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Title of Presentation: Leishmania release exosomes containing short RNA sequences

Abstract

During an infection with leishmania, impairment of macrophage microbicidal function has been linked to transport of parasite virulence factors into macrophage cytosol. Our group has identified the release of small membrane vesicles, also known as exosomes, as a mechanism for delivery of leishmania proteins to host macrophages. Moreover, we have shown that leishmania-derived exosomes can modulate innate and adaptive immune responses through effects on host monocytes and dendritic cells. Recent studies with mammalian exosomes have shown that these vesicles can shuttle RNA between cells. We thus hypothesized that exosomes released by leishmania, in addition to proteins, also contain parasite RNAs, and that these molecules may play a role in pathogenesis.

Indeed, our recent findings clearly show that exosomes released by *Leishmania donovani* and *Leishmania braziliensis* contain RNA sequences. Interestingly, in both species the size range of exosomal RNA was considerably narrower than that of total cellular RNA, with the majority of sequences being shorter than 200 nt. To characterize leishmania exosomal RNA in more detail, RNA extracts were processed for paired end sequencing with Illumina MiSeq. When aligning the *L. donovani* and *L. braziliensis* exosome libraries with their respective reference genomes, the vast majority of reads mapped to known non-coding RNA, such as transfer RNA (tRNA) and ribosomal RNA. Only a small number of reads mapped to annotated protein coding sequences; and these were present in a very low abundance, suggesting that leishmania exosomes do not contain messenger RNA. We found evidence for the presence of tRNA-derived fragments, a phenomenon recently described in other Trypanosomatids (*Trypanosoma cruzi*). In addition, a number of sequences mapped to regions of the genome that currently lack annotation, suggesting that leishmania exosomes may contain novel, previously uncharacterized transcripts. Northern blotting with probes for tRNA-derived fragments and novel transcripts revealed that several of these seem to be enriched in exosomes versus total leishmania RNA.

In conclusion, this is the first report to show that leishmania secrete RNA within extracellular vesicles. Our data suggests that RNA in leishmania exosomes is subject to selective packaging rather than random cytosolic sampling. Current experiments are focused on understanding the function of these RNA sequences in leishmania biology, particularly pathogen-host interactions.