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Research Paper

Pan-Nematoda Transcriptomic Elucidation of Essential Intestinal Functions and Therapeutic Targets With Broad Potential

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ABSTRACT

The nematode intestine is continuous with the outside environment, making it easily accessible to anthelmintics for parasite control, but the development of new therapeutics is impeded by limited knowledge of nematode intestinal cell biology. We established the most comprehensive nematode intestinal functional database to date by generating transcriptional data from the dissected intestines of three parasitic nematodes spanning the phylum, and integrating the results with the whole proteomes of 10 nematodes (including 9 pathogens of humans or animals) and 3 host species and 2 outgroup species. We resolved 10,772 predicted nematode intestinal protein families (IntFams), and studied their presence and absence within the different lineages (births and deaths) among nematodes. Conserved intestinal cell functions representing ancestral functions of evolutionary importance were delineated, and molecular features useful for selective therapeutic targeting were identified. Molecular patterns conserved among IntFam proteins demonstrated large potential as therapeutic targets to inhibit intestinal cell functions with broad applications towards treatment and control of parasitic nematodes.

1. Introduction

Parasitic nematodes comprise a major group of pathogens that infect nearly one third of the human population, and compromise, or threaten, the health and productivity of most agricultural animals and plants throughout the world. The selection of anthelmintics (nematicides) that can be used for treatment and control of these pathogens is relatively limited, and acquired resistance to anthelmintics by parasitic nematodes is a growing problem (Albonico et al., 2004; Awadzi et al., 2004; Bourguinat et al., 2008; Osei-Atweneboana et al., 2007; Vercruysse et al., 2012). Consequently, a need exists to identify new parasite targets for therapeutic intervention and the nematode intestine provides a tissue to investigate for this purpose. The intestine of parasitic nematodes, and nematodes in general, is sited at an internal interface that is continuous with the outside environment. As such, the intestine is accessible to anthelmintics that can interfere with intestinal cell functions essential for survival of nematodes. The intestine has a relatively simple tubular design formed by a single cell layer of intestinal cells that resemble polarized epithelial cells (Yin et al., 2008). This single cell layer serves as a physical separation between the environment and the pseudocoelomic body cavity. Evidence indicates that a wide range of functions involved in nutrient acquisition (McGhee, 2007; Yin et al., 2008; Jasmer et al., 2015; Rosa et al., 2015), physiological homeostasis and basic defense against environmental toxins (Park et al., 2001) are all sited at the apical intestinal membrane. Intestinal cells are also a primary site for synthesis of yolk proteins that are eventually incorporated into ova (Chotard et al., 2010). Nematode intestinal cells Protein families (orthologous groups) were defined utilizing the Markov cluster algorithm (Enright et al., 2002) using the OrthoMCL also have clear importance as targets for anthelmintic therapies related to vaccines (Jasmer et al., 1993; Smith, 1993; Pearson et al., 2010), contemporary anthelmintics (Jasmer et al., 2000) and biotoxins (Wei et al., 2003). Nevertheless, the limited knowledge on biological properties of nematode intestinal cells and the extent to which those properties are conserved among parasitic species has impeded research aimed at developing new therapeutic methods directed at this tissue. Indeed, the challenges related to research on the nematode intestine extend to virtually every tissue in parasitic nematodes. Hence, methods related to solving challenges related to the intestine have application to other tissues of nematode pathogens.

Nematode species display remarkable versatility in relation to the diverse trophic niches that they occupy and display substantial diversity at morphological levels of the intestine (Munn and Greenwood, 1984). Hence, while many basic intestinal cell characteristics are likely to be broadly conserved, other characteristics may markedly vary among different nematode lineages and species. Orthologous protein families

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conserved among phylogenetically diverse nematode species have been investigated (Mitreva et al., 2011), while only modest attempts have been made to deduce characteristics of nematode intestinal cells that are conserved among, and potentially ancestral to, all nematodes (Yin et al., 2008).

We initially sought to develop computational platforms to distinguish between subsets of intestinal proteins that are essential to all nematodes and those that may be related to success of individual lineages or species. A comprehensive assessment of this kind will provide a knowledge base that will drive development of research hypotheses and support research to elucidate biological functions essential for the survival of many, or all parasitic nematodes.

The selection of species investigated was critical for success in this research (Fig. 1). Using RNA from dissected intestines we directly identified the genes expressed in the adult intestine of three parasitic nematode species of livestock: *Trichuris suis*, *Ascaris suum* and *Haemonchus contortus*. Each of these is a soil transmitted pathogen with model applications to other phylogenetically related pathogens of humans and animals. Each of these species, referred to here as 'core species', is a member of a different and diverse nematode taxonomic clade (I, III and V, respectively (Blaxter et al., 1998)), and each core species occupies a distinct trophic niche (epithelial layer of the swine cecum, swine small intestinal lumen and blood feeder at the abomasal mucosa of small ruminants, respectively), likely requiring diverse adaptations for success (Fig. 1). Therefore, intestinal cell proteins and functions conserved among these phylogenetically and biologically diverse nematodes are likely to reflect basic features ancestral to many or all nematodes. Beyond these considerations, the relatively large size of the adult worms of each of the core species supports dissection to obtain intestinal tissue and RNA, which is otherwise difficult for most nematode species.

We sought to identify nematode intestinal cell characteristics based on direct genome-wide intestinal transcript evidence for species that span the phylum Nematoda. Transcript expression data was coupled with predicted or known intestinal proteins to deduce functional characteristics that are broadly conserved among (and potentially ancestral to) all nematodes. A platform was also established to assess variation in intestinal cell characteristics that may have contributed to the diversity among nematode lineages and species, at a whole-protein sequence level. Comparisons of deduced functions assessed the determinants of evolutionary success at the phylum, lineage and species levels. Data distinguishing conserved and variable characteristics were then used to explore molecular patterns of proteins that may have broad application to chemical intervention of nematode intestinal cell functions. Importantly, the research also established a general approach that can be used to derive proteins and functions of ancestral importance to other nematode tissues such as neuromuscular, hypodermal and reproductive tissues, to name a few.

2. Materials & Methods

2.1. Ethics Statement

The research involving use of swine was reviewed and approved by the Washington State University Institutional Animal Care and Use Committee, protocol #04097-004, approved on 12/19/2013. Guidelines are provided by the Federal Animal Welfare Act, USA.

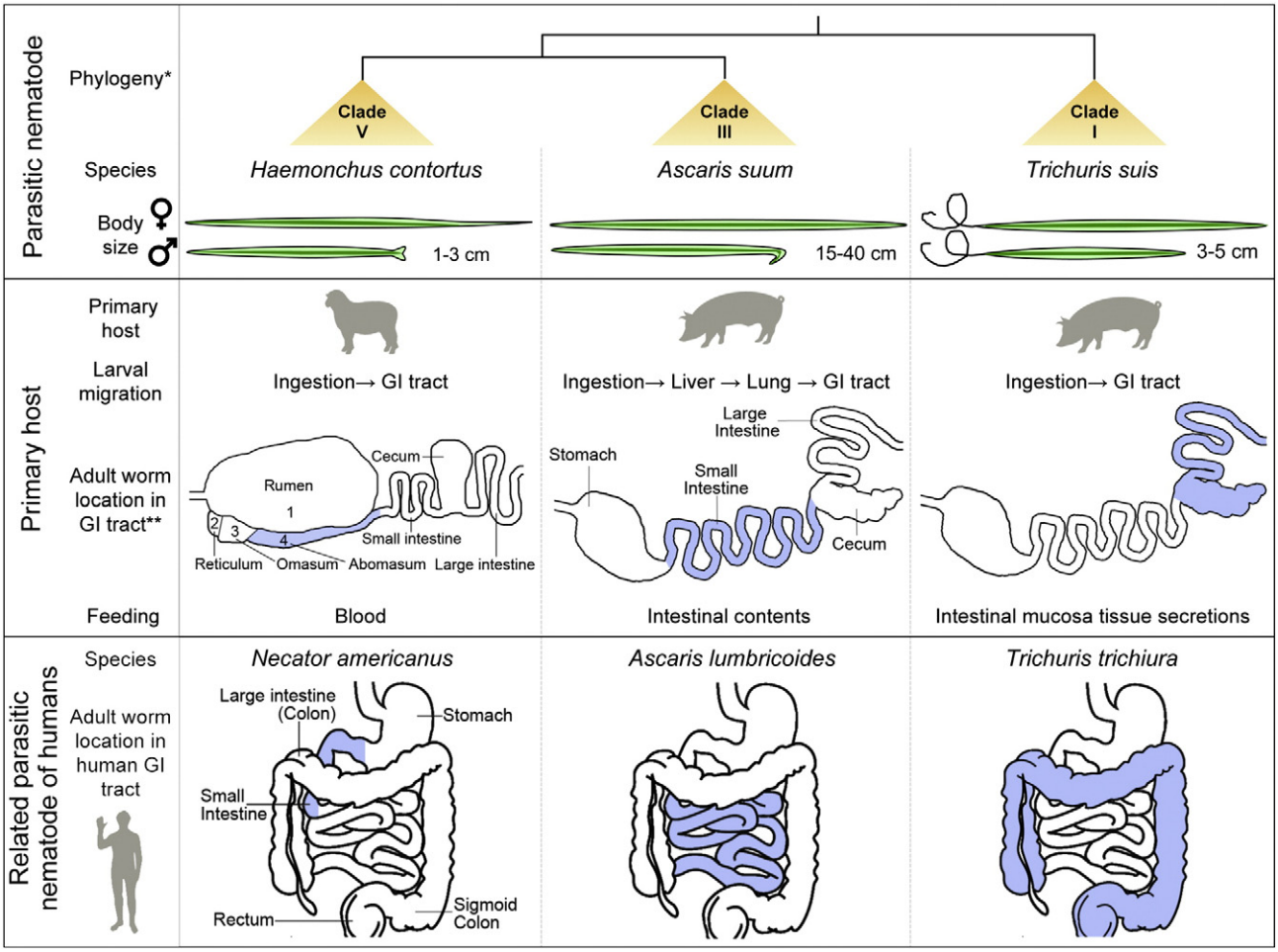

Fig. 1. Characteristics of core species: phylogeny, host location and trophic ecology. *Phylogeny based on Blaxter et al., 1998. ** GI tract, gastro-intestinal tract.