Name(s)

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Project Number

S1507

Project Title

The Role of Tetraspanins in the Uptake of Candida albicans by Host Cells: Year 2

Abstract

Objectives

The fungus, Candida albicans grows on the skin and in the GI tract of healthy people as part of the normal microbiota. However, in hospitalized patients, the fungus can enter the bloodstream, where it is carried throughout the body, causing a severe infection called disseminated candidiasis that kills 40% of those who develop this infection. For C. albicans to escape from the blood vessels and infect the deep tissues, it must invade the endothelial cells that line the inside of the blood vessels. Previous studies showed that C. albicans invades an endothelial cell by binding to a receptor, N-cadherin, which induces the cell to engulf the organism and pull it inside. Last year, I discovered that CD9 and CD63, which are endothelial cell membrane proteins called tetraspanins, are required for C. albicans to invade endothelial cells. My current hypothesis is that CD9 is required for N-cadherin to mediate the endocytosis of C. albicans by endothelial cells, and my goal was to use CD9 siRNA to test this hypothesis.

Methods

The HUVEC-TERT endothelial cell line was grown in tissue culture and transfected with either control or CD9 siRNA using Lipofectamine 2000. The effects of siRNA on CD9, CD63, and N-cadherin protein levels were determined by Western blotting. The capacity of the CD9 siRNA and anti-CD63 antibodies to inhibit the uptake of C. albicans by endothelial cells was determined using a differential fluorescence assay. The accumulation of N-cadherin and CD9 around C. albicans in the endothelial cells was detected by indirect immunofluorescence using specific primary antibodies. Each antibody was detected with fluorescent labeled secondary antibodies, and the cells were imaged by confocal microscopy.

Results

By Western blotting, it was found that the CD9 siRNA knocked down CD9 protein levels by 82%, relative to endothelial cells transfected with control siRNA. The CD9 siRNA also increased CD63 levels by 25%, and reduced N-cadherin levels by 23%. The overall effect of the CD9 siRNA was to reduce the endocytosis of C. albicans by 42% 17%. Combining the CD9 siRNA with a specific monoclonal antibody against CD63 did not further reduce endocytosis. By confocal microscopy, CD9 and N-cadherin were observed to accumulate around C. albicans hyphae in endothelial cells that were transfected with control siRNA. When the cells were transfected with CD9 siRNA, very little CD9 was detected and N-cadherin did not accumulate around the organisms.

Conclusions

These results indicate that the tetraspanin, CD9 is required for C. albicans to invade endothelial cells. The

Summary Statement

I discovered that the tetraspanin, CD9 is required for N-caderin to function as an endothelial cell receptor for Candida albicans.

Help Received

Dr. Hong Liu provided access to lab facilities and scientific guidance.