Introduction

Breast cancer is the leading cause of cancer deaths among women worldwide. While many risk factors for breast cancer are known, viral infection has emerged as a potential contributor to tumor development. Human Cytomegalovirus (HCMV) generally causes mild or asymptomatic infection; however, recent evidence links HCMV to breast cancer. Several studies have detected HCMV DNA and proteins in breast tumor biopsies. Of particular interest is the viral cytokine cmvIL-10, a potent immunosuppressive protein encoded by the HCMV UL111A gene, which promotes proliferation, migration, and invasion of breast cancer cells in culture (1, 2). Since HCMV is transmitted via human breast milk, we hypothesized that breast tumor cells could become infected and produce cmvIL-10. Here, we investigated whether breast tumor cells support productive virus infection.

Materials and Methods

1. Cell Lines. Four human breast tumor cell lines were used, each with a unique expression profile for the key receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Additionally, each cell line is representative of a diagnostic tumor classification. Necrotic human foreskin fibroblasts (NuFF) cells were used as a control (Table 1).

2. Virus infection, viral entry assays, and analysis of viral gene and protein expression. Breast tumor cells were infected with the HCMV clinical strain TB40/E-wildtype, which is tagged with green fluorescent protein (GFP). NuFF cells were used as a control (2). RNA was extracted and cDNA synthesized. Viral gene expression was analyzed using polymerase chain reaction (PCR). Additional mock infected and infected breast tumor cells were harvested for analysis of viral protein expression by Western blot. NuFF cells were used as a control for infection.

3. Virus infectivity assays. NuFF cells were treated with supernatant (SN) from mock infected and HCMV-infected MCF-7 or MDA-MB-231 breast tumor cells. Mock infected and HCMV-infected NuFF SN was used as an additional control. Cells were monitored for signs of infection for 5 days using fluorescence microscopy.

4. HCMV-infected breast tumor cells express viral proteins. Lysates from mock infected or infected breast tumor cells were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotted using plasma from a representative HCMV-positive donor. Lysates from HCMV-infected breast tumor cells showed significantly stronger responses to the HCMV-positive patient serum as compared to the lysates from mock-infected breast tumor cells. Lysates from mock-infected and infected NuFF cells were run as a control for infection status. The IE1 protein can be seen in the infected cell lysates at 72 h, while the IE2 protein can be seen at 60 h. The cmvIL-10 protein can be seen in the infected cell lysates at 20 kDa. No viral proteins were detected in mock infected cell lysates. This data indicates that HCMV proteins are expressed by infected breast tumor cells. In combination with the viral entry and gene expression data these results provide additional supporting evidence that breast tumor cells support HCMV infection in vitro.

5. Conclusions

• Human breast tumor cells permit HCMV entry in vitro. This supports the hypothesis that breast tumor cells support HCMV infection, and suggests support for in vivo infection of breast tumor cells.
• HCMV proteins are expressed by breast tumor cells in vitro. Providing further support for the hypothesis that breast tumor cells support HCMV infection.
• Infectious viral progeny are produced during infection of breast tumor cells, which suggests that breast tumor cells are productively infected.
• Future work will include additional detection of cmvIL-10 by ELISA and investigation of the impacts of HCMV infection on breast tumor cell surface receptor expression patterns.

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References