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# The crucial role of hepatocyte growth factor receptor during liver-stage infection is not conserved among *Plasmodium* species

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## To the Editor

Protozoan parasites of the genus *Plasmodium* are the vector-borne causative agents of malaria, responsible for more than 350 million clinical cases and one million deaths annually. Studies using rodent malaria models have shown that during the bite of an infected *Anopheles* mosquito, *Plasmodium* sporozoites are transmitted and subsequently traverse a variety of cell types, wounding their membranes on the way[1]. This allows sporozoites to travel through the dermis into the bloodstream and then pass through the cell layer that lines blood vessels in the liver as well as several hepatocytes before taking up residence in host hepatocytes for further development as exoerythrocytic forms (EEFs)[2]. Although cell traversal provides a means to cross tissue barriers, it is unclear whether this constitutes its only biological role. A novel function for parasite host cell traversal was proposed by Carrolo *et al.*[3], who showed that cell traversal by sporozoites of the rodent malaria parasite *Plasmodium berghei* induces secretion of hepatocyte growth factor (HGF). HGF is a soluble factor that activates the receptor tyrosine kinase MET, which is capable of initiating a cascade of signaling events that result in hepatocyte proliferation and survival. Carrolo *et al.*[3] further showed that this signaling cascade is crucial to promote early development of EEFs and thus substantially contributes to transmission success. More recently, the same group demonstrated, again using *P. berghei*, that MET signaling events are important for the parasite to protect itself from host hepatocyte apoptosis[4] and subsequently showed that a dietary supplement, genistein, which inhibits MET activation, can treat liver-stage malaria infection[5]. A fundamental question, then, is whether this unique function for cell traversal is broadly conserved among *Plasmodium* species and whether it is found in human malaria parasites.

To test the ability of another rodent malaria parasite species, *Plasmodium yoelii*, and the human malaria parasite, *Plasmodium falciparum*, to activate MET by cell traversal, we incubated sporozoites of each species with HepG2-CD81 hepatoma cells for 1 h to allow traversal and then collected cellular lysates. Using an antibody specific to the phosphorylated activation loop of MET, we probed the lysates for MET activation. As a negative control, we used lysates from cells that had been incubated with salivary gland extracts from uninfected mosquitoes (Supplementary Methods). We found that cell traversal by *P. berghei* sporozoites but not *P. yoelii* or *P. falciparum* sporozoites led to the activation of MET (Fig. 1a). To ensure that *P. falciparum* does not fail to activate MET because it is being assayed in a cell type that does not support its EEF development, we allowed *P.*

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*falciparum* sporozoites to traverse HC04 cells, a hepatocyte-derived cell line that does support *P. falciparum* EEF development[6], but, again, we did not detect MET activation (Fig. 1b). To ensure that differences in MET activation were not due strictly to differences in traversal rates, we incubated sporozoites with HepG2-CD81 cells in the presence of labeled dextran. Given that cells that are wounded by traversal take up the labeled dextran, we used FACS to identify traversed cells (Supplementary Methods). We observed comparable rates of traversal for each of the three species. Modest differences in traversal rates between species did not correlate with MET activation. Instead, MET activation was only observed for *P. berghei*, which had slightly lower traversal rates than *P. yoelii*, and similar traversal rates when compared to *P. falciparum* (Supplementary Fig. 1).

To assess whether direct MET activation causes substantial differences in parasite invasion or development rates, we infected HepG2-CD81 cells with *P. yoelii* or *P. berghei* after treatment with either HGF or the MET inhibitor SU11274. We found that HGF pretreatment led to an increase in *P. berghei* EEFs, whereas MET inhibition led to fewer *P . berghei* EEFs (Fig. 1c and Supplementary Fig. 2), in agreement with Carrolo *et al.*[3]. However, no difference in *P. yoelii* EEF burden was seen after modulation of MET signaling (Fig. 1c and Supplementary Fig. 2).

Taken together, our data suggest that, unlike *P. berghei*, neither *P. yoelii* nor *P. falciparum* activates MET signaling upon cell traversal. Furthermore, *P. yoelii* host cell invasion and EEF development do not seem to be affected by MET activation or inactivation. All three species traverse HepG2-CD81 cells at comparable rates, suggesting a particular trait unique to *P. berghei* cell traversal that leads to HGF secretion. Furthermore, *P. berghei* invasion, early development or both have a stronger dependency on MET. This is particularly interesting in light of the observations that *P. berghei* is less selective in its host cell than other *Plasmodium* species. For instance, *P. berghei* can readily infect HepG2, Hepa1-6 and Huh7 and nonhepatocytic cells, whereas *P. yoelii* and *P. falciparum* require CD81 to invade cells[7] and only develop in hepatocyte-derived cells. Recently, it has been demonstrated that *P. berghei* EEFs develop fully inside the skin of the mouse[8], but similar evidence for other rodent *Plasmodium* species is less direct[9], and none exists for *P. falciparum*. One possibility is that *P. berghei* is able to use receptor tyrosine kinase signaling, which is available in nearly all cell types and lines, to facilitate invasion and early development, whereas other *Plasmodium* species such as *P. yoelii* and *P. falciparum* require more specialized molecules including but not limited to CD81, combinations of which are only provided in a very small number of hepatocyte-derived cell lines or on primary hepatocytes.

Our findings are consistent with the few instances of published data comparing the impact of host signaling in the two rodent parasite species. Cunha-Rodrigues *et al.*[7] recently showed that genistein, a dietary supplement that acts at least in part by inhibiting MET activation, lowers *P. berghei* liver stage burden in mice. However, higher levels of genistein are required to reduce *P. yoelii* EEFs *in vitro*. Identifying previously unknown signaling proteins in the hepatocyte that are required for invasion and liver stage development remains a crucial point of investigation, but caution should be taken to ensure that findings are broadly applicable to malaria parasites in general, including those infecting humans.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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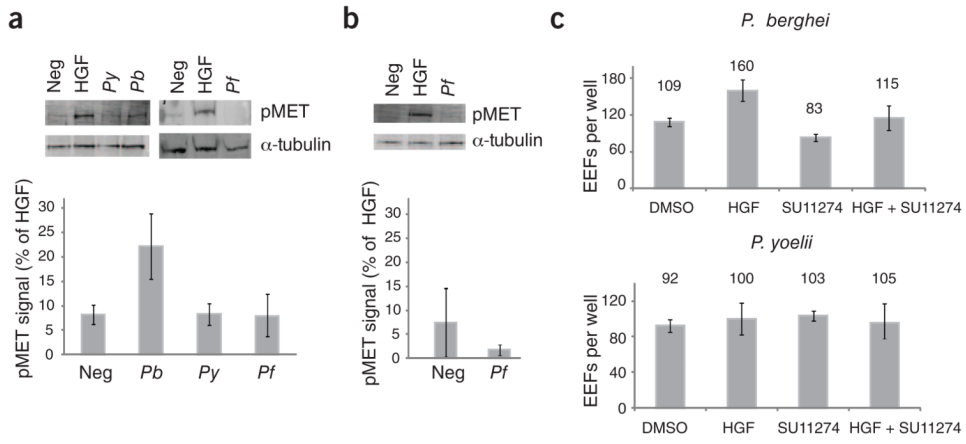


**Figure 1.**

MET signaling is crucial for *P. berghei*(*Pb*) but not *P. yoelii*(*Py*) or *P. falciparum* (*Pf*) hepatocyte infection. (**a,b**) Cells were incubated with *Plasmodium* sporozoites for 1 h. Lysates were probed for the presence of activated MET receptor (pY1234/1235) in either HepG2-CD81 cells (**a**) or HC04 cells (**b**). Signal was detected in triplicate, normalized to human α-tubulin, and signal produced by 1h of HGF treatment was set to 100. Error bars represent s.d. of triplicate measurements. (**c**) HepG2-CD81 cells were treated with either HGF or the MET inhibitor SU11274 for 1 h then infected with $1 \times 10^5$ *P. berghei* or *P. yoelii* sporozoites. Parasites were allowed to develop for 24 h, visualized by immunofluorescence assay and manually counted. Error bars represent s.d. of biological replicate experiments.