Cell signalling during *Schistosoma mansoni* male-female interactions

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Schistosomes are blood flukes that infect approximately 250 million people and kill more than 100,000 annually in Low and Middle-income countries. Unusual among parasitic flatworms, schistosomes are dioecious (separate sexes) with heterogametic females (2n=16, ZW), and homogametic males (2n=16, ZZ). The genome of *Schistosoma mansoni* encodes over 260 protein kinases, well conserved regulatory proteins through the evolution of eukaryotes. To understand protein kinase signal transduction and function during male-female interactions of *S. mansoni*, we investigated the temporal effects of excretory-secretory products (ESPs) produced by adult male worms over 20 h culture on protein kinase activities in female worms, and *vice versa*. Western blotting with anti-phospho tyrosine/serine/threonine antibodies revealed that the phosphorylation status of multiple proteins changed over 60 min in response to ESP molecules released from worms of the opposite sex. Exchange of male/female ESPs between groups of single sex worms resulted in a rapid activation of protein kinase pathways, particularly p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (ERK) pathways where activation was evident as early as 5 min. Phosphorylation (activation) of p38 MAPK and ERK was significantly attenuated by the inhibitors SB203580 and U0126, respectively. Immunofluorescence and confocal laser scanning microscopy revealed that the activation occurred throughout different tissues including the parasite tegument. In addition, the motility of the parasites was enhanced in response to opposite sex ESPs, and expectedly, the inhibition with SB203580 and U0126 for 1 h prior to exposure to opposite sex ESPs profoundly reduced worm motility. Using biotinylated-ESPs and fluorescence confocal laser scanning microscopy we observed that the adult male ESP bound to the surface membrane of female worms and *vice versa*. Finally, cell proliferation was investigated using Click-it EdU and Alexa Fluor staining. Whereas single sex adult worm in culture showed low levels of cell proliferation over six days, there was a striking cell proliferation increase in the testes, ovaries and surface layer of worms when exposed to opposite sex ESPs. The male/female ESPs were further investigated by proteomics and 896 and 469 proteins were identified in crude and extracellular vesicle-depleted preparations, respectively. To the best of our knowledge, this research represents the first report on male-female ESPs activating signal transduction in opposite sex adult worms and driving cell proliferation in both reproductive and somatic tissues. These findings may expose tentative targets to develop novel strategies for schistosomiasis control.