Transgenic T. gondii as a platform for MeCP2 protein delivery to the CNS

Lilach Sheiner, PhD
University of Glasgow
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Although Rett Syndrome was shown to be reversible by the supplementation of MeCP2 (Guy et al 2007, Robinson et al 2012, Garg et al 2013, Gadalla et al 2013, Gadalla et al 2017), the lack of robust methods for the delivery of proteins to the CNS is a major obstacle in the application of protein-based therapies to Rett Syndrome. To address this need, we developed a protein delivery system that derives its specificity and efficiency from the protozoan Toxoplasma gondii. Through co-evolution with its hosts, T. gondii has developed the ability to pass through biological barriers and reach the brain. When in the brain T. gondii invades mainly neuronal cells, into which it secretes effector proteins that mediate its innocuous persistence. Through engineering T. gondii to express and secrete heterologous proteins, we utilize its endogenous secretion mechanisms to serve as a therapeutic protein delivery platform.

T. gondii is an obligate intracellular parasite that infects many warm-blooded animals, including human (Feustel et al. 2012). Chronic infection with T. gondii are mostly asymptomatic (Montoya & Liesenfeld 2004). Under immune pressure, T. gondii generates quiescent, non-immunogenic, cysts which are found mostly in the brain and persist for the lifetime of the infected person. T. gondii resides in several cell types of the central nervous system including astrocytes, microglia and neurons (Carruthers & Suzuki 2007), but evidence from mice suggest that in vivo the cysts reside mostly in neuronal cells (Cabral et al. 2016). Most importantly, T. gondii continues to secrete effector proteins into these brain cells, both when entering them and while intracellular.

The use of infectious agents to facilitate medical treatments is an emerging approach in biomedical engineering. Research is increasingly turning to parasites that co-exist with humans to treat chronic diseases. Evidence supporting the potential of this approach include the use of parasitic worms to treat immune disorders (Elliott & Weinstock 2009); immunotherapy with engineered bacteria (Rothman & Paterson 2013; Chorobik et al. 2013; Wood & Paterson 2014); and microbiome engineering (Cano-Garrido et al. 2015; Kali 2015). Attenuated T. gondii is already proposed as cancer therapy as it naturally induces immune responses that cause tumor remission and increased survival in mouse models (Fox et al 2013, Fox et al 2015), highlighting the feasibility of using live T. gondii in a therapeutic setting.

In summary, the combination of T. gondii's ability to cross biological barriers, synthesize proteins locally and deliver them into mammalian brain cells offers a unique platform to develop a synthetic biology-based solution to the challenges of protein delivery for Rett Syndrome therapy. We engineering a strain of T. gondii that expresses MeCP2 fused to an endogenous parasite secretory sequence. This transgenic line synthesizes and delivers MeCP2 into neuronal cells in culture. We showed that this parasite MeCP2 behaves like a functional MeCP2. Under this grant we propose to investigate the effects that MeCP2-secreting T. gondii have on mice models of Rett Syndrome and assess their potential as a therapeutic strategy for the treatment of Rett symptoms by MeCP2 delivery.