Elucidating the Significance of Rhoptry Proteins Associated with the Survival Strategy of *Toxoplasma gondii* in the Host

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Table of Contents

Abstract

Most intracellular pathogens modify host cell functions to avoid host's defense system and replicate in the host. *Toxoplasma gondii*, which is an intracellular apicomplexan parasite, also changes the function of host cells. These modifications are represented by the inhibition of parasite clearance, interpretation of host gene expression and recruitment of host organelles. Rhoptry kinase family proteins (ROPs), which are secreted into host cells from apicomplexan specialized organelles rhoptries, are involved in clearance inhibition of parasites and genetic interpretation in host nuclei. In this study, I analyzed ROPs associated with the functional modifications of host cells in Japanese isolates of *T. gondii* and searched ROPs participated in host mitochondrial recruitment. I could explain abnormal virulence of Japanese *T. gondii* in mice by revealing genetic structure of the ROP5, ROP16 and ROP18, which are related to virulence of parasites by inhibiting clearance of parasites and controlling host gene expression, and identify ROP39 as the novel factor of host mitochondrial recruitment. ROPs are possibly promising drug targets of *T. gondii* considering the importance in *T. gondii* infections.

Chapter 1. General Introduction

Toxoplasma gondii is an apicomplexan parasite that can infect almost all warm-blooded animals, and nearly one-third of the world's population is infected with this parasite (Grigg and Sundar, 2009). *T. gondii* causes a latent infection in most humans, but leads to lethal diseases, including encephalitis, only in immunosuppressed people due to acquired immunodeficiency syndrome (AIDS) or organ transplantation. In pregnant women, initial infection with *T. gondii* may cause fetuses to encounter the parasites through vertical transmission, and this can result in serious symptoms such as retinochoroiditis, hydrocephalus and psychomotor retardation (Montoya and Liesenfeld, 2004). When *T. gondii* invades host cells, it secretes rhoptry kinase family proteins (ROPs) from rhoptry organelles into the cells. Secreted ROPs are localized and function in host nuclear or on parasitophorous vacuole (PV), which contains an intracellular parasite. About 60 ROPs have been identified and they commonly possess a N-terminal signal peptide and a serine/threonine kinase domain. *T. gondii* modifies various functions of host cells possibly for establishing ideal environment to replicate in the cells. The inhibition of parasite clearance, interpretation of host gene expression and recruitment of host organelles are representative of the functional modification. ROP18 phosphorylates immunity-related GTPases (IRGs) on PV with the aid of ROP5 to inhibit parasite clearance (Fentress *et al*., 2010; Behnke *et al*., 2012). ROP16 interprets host gene expression associated with immune response through the phosphorylation of STAT3 and STAT6 (Saeij *et al*., 2007). The phenotypes of *T.*

gondii is classified into three types (type I, type II and type III). *T. gondii* of each type shows different virulence to mice (Sibley and Boothroyd, 1992). The differences of these virulences are attributed to distinct genetic variations of ROP5, ROP16 and ROP18 in the parasites' genome (Saeij *et al*., 2006; Taylor *et al*., 2006; Saeij *et al*., 2007; Behnke *et al*., 2011; Reese *et al*., 2011). Elucidating molecular mechanism associated with survival strategy of *T. gondii* in the host, I dissected the function of ROPs, which are thought to be essential factors for parasite's survival.

4. General Discussion

The identification of TgCatJpOk3 and TgCatJpOk4 showed that traditional phenotyping based on type I-III could not estimate the virulence of parasite accurately. In this study, the analysis for ROPs enhancing virulence of *T. gondii* worked well to reveal the cause of high virulence of TgCatJpOk4. Therefore, the information of ROPs are thought to be effective for predicting virulence of *T. gondii*.

The potency of host mitochondrial recruitment varies in type I-III parasites, in order of type I ≥ type II I≥ type II. Considering that the genetic structures of ROP5, ROP16 and ROP18 contribute to virulence of parasites in mice, I reasoned that the genetic structure of ROP39 in each type of *T. gondii* might contribute to different strength of the mitochondrial recruitment between type I-III strains. I searched the amino acid sequence of type I-III ROP39 and found the amino acid sequence identities are 95.07% (type I vs type II), 95.24% (type II vs type III) and 99.83% (type III vs type I), which indicates the amino acid sequence of type II is different from those of type I and III (Fig. 14). Furthermore, I discovered that transcription level of ROP39 was lower in type II strains than in type I and type III strains by referring ToxoDB (http://toxodb.org), which indicated that transcription levels of ROP39 correlated with the ability of host mitochondrial recruitment. Taken together, amino acid substitutions or/and transcription levels of ROP39 may determine the strength of host mitochondrial recruitment.

ROPs are participated in various process indispensable for establishing parasite infection. There are still a lot of functionally unknown ROPs. Therefore, understanding comprehensively the function of ROPs in host cells may lead to elucidating the mechanism to establish the infection of *T. gondii*. ROPs are fascinating drug targets because human beings do not have these molecules. In fact, inhibitors of kinase activity of ROPs are studied to be used for the treatment of *T. gondii* (D. Sibley, personal communication). Therefore, the accumulation of knowledge related to ROPs may provoke a development of effective drugs against *T. gondii*.

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