Human Breast Tumor Cells Support Productive HCMV Infection

Kaitlin Branch and Juliet V. Spencer

Herpes Virus Research Laboratory, Department of Biology, University of San Francisco

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.

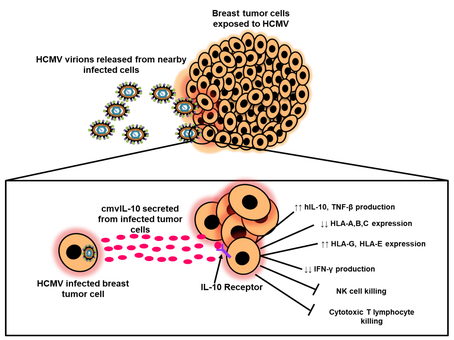


Figure 1. HCMV-infected breast tumor cells may serve as the source of cmvIL-10 in the tumor microenvironment. Breast tumor cells exposed to HCMV become infected and secrete cmvIL-10 into the tumor microenvironment. The cmvIL-10 protein secreted by HCMV-infected breast tumor cells binds the IL-10R on nearby breast tumor cells and initiates modulation of expression of immune recognition receptor proteins and immune effector molecules.

Materials and Methods

1. Cell Lines. Four human breast tumor cell lines were used, each with a 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. Neonatal human foreskin fibroblasts (NuFF) cells were used as a control (Table 1).

Table 1. Representative breast tumor and fibroblast cell lines used.

Cell Line	Diagnostic Classification	ER	PR	HER2
MCF-7	Luminal A	+	+	-
BT474	Luminal B	+	+	+
MDA-MB-231	Claudlin-low	-	-	-
SKBR3	HER2	-	-	+
NuFF	Fibroblast	-	-	-

*** NuFF =** Neonatal human foreskin fibroblasts, not breast tumor cells. NuFF cells used as a control for infection.

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 (3). 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) harvested 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.

Results

Breast tumor cells permit HCMV entry

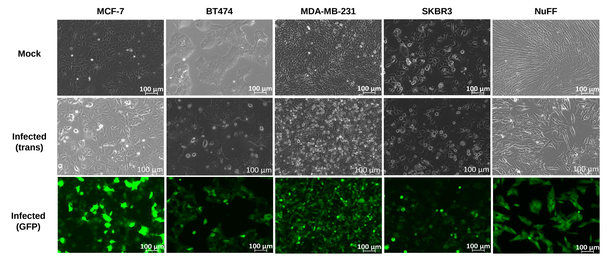
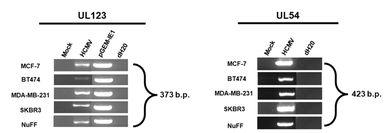


Figure 2. Breast tumor cells permit HCMV entry regardless of ER/PR/HER2 Status. Breast tumor cells were exposed to HCMV clinical strain TB40/E-wildtype tagged with green fluorescent protein (GFP) for 72 hours and monitored for viral entry by fluorescence microscopy. All breast tumor cell lines exposed to HCMV were positive for viral entry as demonstrated by green fluorescence. NuFF cells exposed to HCMV were also positive for viral entry. Mock infected breast tumor cells and NuFF cells were all negative for viral entry as expected. Results indicate that viral entry occurs independent of ER/PR/HER2 receptor interactions since all four breast tumor cell lines have distinct ER, PR, and HER2 receptor expression profiles. .

Breast tumor cells support viral gene expression



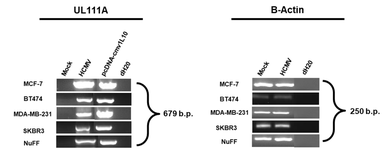


Figure 3. Breast tumor cells exposed to HCMV express immediate-early, early, and late viral genes. RNA harvested from mock infected and infected breast tumor cells was reverse-transcribed to cDNA and analyzed for HCMV immediate-early, early, and late viral gene expression using RT-PCR. Mock infected breast tumor cells were negative for UL123 (immediate-early), UL54 (early), and UL111A (late) HCMV gene expression. Breast tumor cells exposed to HCMV were positive for UL123 (immediate-early), UL54 (early), and UL111A (late) HCMV gene expression. These results indicate that breast tumor cells support HCMV infection in vitro.

HCMV-infected breast tumor cells express viral proteins

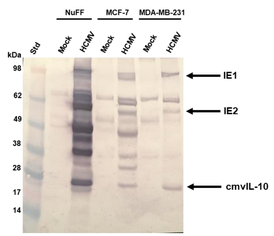


Figure 4. HCMV-infected breast tumor cells express HCMV proteins. Lysates from mock infected or infected breast tumor cells were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotted using plasma from a representative HCMV-positive donor. Lysates from HCMV-infected breast tumor cells showed a significantly stronger response 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 78 kDa, while the IE2 protein can be seen at 60 kDa. 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.*

Viral progeny are produced during infection of breast tumor cells

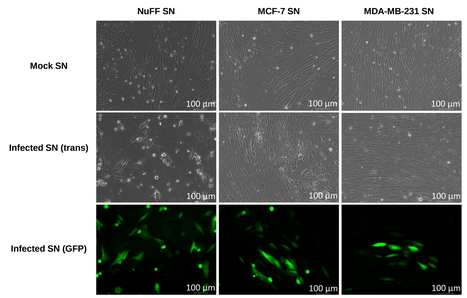


Figure 5. Infected breast tumor cells produce infectious viral progeny. Supernatant (SN) from mock infected and infected breast tumor cells was harvested and used to treat NuFF cells in order to determine if infected breast tumor cells produce infectious viral progeny. SN from mock infected and infected NuFF cells was used as a control. Fluorescence microscopy results demonstrated that NuFF cells treated with SN from infected MCF-7 or MDA-MB-231 cells were positive for infection as indicated by areas of green fluorescence. NuFF cells treated with SN from infected NuFF cells were positive for infection as well. NuFF cells treated with SN from mock infected MCF-7, MDA-MB-231 or NuFF cells were all negative for infection as indicated by lack of fluorescence. These results demonstrate that HCMV-infected breast tumor cells are producing infectious viral progeny and also suggest that these infected breast tumor cells are undergoing lytic (productive) viral infection.

Conclusions

- Human breast tumor cells permit HCMV entry *in vitro.* This suggests that HCMV may enter human breast tumor cells *in vivo*.
- Human breast tumor cells support immediate-early, early, and late viral gene expression *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 human 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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