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Commun Integr Biol. Author manuscript; available in PMC 2009 January 22.

Published in final edited form as:
Commun Integr Biol. 2008 ; 1(1): 37–39.

A role for vesicular transport of macromolecules across cell walls in fungal pathogenesis

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Abstract

In our recent work, we have shown that fungal species from different phyla produce extracellular vesicles. The vesicles are heterogeneous and morphologically similar to mammalian exosomes, with intact bilayered membranes. Proteomic analyses reveal that the vesicles contain a broad array of macromolecules, many of which are associated with fungal virulence. Further, the biological import of the extracellular fungal vesicles is supported by their presence during murine cryptococcosis and the immunoreactivity of convalescent serum from patients with *Cryptococcus neoformans* or *Histoplasma capsulatum* vesicle protein extracts.

In contrast to most eukaryotic cells, fungi have complex cell walls, that could in theory provide a significant barrier to the secretion of large molecules. The discovery of trans-cell wall vesicular transport in fungi provides a solution to the problem of extracellular transport of macromolecules. Identifying similar vesicles in ascomycetes and basidiomycetes suggest that the shuttle system is ancient, predating the divergence of these branches 0.5–1.0 billion years ago. Importantly, the discovery of this trans-cell wall vesicular transport system also poses new, interesting questions for future investigations.

Keywords

Histoplasma capsulatum; *Cryptococcus neoformans*; vesicles; pathogenesis; virulence; cell transport

Eukaryotic and prokaryotic organisms have several pathways for secretion.[1] However, the best understood mechanism in eukaryotes utilizes vesicular migration from the endoplasmic reticulum to the trans face of the Golgi followed by loading into the trans-Golgi reticulum where compounds are sorted into transport vesicles that subsequently move to and fuse with

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Addendum to: Rodrigues ML, Nakayasu ES, Oliveira DL, Nimrichter L, Nosanchuk JD, Almeida IC, Casadevall A. Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. Eukaryot Cell 2008; 7:58–67
and
Albuquerque PC, Nakayasu ES, Rodrigues ML, Frases S, Casadevall A, Zancope-Oliveira RM, Almeida IC, Nosanchuk JD. Vesicular transport in *Histoplasma capsulatum*: an effective mechanism for trans-cell wall transfer of proteins and lipids in ascomycetes. Cell Microbiol 2008; 2008;10:1695–710.

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the plasma membrane where the contents are released by exocytosis.[2] It is well established that fungi can utilize this conventional pathway for secretion.[3]

One alternative mechanism for secretion involves the release of vesicles into the extracellular space. The process is characterized by the formation of small vesicles formed by invagination of the limiting membrane of endocytic compartments (endosomes), and some endosomes develop internal vesicles to form multivesicular bodies. Although multivesicular bodies typically fuse with lysosomes in degradation pathways, they can also fuse with the plasma membrane where the internal vesicles are released extracellularly (exosomes).[4]

Current Knowledge of Fungal Extracellular Vesicles

At the inception of our studies with *Cryptococcus neoformans*, a basidiomyces, we hypothesized that the fungus utilized vesicular transport given that polysaccharide synthesis occurred intracellularly in vesicles[5,6] and that the polysaccharide was a large macromolecule with a mass of 1–7 million Daltons.[7] The ascomycetes *Histoplasma capsulatum, Candida albicans, Candida parapsilosis, Sporothrix schenckii* and *Saccharomyces cerevisiae* are also known to produce diverse exocellular compounds[8,9] and we similarly hypothesized that these fungi utilize vesicles for transport of macromolecules. Our recent work[10-12] demonstrates that these diverse fungi produce heterogeneous extracellular vesicles that contain lipids, carbohydrates and proteins, many of which are associated with fungal virulence.

Glucuronoxylomannan (GXM) is a key factor for virulence in *C. neoformans* and we demonstrated that certain extracellular vesicles contained the polysaccharide.[10] This finding extended the observation by Yoneda and Doering[6] showing that a *C. neoformans* strain defective in the production of Sav1p, a homolog of the *S. cerevisiae* protein Sec4p, accumulated exocytic vesicles containing GXM at the yeast cell septum and bud during cell division. In addition to polysaccharide, well established virulence factors of *C. neoformans* such as glucosylceramide, urease and laccase were also vesicle associated.[11] Further supporting the biological relevance of the vesicles, we observed vesicles within the cell wall and extracellular vesicles associated with the fungal surface during murine cryptococcosis.[10]

However, the most striking finding was the identification of many diverse proteins within the extracellular vesicles. Proteomic analysis of *C. neoformans* and *H. capsulatum* vesicles identified 76 and 206 proteins, respectively.[11,12] Notably, numerous proteins associated with virulence were identified, and heat shock proteins, superoxide dismutase, thiol-specific anti-oxidants and catalases were found in both fungi. Furthermore, roughly a third of the proteins characterized in the fungal vesicles corresponded to proteins previously identified in mammalian exosomes. To test whether such vesicles were made in during infection, we examined whether *H. capsulatum* vesicular proteins were recognized by hyperimmune human serum and found that the serum strongly reacted with diverse vesicular proteins, including the virulence associated proteins Hsp60,[13,14] and histone 2B.[15] Hence, proteins transported via vesicles appear to be involved in host-pathogen interactions.

Future Questions for Consideration

Numerous interesting questions have arisen from the discovery of vesicular trans-cellular transport in fungi, including "*what are the functions, morphologies and contents of the various types of vesicles?*"; "*what is the mechanism for trans-cellular passage?*"; and "*what are the relationships between vesicles and host cells?*". For instance, our microscopy studies suggest that at least four types of vesicles are released by fungi.[10-12] This is especially important due to the potential role of extracellular vesicles as 'virulence factor bags'. We hypothesize that vesicular transport has been developed as a mechanism for efficient delivery of factors that combat host effector responses (such as catalases and superoxide dismutases) and are toxic to

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host cells (such as GXM, urease and laccase) with an end result of the vesicles significantly facilitating fungal pathogenesis. However, capsular synthesis also appears to depend on vesicular delivery of GXM, which raises the question of whether the fungus can target vesicles to itself versus host cells. At present, it is not clear whether individual vesicles contain products toxic to host and factors involved in maintenance of the fungal cell (such as GXM or glucosylceramide) or whether they are independent. The current methods we use to isolate vesicles do not permit discrimination of their origin or content, but differences in size, density and enzymatic contents should permit fractionation in future studies. We do not know if vesicles are formed by processes similar to mammalian exosomes or multivesicular bodies or if they derive via membrane budding or other processes. Clearly, the finding of vesicles will lead to the development of new methods for separation and analysis that will shed light on these questions.

As noted above, the fungal cell wall is remarkably complex and the mechanism for vesicle trans-cell wall transport is unknown. Data from Yoneda and Doering[6] suggests that vesicles with GXM are directed to the cell surface in post-Golgi vesicles or through endosome recycling. More recently, Hu et al.[16] showed that *C. neoformans* lacking Vps34 (vacuolar protein sorting 34) had marked reduction in melanin formation suggesting that laccase-containing vesicles derive from the endocytic pathway. *S. cerevisiae* expresses the molecular machinery associated with multivesicular body formation and sorting,[17] but their role in extracellular vesicle production has not been shown. Notably, we and others have reported the presence of pores in fungal cell walls that can be sufficient to allow the shuttling of vesicles.[18] Myosin and similar motor proteins can be involved in vesicle transportation[19] and a myosin analog has identified in the cell wall of *Aspergillus fumigatus*.[20] However, there is no information regarding the functionality of motor proteins within a fungal cell wall. This area of study is particularly important as the transport mechanisms are potential targets for novel antifungal drugs or vaccines, especially if the pathway is broadly conserved in fungi. Given that the fungal cell wall is significantly different from mammalian cellular structures, it is suspected that such therapeutics would have high specificity for fungal cells and low host toxicity.

Summary

We have demonstrated that diverse fungi produce extracellular vesicles that contain numerous carbohydrates, lipids and proteins.[10-12] Concentration of pathogenic determinants into vesicles appears to represent an efficient mechanism for the release of virulence factors into host tissues. Pathogenic mechanisms and secretory processes in microbes are closely associated and we advocate that the understanding of secretory mechanisms and their regulation in microbial pathogens may represent a promising strategy for the design of new drugs and prophylactic agents. The discovery of vesicles in cryptococcal culture supernatants has opened an exciting, broad avenue for exploration of fungal cell biology that impacts our understanding of macromolecular export and pathogenesis.

Acknowledgements

Marcio L. Rodrigues and Leonardo Nimrichter are supported by grants from the Brazilian agencies FAPERJ and CNPq. Leonardo Nimrichter is supported in part by an Interhemispheric Research Training Grant in Infectious Diseases, Fogarty International Center (NIH D43-TW007129). Arturo Casadevall is supported by NIH grants AI033142, AI033774, AI052733 and HL059842, and Joshua D. Nosanchuk by NIH AI52733 and AI056070-01A2.

References

1. Wickner W, Schekman R. Protein translocation across biological membranes. Science 2005;310:1452–6. [PubMed: 16322447]

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2. Glick BS, Malhotra V. The curious status of the Golgi apparatus. Cell 1998;95:883–9. [PubMed: 9875843]
3. Schekman R. Lasker Basic Medical Research Award. SEC mutants and the secretory apparatus. Nat Med 2002;8:1055–8. [PubMed: 12357231]
4. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: from biogenesis and secretion to biological function. Immunol Lett 2006;107:102–8. [PubMed: 17067686]
5. Feldmesser M, Kress Y, Casadevall A. Dynamic changes in the morphology of *Cryptococcus neoformans* during murine pulmonary infection. Microbiology 2001;147:2355–65. [PubMed: 11496012]
6. Yoneda A, Doering TL. A eukaryotic capsular polysaccharide is synthesized intracellularly and secreted via exocytosis. Mol Biol Cell 2006;17:5131–40. [PubMed: 17021252]
7. McFadden DC, De Jesus M, Casadevall A. The physical properties of the capsular polysaccharides from *Cryptococcus neoformans* suggest features for capsule construction. J Biol Chem 2006;281:1868–75. [PubMed: 16278213]
8. Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev 2003;67:400–28. [PubMed: 12966142]
9. Fisher KL, Deepe GS Jr, Woods JP. *Histoplasma capsulatum* strain variation in both H antigen production and beta-glucosidase activity and overexpression of HAG1 from a telomeric linear plasmid. Infect Immun 1999;67:3312–6. [PubMed: 10377107]
10. Rodrigues ML, Nimrichter L, Oliveira DL, Frases S, Miranda K, Zaragoza O, Alvarez M, Nakouzi A, Feldmesser M, Casadevall A. Vesicular polysaccharide export in *Cryptococcus neoformans* is a eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell 2007;6:48–59. [PubMed: 17114598]
11. Rodrigues ML, Nakayasu ES, Oliveira DL, Nimrichter L, Nosanchuk JD, Almeida IC, Casadevall A. Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. Eukaryot Cell 2008;7:58–67. [PubMed: 18039940]
12. Albuquerque PC, Nakayasu ES, Rodrigues ML, Frases S, Casadevall A, Zancope-Oliveira RM, Almeida IC, Nosanchuk JD. Vesicular transport in *Histoplasma capsulatum*: an effective mechanism for trans-cell wall transfer of proteins and lipids in ascomycetes. Cell Microbiol. 2008Epub
13. Gomez FJ, Allendoerfer R, Deepe GS Jr. Vaccination with recombinant heat shock protein 60 from *Histoplasma capsulatum* protects mice against pulmonary histoplasmosis. Infect Immun 1995;63:2587–95. [PubMed: 7790073]
14. Deepe GS Jr, Gibbons RS. Cellular and molecular regulation of vaccination with heat shock protein 60 from *Histoplasma capsulatum*. Infect Immun 2002;70:3759–67. [PubMed: 12065519]
15. Nosanchuk JD, Steenbergen JN, Shi L, Deepe GS Jr, Casadevall A. Antibodies to a cell surface histone-like protein protect against *Histoplasma capsulatum*. J Clin Invest 2003;112:1164–75. [PubMed: 14561701]
16. Hu G, Hacham M, Waterman SR, Panepinto J, Shin S, Liu X, Gibbons J, Valyi-Nagy T, Obara K, Jaffe HA, Ohsumi Y, Williamson PR. PI3K signaling of autophagy is required for starvation tolerance and virulenceof *Cryptococcus neoformans*. J Clin Invest 2008;118:1186–97. [PubMed: 18259613]
17. Hurley JH, Emr SD. The ESCRT complexes: structure and mechanism of a membrane-trafficking network. Annu Rev Biophys Biomol Struct 2006;35:277–98. [PubMed: 16689637]
18. Eisenman HC, Nosanchuk JD, Webber JB, Emerson RJ, Camesano TA, Casadevall A. Microstructure of cell wall-associated melanin in the human pathogenic fungus *Cryptococcus neoformans*. Biochemistry 2005;44:3683–93. [PubMed: 15751945]
19. Schliwa M, Woehlke G. Molecular motors. Nature 2003;422:759–65. [PubMed: 12700770]
20. Esnault K, el Moudni B, Bouchara JP, Chabasse D, Tronchin G. Association of a myosin immunoanalogue with cell envelopes of *Aspergillus fumigatus* conidia and its participation in swelling and germination. Infect Immun 1999;67:1238–44. [PubMed: 10024566]