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Thesis abstract

Quantitative proteomic analyses of isolate variation and virulence in *Giardia duodenalis*

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Giardia duodenalis is a parasitic protozoan with a global human infection burden of 250 million, and is therefore the largest parasitic cause of diarrheal disease worldwide. Though some cases are asymptomatic, giardiasis can be acute and chronic, with post-infection sequellae including irritable bowel syndrome, chronic fatigue, obesity and type II diabetes. Importantly, *Giardia* is problematic in children under the age of five, causing ill-thrift and failure-to-thrive. In addition, diarrheal diseases including *Giardia* constitute the second-leading cause of mortality for this age category. *Giardia* has a direct life cycle, where infective, tetranucleated cysts are transmitted via the faeco-oral route, and then excyst in the duodenum into virulent, flagellated trophozoites. The prevalence of the parasite is also due to its wide host range, with zoonotic transfer from wild, livestock and domestic animal species to humans. Efforts continue to define the mechanisms of virulence and pathophysiology, as more research is needed to elucidate the relationship between host and parasite factors.

Advances in genetic epidemiology have defined clear assemblages that segregate phylogenetically according to host range, and multiple assemblage and subassemblage genome sequences are now available. These genome sequences have provided the databases necessary for bottom-up, or shotgun, proteomics, and as such have expanded possibilities for quantitative analyses in this parasite. This thesis aimed to provide a thorough quantitative proteomic foundation to enhance the *Giardia* research field both biologically and technically. To achieve this, the thesis consists of four experimental investigations into aspects of parasite variation and virulence, all of which have generated quantitative proteomic data.

Firstly, two different protein sample preparation and fractionation methods were compared for label-free quantitative proteomics. These were applied to two *G. duodenalis* assemblage A1 isolates with different phenotypes, in order to investigate possible sources of isolate variation. The optimised protocol generated from this initial investigation was applied in later studies, which are also contained within this thesis. In addition, phenotypes associated with pathogenicity correlated with up-regulation of known virulence factors in *Giardia*.

Following this initial investigation, quantitative data was generated using the same label-free approach for eight assemblage A isolates, which constituted the first comprehensive proteomic baseline for this taxonomic group. Isolates of diverse host,

geographic and subassemblage origins were analysed using mass spectrometry to characterise their common proteomes and isolate-specific variations. In addition, both the A1 and A2 subassemblage genome databases were evaluated for peptide to spectrum matching, which demonstrated the importance of subassemblage databases to improve identifications from the *Giardia* variable genome.

The third study investigated isolate variation in the biological context of the process of differentiation in *G. duodenalis*. Label-free quantitative proteomics was used to analyse the proteomes of cysts and trophozoites from two genome-alternate subassemblage A1 isolates. This is the first post-genomic analysis of the life cycle beyond the genome isolate, WB. A range of isolate-independent, universal encystation markers were identified, as well as several indications of isolate-specific life-cycle adaptations which may impact reinfection success in subsequent generations.

Finally, the last experiment in this thesis investigated disease induction using in vitro host-parasite interaction models between intestinal epithelial cell (IEC) lines and trophozoites. We used isobaric Tandem Mass Tags (TMT) to sensitively quantitate changes in trophozoites which were either allowed to attach to host-cell monolayers, or were exposed to host-cell secretions alone. This is the first use of TMT label technologies for quantitative proteomics in *Giardia*. This has demonstrated that distinct protein cascades are induced by both levels of host-signals, and also that induction of virulence factors is not dependent on parasite attachment to host cells.

Through these experiments, this thesis demonstrates that a range of quantitative proteomic approaches are suitable for *G. duodenalis*, all of which are capable of providing important insight into key aspects of parasite biology. These studies provide an important proteomic complement for genomic and transcriptomic data currently available in the literature, which is necessary for undertaking a systems biology approach to understanding *Giardia*.

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