Human Breast Tumor Cells Support Productive HCMV Infection

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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 UL117A 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. Neoadjuvant human foreskin fibroblasts (NuFF) cells were used as a control (Table 1).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>ER expression</th>
<th>PR expression</th>
<th>HER2 expression</th>
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<tbody>
<tr>
<td>MCF-7</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BT474</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NuFF</td>
<td>-</td>
<td>-</td>
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*NuFF = Neoadjuvant 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, 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 reverse transcriptase polymerase chain reaction (RT-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

1. Breast tumor cells permit HCMV entry

Breast tumor cells were exposed to HCMV clinical strain TB40/E, which is tagged with green fluorescent protein (GFP). Tumor cells were harvested for analysis of viral protein expression by fluorescence microscopy. All breast tumor cell lines 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. These results indicate that breast tumor cells have distinct ER, PR, and HER2 receptor expression profiles.

2. Breast tumor cells support viral gene expression

Breast tumor cells were infected with the HCMV clinical strain TB40/E. RNA was extracted and cDNA synthesized. Supernatant (SN) harvested from mock infected and infected breast tumor cells was reverse transcribed to cDNA and analyzed for HCMV gene expression by reverse transcriptase polymerase chain reaction (RT-PCR). Mock-infected breast tumor cells showed no significant response to HCMV infection. In contrast, infected breast tumor cells showed a significant response to HCMV infection as indicated by areas of green fluorescence. These results suggest that breast tumor cells support HCMV infection.

Conclusions

• Human breast tumor cells permit HCMV entry in vitro. This suggests that HCMV may enter human breast tumor cells.

• 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. For providing further support for the hypothesis that breast tumor cells support HCMV infection.

• Infection of virus progeny 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 ELISAs and investigation of the impacts of HCMV infection on breast tumor cell surface receptor expression patterns.

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References

