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

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Abstract

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 due 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.

Keywords

urogenital schistosomiasis; bladder cancer; Schistosoma haematobium; urinary schistosomiasis; schistosomiasis haematobia

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

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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 [17,18]). 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 non-endemicity, including nitrosamines and other chemical exposures from industrial and agricultural sources, as well as from tobacco smoking (reviewed in [19,20]). Thus, multiple factors may intersect to confer increased risk for bladder cancer associated with *S. haematobium* infection in humans.

Though the association between *S. haematobium* infection and bladder cancer is strong, identification of the underlying mechanisms has progressed slowly and likely hampers the diagnosis and successful treatment of urogenital schistosomiasis–associated bladder cancer. The interrelated reasons for slow progress in this field include: 1) lack of a tractable animal model to study the progression of urogenital schistosomiasis; 2) until recently, a paucity of genomic information about *S. haematobium*; 3) few genetic tools to manipulate life stages of *S. haematobium*; and 4) an incomplete catalog of mutations that may be unique to human schistosomal bladder cancer compared to other bladder cancers. Over the past few years, new research efforts have directly targeted some of these key roadblocks [21–23], opening new avenues to investigate *Schistosoma 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, with potential to test broader questions regarding the role of inflammation in epithelial cancers.

The inflammatory environment during acute and chronic *S. 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 [24,25]. 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 [26–28]. However, the potentially unique properties of inflammatory responses in the bladder mucosa [29] 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 [30]. 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 site of human pathology [31]. Infection of hamsters and non-human primates with *S. haematobium* cercariae leads to worm maturation and oviposition [32]. Hamsters, however, exhibit low rates of pelvic organ infection, and instead develop predominantly hepato-enteric schistosomiasis. In 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 [33–35]. Though 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 [36].

Several years ago we sought to help overcome this impasse in animal models of urogenital schistosomiasis. By micro-injecting a bolus of *S. haematobium* eggs into the bladder wall of mice [37], 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 [21]. 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 [38]; these processes of de-differentiation may be relevant to bladder carcinogenesis and cancer progression [39]. 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 [40]). 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 [41–44].

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 [45]. When taking up residence within particular host tissues, microbes and macro-organisms may initiate inflammation that can lead to eventual cancer formation [9,46,47]. While 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 [48–52]. 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 likely receives dramatically different inflammatory signals compared to the comparatively 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 [53], while it is arguable whether any substantial lymphoid structures exist in the human bladder save when bladder inflammation, or cystitis, is present [54]. Importantly, *S. haematobium* infection leads to profound and persistent aggregations of lymphoid cells in subepithelial regions of the bladder [55–57]. Thus, studying urogenital schistosomiasis may lead to a broader understanding of how chronic cystitis results in bladder carcinogenesis (reviewed in [58]).

To date, the effects of inflammatory signaling on urothelial cells remain poorly characterized. The transcriptional innate immune response of the urothelium to bacterial infection has been examined [59,60], but it remains to be seen what effects specific cytokines exert on the urothelium. This is relevant to understanding schistosomal bladder cancer considering that *S. haematobium* eggs are powerful inducers of host cytokine responses. STAT3 is a key transcription factor activated within cells by inflammatory signals and has been implicated in malignant transformation of the colon as a result of IL-6 signaling [61–64]. Constitutively active STAT3 in urothelial cells has been demonstrated to lead to invasive bladder cancer in a mouse model [65], but the inflammatory cues that might precipitate such signaling remain to be determined.

The effects of inflammation on epithelial regeneration and carcinogenesis are relevant to understanding epithelial neoplasms in general. Consequently, the unique structure of the intestinal crypt has been probed with lineage tracing tools to follow the contribution of

various cell populations to epithelial regeneration in this tissue site. Recently, some approaches previously used to investigate the intestinal epithelium have been adapted to characterizing urothelial regeneration, resulting in important new insights. Mysorekar and colleagues demonstrated that bone morphogenetic protein 4 (Bmp4) plays an indispensable role in replenishment of bladder urothelium during bacterial injury, but not chemical injury with protamine sulfate [66]. Using inducible reporter systems to mark progenitor cells and their progeny, sonic-hedgehog (*Shh)* expressing basal cells were shown to reconstitute the urothelium after both bacterial infection and chemical injury [67]. We have performed lineage tracing experiments using multi-color reporter systems that suggest that clonal bladder tumors induced by chronic exposure to the compound N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) arise exclusively from the *Shh*-expressing cell population [68]. In a different injury model based on use of cyclophosphamide, suprabasal ‘intermediate’ cells that form a portion of the *Shh*-expressing bladder cell population can contribute substantially to reconstitution of the adult urothelium [69], though it is untested whether these intermediate cells would contribute to tumor formation after BBN treatment.

Nevertheless, it is now becoming clear that the cells involved in the processes of homeostatic maintenance (turnover) and regeneration after epithelial injury can vary by tissue and type of injury (reviewed in [70]). Identifying the cell of origin involved in schistosomal bladder cancer could help answer the question of why *S. haematobium* infection is predominantly, though not exclusively, associated with squamous cell carcinoma (SCC) of the bladder, rather than the urothelial carcinoma type that is more prevalent worldwide and arises later in life in populations not exposed to *S. haematobium* 9,20]. It is not understood whether transdifferentiation of the urothelium to squamous metaplasia occurs prior to or subsequent to transformation [71–73]. Interestingly, squamous metaplasia is often observed in *S. haematobium*-infected bladders featuring SCC (Figure 2), providing some plausible support in favor of metaplasia being part of the neoplastic sequence (Figure 3). Defining this progression in the setting of urogenital schistosomiasis may be an important basis for improving diagnosis and treatment options.

One pathway potentially involved in this progression involves retinoic acid (vitamin A), a lack of which results in squamous metaplasia of the bladder urothelium [72]. Indeed, regions endemic for schistosomiasis overlap extensively with areas known for widespread vitamin A deficiency. Thus, we speculate that many people exposed to *S. haematobium* may have vitamin A deficiency-associated squamous metaplasia of the bladder. The requirement for retinoic acid receptors in urothelial cells for the processes of urothelial formation and regeneration was recently demonstrated [69]. Further, the immune system deploys retinoic acid receptors for diverse, important functions such as control of trafficking of dendritic cell precursors to the intestine [74] and establishment of secondary and tertiary lymphoid organs by lymphoid tissue inducer (LTi) cells [75,76]. These diverse roles of vitamin A imply that it may be insightful to study how it shapes the relationship between the immune system and urothelial regeneration and differentiation in the context of *S. haematobium* infection. Thus, continued work on the dynamics of bladder urothelial regeneration may suggest experiments to explore how particular urothelial and immune cell populations interact in the context of *S. haematobium* egg-associated inflammation and carcinogenesis.

The role of co-infections in carcinogenesis

Akin to the challenge presented by a developing tumor, managing an active helminth infection presents the host with potential survival tradeoffs between the resistance and tolerance mechanisms available [77,78]. For example, the immunoregulatory dimension (IL-10 and TGF-β) of an established T helper 2 (Th2, type 2) response to helminths may delay or prevent clearance of viruses or bacteria that otherwise requires full initiation of T helper 1 (Th1, type 1) effector molecules and immune cells [79,80]. Schistosome infection may thus significantly alter susceptibility and progression of viral, bacterial or protozoan infections both locally and elsewhere in the body [81,82].

Viral and bacterial infections have been linked to initiation of cancers at a variety of different organ sites. Specific pathogenesis mechanisms used by these bacteria and viruses to invade or otherwise modulate host cells can facilitate transformation by destabilizing the host genome [83]. The inflammatory environment established by the presence of *S. haematobium* eggs in the bladder lamina propria, and especially the tolerizing influence of IL-10 and TGF-β during chronic phases of helminth infection more broadly [80], may counteract successful clearance of viruses or bacteria that take up residence in tissues such as the bladder.

In addition to the impact of viral integration on the host genome, viruses can promote cancer through expression of viral oncoproteins that may alter rates of cellular proliferation, suppress apoptosis, and increase genomic instability through interference with cell cycle regulation and DNA repair mechanisms. Several groups have investigated a possible role for human papillomavirus (HPV) involvement in schistosomal bladder cancer, with some studies finding potential associations [11,84,85] whereas samples from patient groups from other geographic regions do not show evidence for the HPV strains studied [86]. The definitive demonstration of HPV or other viral involvement in the etiology of schistosomal bladder cancer may be complicated by the possibility of “hit-and-run” scenarios where viruses transiently affect the genetic or epigenetic states of cells in a pro-tumorigenic manner, yet may be lost from subsequent tumors if viral persistence is not necessary for tumor survival [87]. Nevertheless, deep sequencing could be an unbiased approach to identifying persistent viruses that may be associated with schistosomal bladder cancers [88].

Some studies have observed an association in humans between *S. haematobium* infection and bacterial urinary tract co-infections, with evidence that levels of procarcinogenic N-nitrosamines in urine are increased [89]. Additional work by Hicks and colleagues suggest that sub-carcinogenic doses of nitrosamines are not sufficient to induce bladder cancer in baboons, except for those infected with *S. haematobium* 90]. This has implications for the potential role of nitrosamines in schistosomal bladder cancer. Specifically, if *S. haematobium*-bacterial uropathogen co-infection is common in humans and bacterial metabolism indeed leads to higher nitrosamine levels in the bladder, is bacterial co-infection sufficient in and of itself to induce bladder cancer in some individuals? Or do these individuals need exposure to additional nitrosamine sources, for example through tobacco products? Clearly, any application of the rodent model of nitrosamine-induced bladder cancer to animal models of urogenital schistosomiasis must be undertaken carefully. These

issues underscore the potential complexity of the role of nitrosamines and microbial co-infection in schistosomal bladder cancer.

Carcinogenic effects of products from *Schistosoma haematobium* eggs

Schistosome eggs are well known for their ability to influence surrounding host tissues. For instance, soluble egg antigen (SEA) preparations from *S. mansoni* eggs have been studied for their effects on a variety of host cell types. Omega-1 protein from *S. mansoni* eggs shapes dendritic cell promotion of Th2 activation via protein internalization by the mannose receptor and subsequent degradation of messenger and ribosomal RNAs [91]. Another *S. mansoni*-derived egg protein with similar potential for entry into host cells is IPSE/alpha-1 [92], which also features other immunomodulatory properties [93]. Botelho and colleagues showed that SEA preparations from *S. haematobium* may have proliferative and genotoxic effects on urothelial cell cultures [94], potentially through effects on estrogen receptor activity [95]. Interpretation of these results, however, must be tempered by the knowledge that immortalized urothelial cells were used in this work, which by definition differs from normal urothelium. This work has similarities to observations made by Smout *et al*., who demonstrated that the carcinogenic liver fluke *O. viverrini* produces a homolog for granulin, a potent human growth factor implicated in cell proliferation and wound healing, that drives proliferation of host cells [96].

The recent genomic and transcriptomic characterization of *S. haematobium* adult worms and eggs [23] may greatly accelerate identification and characterization of *S. haematobium*-specific factors that may be involved in cancer induction. Ongoing improvements in the annotation of the genome will further drive a deeper understanding of procarcinogenic schistosome products. Co-culture of these parasite products with urothelial cells will facilitate screening assays for proliferative, transcriptional, epigenetic, and metabolic endpoints consistent with cancer phenotypes. Targets worthy of deeper scrutiny could then be tested for *in vivo* activity by injecting transgenic eggs [22] into the mouse bladder wall [21]. Physiologic validation of candidate procarcinogenic schistosome products will be essential to understanding schistosomal bladder cancer.

Mapping the genetic and transcriptional landscape of human schistosomal bladder cancer

Bladder cancers are generally understood to group into somewhat exclusive phenotypes based on *FGFR3* and *TP53* mutations. Whereas the former cancers tend to be recurrent but superficial and non-muscle invasive, the latter set of neoplasms exhibit a propensity for rapid development of carcinoma *in situ* and progression to muscle invasion [97,98]. Most of this body of research has been based on investigation of non-schistosomal bladder cancer cases in developed countries. The association of decreasing prevalence of squamous cell carcinoma (SCC) of the bladder in Egypt with falling schistosome infection rates suggests a link between urogenital schistosomiasis and bladder cancer histology [99]. Shifts in agricultural employment, pesticide and urbanization may be increasingly powerful factors in observed changes in bladder cancer risk and histology within Egypt [100], underscoring the complex interactions involved in schistosomal bladder cancer.

Our knowledge to date about the particular pathways altered in schistosomal bladder cancer has been based on immunohistochemical analyses of formalin-fixed, paraffin-embedded tumor specimens. Examination of markers involved in SCC (schistosomal and non-schistosomal) have suggested possible discriminatory power for p53, p27, and Ki67, along with various cyclins and cytokeratins [101–104].

Though informative, these immunohistochemistry approaches are low-throughput, require validated reagents, and rely on prior knowledge about potentially relevant pathways. Moreover, these analyses typically focus on well-established, end-stage cancers that have accumulated numerous mutations, with little or no evidence of causality or sequence. Significant molecular complexity may remain unobserved. The efforts to uncover this complexity has driven the formation of research consortia to apply high-throughput sequencing to analyze the exomic, transcriptomic, epigenomic, and genetic architecture of a broad range of cancers (i.e., the Cancer Genome Atlas: http://cancergenome.nih.gov/ and the International Cancer Genome Consortium: https://www.icgc.org/), including urothelial cell carcinoma of the bladder [105]. Sadly, as of this review, schistosomal bladder cancer is not currently one of the diseases targeted by these consortia. Applying high-throughput sequencing to non-schistosomal urothelial carcinomas of the bladder has already suggested new markers that may aid in prognosis and guide treatment [106–109]. Comparing squamous and urothelial forms of schistosomal bladder cancer with bladder cancers of other etiologies could provide important new insights into the relative contributions of *S. haematobium* products, possible viral or bacterial co-infections, and inflammation on cancer induction or progression. Further, genomic sequences of schistosomal bladder cancers could be compared to squamous cell carcinomas from other tissues [110–112], which may suggest common and unique pathways at work in these aggressive cancers. Earlier sequencing technologies were applied to cholangiocarcinomas associated with *O. viverrini* infection and have documented many previously unrecognized mutations in this form of cancer [7]. With new sequencing and analysis approaches allowing comprehensive measurements of complex genetic rearrangements [113], it is now possible to gain useful insights into the myriad changes occurring in tumors such as schistosomal bladder cancers [114].

Concluding remarks and future perspectives

The initiation and progression of schistosomal bladder cancer is almost certainly a complex and multi-step process, involving the actions of the parasite, the host immune and tissue regenerative response, and possible participation of co-infections, micronutrients (e.g., vitamin A), and environmental exposures to carcinogens. Superimposed on these processes are background factors consisting of parasite genetic diversity, individual variations in intensity and recurrence of infection, host genetic diversity (including polymorphic loci that affect immune responses or susceptibility to cancer), and sex differences in immune and urogenital biology. Though animal models will never recapitulate all aspects of these complex processes, appropriate application of such models will play an important role in efforts to isolate and characterize the pathways involved in schistosomal bladder cancer.

Unbiased molecular approaches will be critical to generating hypotheses about the transformation processes that gives rise to human bladder cancers associated with urogenital

schistosomiasis. The sequencing approaches described above, while useful in addressing this need, face the ongoing challenges of reliably reducing false positives and correctly identifying driver versus passenger mutations [115]. Again, improved animal models of urogenital schistosomiasis will play an important role in demonstrating the relevance of candidate cancer mechanisms.

Further investigation into the immunological properties of the mammalian bladder will clarify its shared and unique properties with the immunologically distinct epithelial sites of the skin, gut, and lung. By investigating the behavior of key morphogenetic pathways (Wnt, Notch, Shh) in progenitor cell populations during inflammatory responses in the bladder, we may begin to characterize how urothelial regeneration becomes neoplastic during chronic *S. haematobium* infection, with implications for our understanding of other inflammation-associated cancers that arise from epithelial cells.

In contrast to the perception of urogenital schistosomiasis being an exotic infectious disease of marginal importance, *Schistosoma haematobium* may infect nearly 200 million people worldwide [25]. Given that bladder cancer may be the most expensive neoplasm to manage from diagnosis to death, in large part due to its recurrent nature [116], it makes sense from both the economic and human perspectives to invest significant, further effort in understanding schistosomal bladder cancer in order to improve diagnostic and therapeutic approaches.

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Highlights

- The causal link between *S. haematobium* infection and bladder cancer is accepted
- The mechanisms of schistosomal bladder cancer (SBC) are poorly understood
- Newly identified bladder regeneration pathways may be relevant mechanisms to SBC
- New mouse models and genomics methods may expand knowledge of SBC

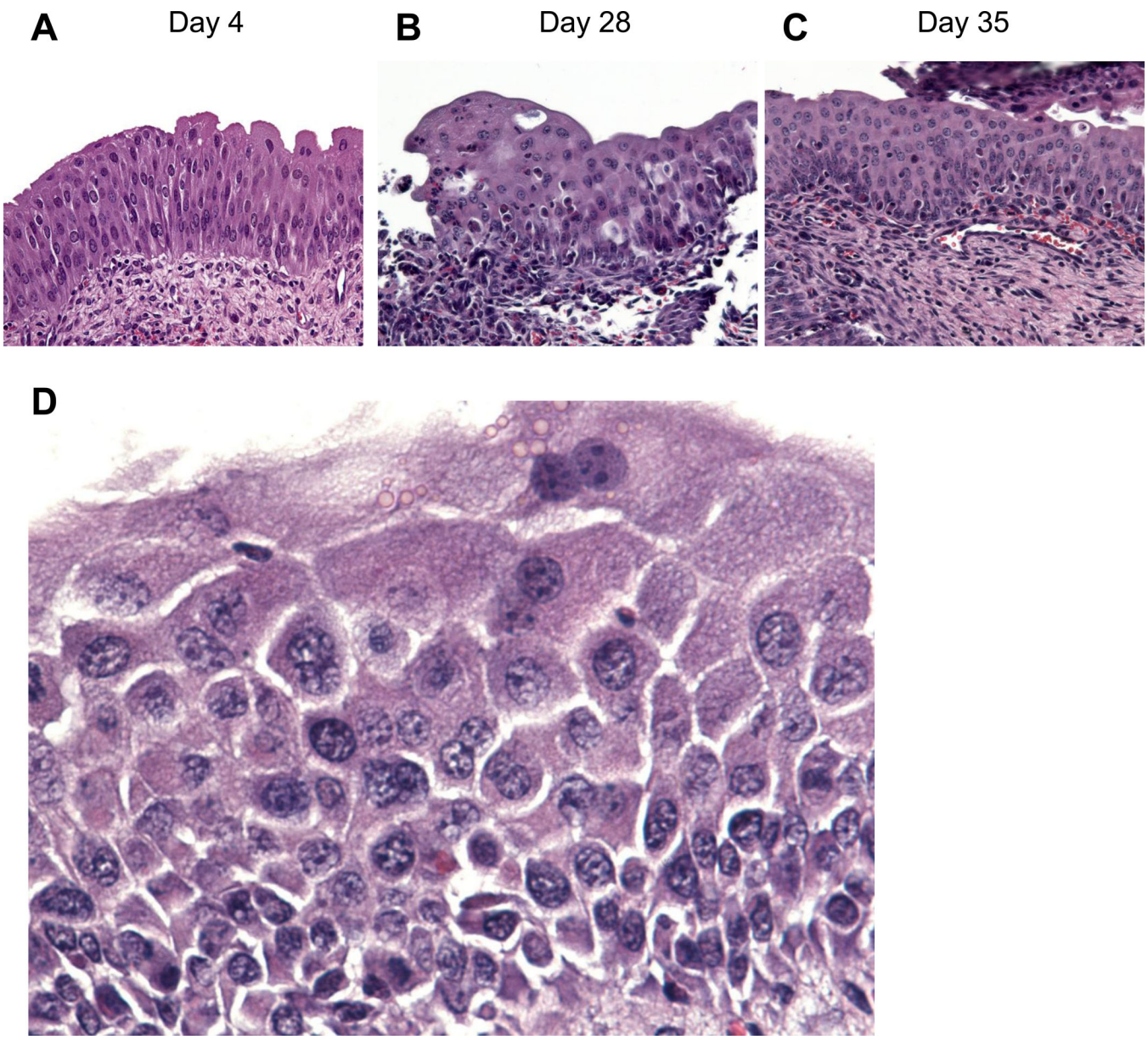

Figure 1.

Schistosoma haematobium eggs may induce mouse bladder preneoplasia. (A-C) egg injection of the mouse bladder wall induces early and sustained urothelial hyperplasia (urothelium >3 cells thick) with reactive nuclear changes. Adapted from Fu et al. [117]. (D) egg injection of the mouse bladder wall also induces squamous metaplasia, with typical signs of very fine, stratum spinosum-like spiny projections between cobblestone-resembling urothelial cells with foamy cytoplasm. Urothelial hyperplasia and squamous metaplasia may be preneoplastic lesions, in that they may be necessary but not sufficient for frank bladder carcinogenesis.

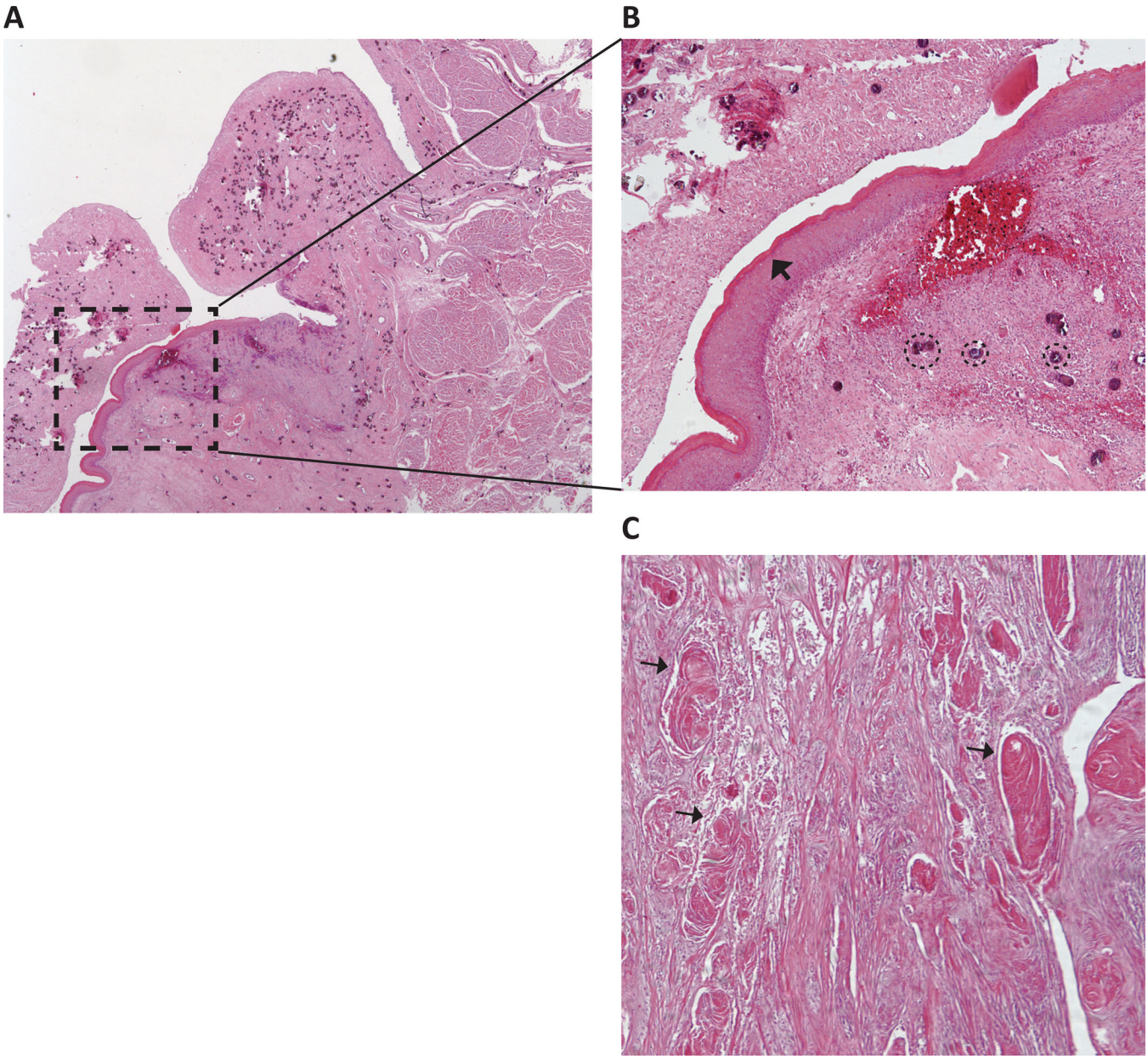

Figure 2.

Schistosoma haematobium-associated bladder squamous metaplasia and carcinoma. Micrographs from stained sections of a bladder with keratinized, moderately differentiated squamous cell carcinoma associated with urogenital schistosomiasis. (A) Low power view of bladder section. (B) Higher power view of area indicated by arrow in (A) demonstrating squamous metaplasia of the urothelium with infiltration of the lamina propria by a large number of *S. haematobium* ova (several eggs are circled as examples). In this specimen, the squamous metaplasia is evident as a hyperkeratotic squamous epithelium (arrowhead) lining the bladder lumen. (C) Another region of the same bladder exhibits abundant keratin pearls (examples indicated by arrows), a classic sign of squamous cell carcinoma.

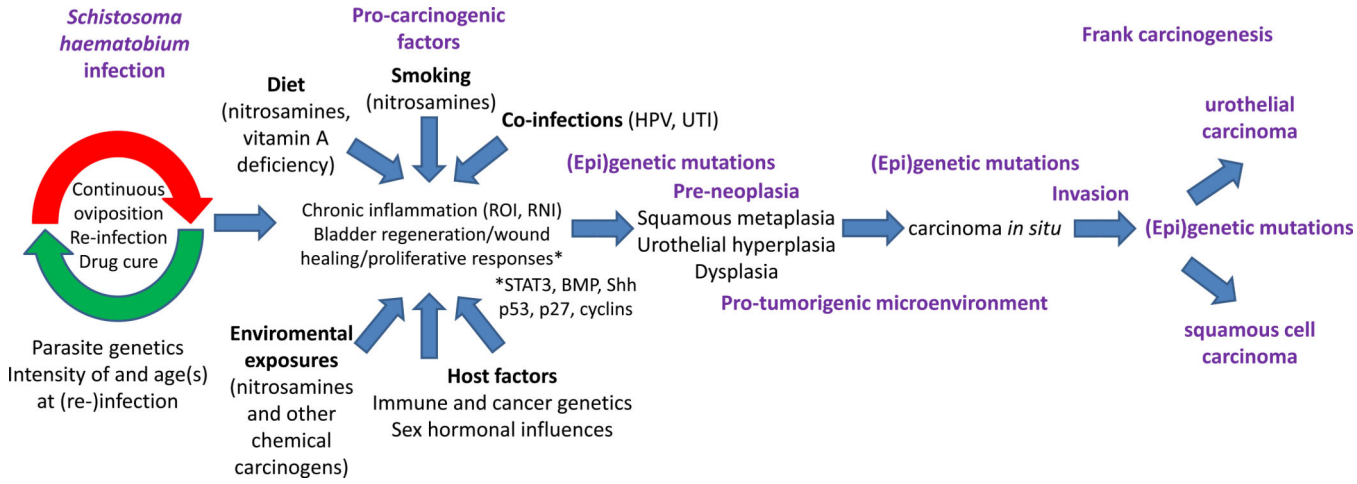

Figure 3.

Possible risks and pathways for schistosomal bladder cancer. Long-lived *S. haematobium* worms and re-infection of hosts by new parasites results in long-term egg deposition in the bladder. Oviposition kinetics are subject to modulation by *S. haematobium* strain genetics, intensity and host age(s) at parasite exposure, anthelminthic chemotherapy, and host immunity. Egg deposition in the bladder leads to chronic inflammation and host regenerative, proliferative, and wound healing responses (possibly STAT3, BMP, sonic hedgehog [Shh], p53, p27, and cyclin-mediated), all of which are modified by numerous cancer risk factors, such as diet (nitrosamines in beer and other fermented foods, micronutrient deficiencies), smoking (another source of nitrosamines and other carcinogens), co-infections by human papillomavirus (HPV) and/or bacterial uropathogens (urinary tract infections, UTI), exposure to environmental carcinogens (including nitrosamines and other occupational chemicals), and host polymorphisms in tumor suppressor genes, oncogenes, and immune function-related genes. Gender, in the form of sex hormones, may also be an important risk factor, given that males are more prone to bladder cancer in general. Genetic mutations and epigenetic alterations (i.e., DNA methylation, histone modification) accumulate in bladder urothelial cells, driving pre-neoplastic lesions such as squamous metaplasia, urothelial hyperplasia, and dysplasia. At the same time, a pro-tumorigenic microenvironment (both stromal and immunologic) arises in this context. As additional genetic and epigenetic abnormalities continue to develop, carcinoma *in situ* ensues, with eventual invasion and onset of urothelial or squamous cell carcinoma.