers of another epithelial organ, the intestinal tract. Given the prominence of lymphoid tissue structures found in association with the intestine and the substantial residential microbial community present in the intestinal lumen, the intestinal epithelium probably receives dramatically different inflammatory signals compared with the relatively sterile bladder urothelium. The Peyer's patches of the intestine are lymphoid structures which have recently been observed as preferential sites of egress for *S. mansoni* eggs to join the fecal stream [51], while it is arguable whether any substantial lymphoid structures exist in the human bladder save when bladder inflammation, or