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Review

Recent advances in *Echinococcus* genomics and stem cell research

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ABSTRACT

Alveolar and cystic echinococcosis, caused by the metacestode larval stages of the tapeworms *Echinococcus multilocularis* and *Echinococcus granulosus*, respectively, are life-threatening diseases and very difficult to treat. The introduction of benzimidazole-based chemotherapy, which targets parasite β-tubulin, has significantly improved the life-span and prognosis of echinococcosis patients. However, benzimidazoles show only parasitostatic activity, are associated with serious adverse side effects and have to be administered for very long time periods, underlining the need for new drugs. Very recently, the nuclear genomes of *E. multilocularis* and *E. granulosus* have been characterised, revealing a plethora of data for gaining a deeper understanding of host-parasite interaction, parasite development and parasite evolution. Combined with extensive transcriptome analyses of *Echinococcus* life cycle stages these investigations also yielded novel clues for targeted drug design. Recent years also witnessed significant advancements in the molecular and cellular characterisation of the *Echinococcus* 'germinative cell' population, which forms a unique stem cell system that differs from stem cells of other organisms in the expression of several genes associated with the maintenance of pluripotency. As the only parasite cell type capable of undergoing mitosis, the germinative cells are central to all developmental transitions of *Echinococcus* within the host and to parasite expansion via asexual proliferation. In the present article, we will briefly introduce and discuss recent advances in *Echinococcus* genomics and stem cell research in the context of drug design and development. Interestingly, it turns out that benzimidazoles seem to have very limited effects on *Echinococcus* germinative cells, which could explain the high recurrence rates observed after chemotherapeutic treatment of echinococcosis patients. This clearly indicates that future efforts into the development of parasitocidal drugs should also target the parasite's stem cell system.

Contents

1. Introduction

The term echinococcosis is used to describe a group of zoonotic diseases caused by infection with the metacestode larvae of tapeworms of the genus *Echinococcus* (Cestoda: Taeniidae). From the

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medical and veterinary point of view, the two most important species of this genus are *Echinococcus granulosus* (the "dog tapeworm") and *Echinococcus multilocularis* (the "fox tapeworm"), the causative agents of cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively (reviewed by Eckert and Deplazes (2004)).

The life cycle of *Echinococcus* spp. is complex and involves two mammalian hosts (Eckert and Deplazes, 2004; Thompson, 1986). The adult tapeworm develops attached to the small intestine of the definitive hosts, which are typically dogs for *E. granulosus*, and red or arctic foxes for *E. multilocularis*. The adults of *Echinococcus* spp. show a relatively typical cestode morphology, with an anterior scolex containing the attachment organs, and a neck region that generates a short chain of segments (proglottids), each one containing a complete set of male and female reproductive organs. The mature proglottids, containing infective eggs, are released with the faeces of the definitive host. Each egg contains an oncosphere (the first larval stage) that is infective if ingested by the intermediate host. Several ungulate species serve as intermediate hosts for the different lineages of the *E. granulosus* species complex, most of them being domestic species (Eckert and Deplazes, 2004). In contrast, several wild rodent species are the natural intermediate host for *E. multilocularis* (Craig, 2003; Eckert and Deplazes, 2004; Rausch, 1954). Humans can also be an accidental host for *E. granulosus* and *E. multilocularis*, although they are a dead-end for the life cycle of the parasite.

When *Echinococcus* eggs are ingested by the intermediate host, the oncospheres hatch in the intestine, penetrate the intestinal wall and are transported by the bloodstream. Most commonly, primary infections develop in the liver (Brunetti et al., 2010; Eckert and Deplazes, 2004), where the oncosphere metamorphoses into the next larval stage, the metacestode. The metacestodes develop as fluid-filled vesicles, comprising a thin layer of tissue (the germinal layer) covered by a syncitial tegument that secretes an acellular, carbohydrate-rich external layer (the laminated layer). Within the germinal layer, thickenings occur that invaginate into the vesicle, resulting in the formation of brood capsules (Koziol et al., 2013; Leducq and Gabrion, 1992), where eventually protoscoleces are formed, which are the infective larvae for the definitive host. The development of metacestodes as fluid-filled cysts that generate protoscoleces asexually is an evolutionary novelty, and asexual reproduction is very rare among cestodes (Freeman, 1973; Slais, 1973; Trouvé et al., 2003). Protoscoleces remain quiescent, with the scolex invaginated within a small posterior body. When the definitive host ingests an infected intermediate host, it also ingests the protoscoleces, which then evaginate their scolex and attach to the intestine, thus closing the life cycle.

E. multilocularis and *E. granulosus* differ in the morphology and development of the metacestode (Eckert and Deplazes, 2004; Thompson, 1986). In the case of *E. granulosus*, each oncosphere develops into a single vesicle ("unilocular development") that can grow to huge dimensions, exceeding 20 cm in diameter. In the case of *E. multilocularis*, new metacestode vesicles are generated by exogenous budding of the metacestode, which therefore develops as a multilocular labyrinth of interconnected vesicles (Eckert et al., 1983; Rausch, 1954; Sakamoto and Sugimura, 1970; Tappe et al., 2010). In human AE (alveolar echinococcosis), the *E. multilocularis* metacestode tissue therefore grows infiltratively like a malignant tumor into the surrounding liver tissue and, in later stages of the disease, can even form metastases in secondary organs. If not adequately treated, parasite expansion will eventually lead to organ failure and death (Brunetti et al., 2010; Eckert and Deplazes, 2004; Kern, 2010). Although the *E. granulosus* metacestode (the 'hydatid cyst') grows more 'benign' (*i.e.* not infiltratively), steady growth of hydatid cysts within the liver, lungs or the brain can also lead to mechanical pressure and to pathological changes associated with compression or obstruction (Brunetti et al., 2010; Eckert and Deplazes, 2004).

Cure of the disease can only be achieved by surgical removal of metacestode tissue in combination with anti-parasitic chemotherapy or, as possible in some cases of CE (cystic echinococcosis), inactivation of hydatid cysts by minimal invasive procedures such as PAIR (punctuation, aspiration, injection, reaspiration), combined with chemotherapy (Brunetti et al., 2010). However, and particularly in AE, complete surgical removal of parasite material is in most cases not possible due to the fact that the disease has been diagnosed at a late stage so that large regions of the liver are affected. In these cases, chemotherapy is the only treatment option. Currently, both anti-AE and anti-CE chemotherapy rely on benzimidazoles (*e.g.* albendazole, mebendazole) which target parasite β-tubulin, thus preventing the formation of microtubuli (Brehm et al., 2000b; Brunetti et al., 2010; Stojkovic et al., 2009). Since its introduction in 1978, benzimidazole-based chemotherapy has significantly improved disease outcome and prognosis of echinococcosis patients. However, due to the fact that host and parasite β-tubulin are highly similar (>90% identical amino acid residues), benzimidazole therapy is associated with significant adverse side effects (Brunetti et al., 2010). Furthermore, only parasitostatic doses can be given to patients so that benzimidazole chemotherapy has to be administered for long periods of time (up to life-long) and is associated with high recurrence rates (Brunetti et al., 2010; Kern, 2010). Altogether, this underlines the urgent need for novel anti-parasitic compounds that can completely inactivate metacestode tissue instead of just diminishing growth.

In addition to a closer understanding of parasite biology, the host-parasite relationship and parasite evolution, the quest for novel chemotherapeutic targets was surely a major rationale for the initiation of whole genome sequencing projects for *E. multilocularis* and *E. granulosus*, about 10 years ago, which culminated in the release of two highly recognised publications in 2013 (Tsai et al., 2013; Zheng et al., 2013). In the following we will briefly outline the characteristics of *Echinococcus* genomes and how this information, when combined with transcriptomics, can be used for the identification of novel drug targets. Furthermore, we will review recent progress in *Echinococcus* stem cell research which should be highly relevant for the development of parasitocidal chemotherapeutics.

2. Echinococcus genomics

In March 2004 a meeting was held at the Wellcome Trust Sanger Institute (Hinxton, UK) that eventually led to the still ongoing '50 helminth genomes initiative' (Holroyd and Sanchez-Flores, 2012). At this time point, no draft genome sequence of a parasitic helminth had been published, although the projects for the trematodes *Schistosoma mansoni*, *Schistosoma japonicum*, and the nematode *Brugia malayi* were in an advanced stage (Brindley et al., 2009). During the meeting, it was decided to complement these efforts by whole genome sequencing projects for additional nematodes, trematodes and (at that time point) at least one cestode. Although worldwide there are more cases of CE than AE, *E. multilocularis* has been chosen as the species for producing a high-quality reference genome due to the fact that it is much more accessible for *in vitro* cultivation (Brehm and Spiliotis, 2008; Hemphill et al., 2010, 2003; Spiliotis et al., 2008, 2004) and, as it later turned out, for genetic manipulation (Mizukami et al., 2010; Spiliotis et al., 2010). By combining classical capillary sequencing with next generation sequencing (NGS) approaches (454, Illumina) and manual curation, a draft genome was assembled in which 89% of the sequence was contained in 9 chromosome scaffolds with very few gaps, and one chromosome (No. 5) was even complete from telomere to telomere (Tsai et al., 2013). Genetic homogeneity due to inbreeding