Do apicomplexan parasites hijack the host cell microRNA pathway for their intracellular development?

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Abstract

RNA silencing plays an important role in development through the action of microRNAs, which fine tune the expression of a large portion of the genome. It is also very important in innate immune responses, especially in antiviral and antibacterial defenses in plants, insects, and animals. Two recent papers now indicate that apicomplexan parasites display the ability to interfere with host microRNA populations.

Introduction and context

The phylum Apicomplexa includes a large group of protozoan parasites responsible for a wide range of animal and human diseases. Among the human pathogens are *Plasmodium falciparum* and *Plasmodium vivax*, the major causative agents of human malaria, as well as *Cryptosporidium parvum* and *Toxoplasma gondii*, which are particularly pathogenic in immunocompromised patients. Apicomplexa are intracellular obligatory parasites that multiply in a so-called parasitophorous vacuole. Although the alterations of the host cell harboring *Toxoplasma* or *Plasmodium* parasites have been extensively documented at the cellular level, still little is known about how the parasite manipulates the host cell at the molecular level, with the notable exception of *Plasmodium* infection of host erythrocytes. So far, most molecular studies on the host-parasite interface have focused on the role of parasite factors that are secreted by the invading parasite, as well as by the resident intracellular parasite, into the host cell [1,2]. These intracellular parasites are expected to profoundly reorganize the host cell for their own needs to ensure safe growth and persistence, and presumably to deploy the most sophisticated mechanisms to this end.

Emerging evidence indicates that viruses and bacteria manipulate the microRNA (miRNA) pathways of the host cells they infect. miRNAs are the most abundant class of small, non-coding, single-stranded RNAs and are involved in regulating gene expression at the post-transcriptional level [3]. *In silico* target prediction suggests that miRNAs may control up to 30% of the translation of the human transcriptome [4]. As such, they govern a variety of fundamental cell functions, including cell proliferation and apoptosis, and are key regulators of cell metabolism. When homeostatic conditions are disrupted – for example, when cells encounter micro-organisms – these regulatory pathways might also contribute to host cell responses/defenses (i.e., the inflammatory response) against the foreign bodies [5]. For example, cell infection by mammalian viruses might be counteracted by cellular miRNAs that target either the virus itself, as in the case of the rhabdoviral vesicular stomatitis virus, or a host factor critical to the virus, as for the lentiviral HIV. Conversely, miRNAs can also act in favor of the micro-organism, either when it is pathogen-encoded (e.g., mammalian virus-encoded miRNAs) or when the micro-organism subverts host miRNAs to its own benefit [6]. Effectors from the bacteria *Pseudomonas syringae* have been recently

shown to suppress transcriptional activation of some miRNAs generated upon sensing of PAMPs (pathogen-associated molecular patterns) by *Arabidopsis* [7].

Major recent advances

Recent data have begun to show how two apicomplexan parasites, *Cryptosporidium* and *Toxoplasma*, are able to target miRNAs in the host cell to alter the cellular environment in ways that favor their intracellular development. *Cryptosporidium* is able to trigger the down-regulation of let-7i (a miRNA with complementarity to Toll-like receptor (TLR)-4 mRNA) in the host cell, leading to the up-regulation of TLR4, a key pathogen recognition molecule that plays a central role in epithelial innate immunity to *Cryptosporidium* infection [8]. Various studies have further substantiated the ability of *Cryptosporidium* to alter miRNA expression in cholangiocytes [9-11]. It is emerging from these studies that following *Cryptosporidium* infection, specific miRNA cluster genes are activated by the binding of the NF- κ B (nuclear factor-kappa B) p65 subunit to their promoter, and that inhibition of these miRNAs increases parasite burden [11]. These results mirror those showing differential alterations in mature miRNA expression profiles in primary human fibroblast cells following *Toxoplasma* infection [12]. Zeiner *et al.* [12] showed that *Toxoplasma* infection specifically increased the transcription of the *miR-17/92* loci by two- to three-fold in human fibroblasts. The effect is apparently a specific response to *Toxoplasma* infection since levels of the mature *miR-17/92*-derived miRNAs remained unchanged upon infection by the closely related parasite *Neospora caninum* [12].

Microarray data comparing the miRNA profiles of cells infected by *Toxoplasma* or *Cryptosporidium* or treated with lipopolysaccharide (LPS) have revealed several important findings. For example, *miR-155/BIC* is up-regulated upon *Toxoplasma* infection but remains unaffected or is down-regulated when exposed to *Cryptosporidium* or LPS, respectively [11]. Of note, *miR-155* has an important role in the mammalian immune system, regulating, at least in part, cytokine production [13]. Two other miRNAs, *miR-198* and *miR-320*, are both up-regulated upon *Toxoplasma* infection whereas they are down-regulated after *Cryptosporidium* infection and unaffected after LPS stimulation [11,12]. These data point to specific modifications of host miRNA profiles upon cell infection by Apicomplexa parasites. Obviously, any change in the host cell miRNA pattern might indicate either a defense mechanism by the cell or a subversion strategy by the parasite, two processes that can be differentiated by evaluating the consequences on parasite growth of disruption or over-expression of the target miRNA pathway.

Future directions

Given the propensity of apicomplexan parasites to co-opt cellular pathways and activities for their benefit, it is perhaps not surprising that these parasites could also reshape their cellular environment by reprogramming the host's RNA interference machinery. Specific host miRNAs could either counteract the intracellular growth of parasites or facilitate it, the two possibilities being not mutually exclusive and depending on the physiological context. These findings open an exciting opportunity to pursue a deep understanding of how the host proteome can be reprogrammed dynamically and reversibly upon Apicomplexa infection.

An additional line of research should explore the upstream regulatory mechanisms, that is, how the parasites directly interfere with RNA silencing pathways and, more specifically, the parasite effectors that are involved in the process. As discussed above, in response to *Cryptosporidium* and *Toxoplasma* infections, the expression of specific miRNA genes is altered at the transcriptional level [11,12]. miRNA are generated through the concerted action of multi-subunit complexes that promote the sequential cleavage, export, and loading of miRNA into silencing complexes. An increasing number of reports suggest that, beyond the transcriptional control of genes that code cluster miRNAs, each of these steps serves as a potential point of regulation, and therefore adds additional complexity to miRNA-dependent gene regulation [3,14,15].

Could parasite regulators of host miRNAs be ribonucleic acids? Unlike in *Cryptosporidium* and *Plasmodium* species, the *Toxoplasma* genome encodes elaborate RNA silencing machinery that generates endogenous small silencing RNAs, including specific miRNAs [16]. Thus, an attractive hypothesis is that *Toxoplasma* has the potential to secrete its own miRNAs to hijack the host cell miRNA defense pathway, similar to what some viruses are able to do. Effectors may also be parasite proteins. It is known that apicomplexan parasites inject various molecules into the host cell resulting in extensive remodeling of the host cell gene expression profile and metabolic pathways [17-19]. The expression of host miRNAs can also be altered in response to parasite recognition by cell surface TLRs [20]. Both intrinsic and extrinsic acting factors could then interfere with target host miRNAs, at any point of the processing of the pri-miRNAs and biogenesis of the miRNAs – transcription, processing, or export.

Abbreviations

LPS, lipopolysaccharide; miRNA, microRNA; TLR, Toll-like receptor.

Competing interests

The authors declare that they have no competing interests.

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