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Title: Investigation of Epstein-Barr virus in breast tumour cells

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Introduction:
Breast cancer is the most commonly diagnosed cancer in women world-wide. Which makes it a significant burden and thus an important area of research. There is intriguing evidence which indicates that exposure to Epstein-Barr virus (EBV) may contribute to the risk and development of breast cancer in humans. More recently a link has been found between breast cancer risk and late exposure to Epstein-Barr virus. For further investigation of the association of EBV and breast tumours, an appropriate laboratory technique needs to be established to detect the virus. Immunohistochemistry (IHC) and polymerase chain reaction (PCR) – based methods have previously been used to identify EBV in breast tumour samples. Unfortunately these methods have limitations, including the non-specificity of antibodies used in IHC, and the inability of PCR methods to determine which cells contain the virus. Therefore, investigation of improved molecular biological techniques is essential for studying viral gene expression and breast cancer. RNA in situ hybridisation is a relatively new technique that uses fluorescently labelled probes to detect messenger ribonucleic acid (mRNA). This method has the potential to detect EBV and quantify viral gene expression, and thus may be a superior method for studying the association between EBV expression and breast cancer formation. Such an approach has not been done before for EBV.

Aim:
The aim of this project was to determine whether RNA in situ hybridisation (or RNAscope) can detect RNA produced by EBV in histological breast cancer tissue sections. More specifically we wanted to identify which cell types within the section, if any, carried the virus.

Impact:
Development and understanding of the use of RNA in situ hybridisation for detecting viruses, such as EBV, is fundamental for the use of this method in future studies and for use as a screening tool.

Method:
RNA in situ hybridisation was carried out on 17 breast cancer tumour sections provided by the Cancer Society Tissue Bank, in order to detect mRNA from EBV. This method uses fluorescently labelled probes which bind to mRNA, which in turn can be detected as fluorescent dots. Using fluorescent microscopy at 40x objective, positivity of EBV was recorded by

counting the number of mRNA molecules detected (red dots) and total cell count (DAPI/blue stained nuclei) in 5 areas from the same section. Furthermore, I assessed which cell types showed positive expression for EBV (for example: adipocyte, stromal, and tumour cells). A lymphoblastoid cell-line, which contains EBV, was used as a positive control. Staining for dapB (a bacterial gene) on breast tumour sections was used as a negative control.

Results:

After optimisation of the RNA in situ hybridisation method, EBV was detected in the histological breast tumour tissue sections from all 17 samples (100%). There was no RNA detected in the negative control. The number of EBV mRNA molecules detected per cell across these sample ranged from 0.1 to 1. I also observed that EBV mRNA was expressed differentially in different cell types. For example, some tumours showed a uniform pattern of one dot per cell, while others showed a more clustered distribution of EBV RNA localised to particular cells. EBV gene expression appeared to not be restricted to any particular cell type as I observed positive expression in the adipocytes, stromal, and tumour cells. To our knowledge this is the first reported example where EBV has been detected in cells other than lymphocytes and epithelial cells.

Conclusion:

For the first time we have detected EBV gene expression in breast tumour cells using RNA in situ hybridisation. The success of this project means that we can accurately define which cells contain the virus, exploit this assay for a larger study, and explore the option of using multiple probes to detect different viruses simultaneously. If a link between viruses and breast cancer can be confirmed, it could lead to the targeted treatment or prevention of a significant proportion of breast cancer.