Understanding the Interactions between Immune Cells and Mammary Tissue in Pre-Cancerous BRCA1 Mutation Carriers

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Introduction

- BCRA1 is a key tumour suppressor gene associated with breast cancer. Carriers of a BRCA1 mutation have a very high risk of developing breast cancer compared to the rest of the population (55% versus 12%)[4].
- BRCA1 deficiency is also linked to the regulation of type-I/type-II interferons resulting in the recruitment of monocytes and macrophages to the tumor[2].
- It is well understood that monocytes and macrophages play an important role in breast tumours, as tumour cells release cytokines that recruit immune cells. These tumour-associated macrophages (TAMs) then release cytokines that lead to upregulation of transcription factors like NF-kB, whose improper regulation is linked to promotion of cancer cell survival and tumour growth[3].
- Although much is known about macrophage interactions in tumours, little is known about the role of immune cells in pre-cancerous tissue. This project investigated immune cell distribution in the mammary tissue comparing non-cancerous BRCA1 mutation carriers with healthy individuals. Paraffin sections of human mammary tissue are studied through immune cell quantification using immunohistochemistry staining of CD68 antigens found on monocytes and macrophages.

Research Question

- **Is there a difference in the monocyte and macrophage cell distribution in the luminal epithelial tissues of the human mammary gland between carriers of a BRCA1 mutation and healthy individuals?**
- The hypothesis is that noncancerous BRCA1 mutated mammary cells release more cytokines than healthy cells, thus recruiting more immune cells.

Methodology

- 6 women ages 35-50 were studied: 3 women with normal BRCA1 status and 3 women identified as BRCA1 mutation carriers. Tissue samples were obtained through prophylactic mastectomies (mutation carriers) and through reduction mammoplasties (normal patients) and were not cancerous.

Immunohistochemistry:

- Sections of formalin-fixed paraffin embedded human mammary tissue were cut via microtone 4µm thick and placed onto slides.
- Sections were deparaffinised and incubated with mouse monoclonal anti-human CD68 (1:200, Thermo Fisher Scientific, Inc.) as the primary antibody. Slides were then incubated with universal anti-mouse (Nichirei Biosciences, Inc.) as the secondary antibody and then developed with DAB visualization (DAKO DAB+ Substrate Chromogen, Agilent Technologies, Inc.). Lastly, slides were counterstained with haematoxylin.
- Stained sections were examined under bright field microscopy with using a Zeiss AxioImager M2 microscope and CD68+ cells were quantified as a function of tissue surface area using photo editing software.
- Since macrophage infiltration into the luminal epithelial cell layers of alveoli and ducts from the stroma[1] is of interest, only these cell layers of these structures were studied, ignoring the surrounding adipose tissue and stroma.

Results

- The immunohistochemistry staining for CD68 antigen was a success, as CD68+ cells are clearly visible and well defined in brown when viewed under the microscope. Alveoli/tubules of the mammary gland were grouped, counted, and plotted separately from large inter/intralobular ducts.

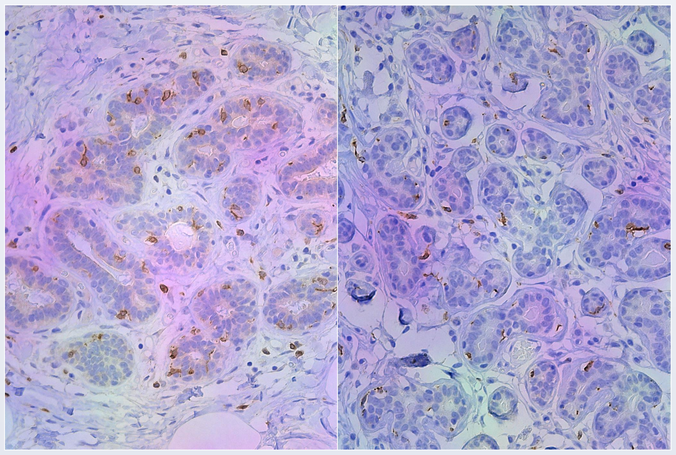

Figure 1. Immunohistochemistry of formalin-fixed paraffin embedded human mammary gland tubules in a BRCA1 mutation carrier (left) and healthy individual (right) stained with anti-Human CD68, followed by DAB chromogen visualization (brown). Nuclei are counterstained with haematoxylin (blue). Image was taken at 200x magnification.

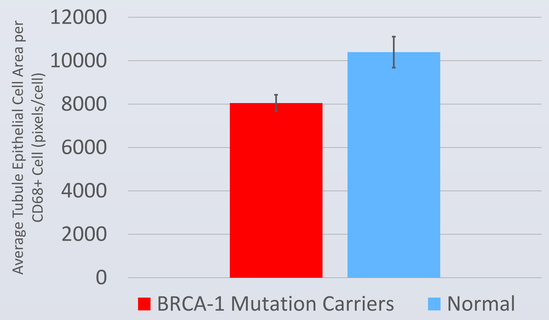

Figure 2. Average luminal epithelial cell area of mammary tubules/alveoli in pixels per IHC identified CD68+ cell in BRCA1 mutation carriers (red) and healthy individuals (blue). Data is expressed as the combined mean of each group ± combined standard error (p-value = 0.007).

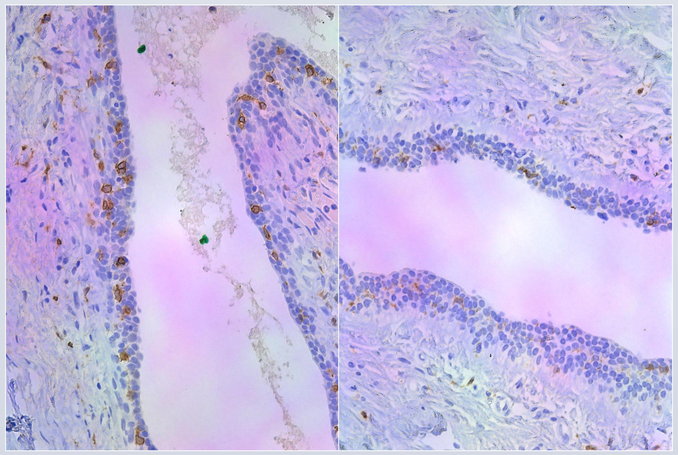

Figure 3. Immunohistochemistry of formalin-fixed paraffin embedded human mammary gland lobular duct in a BRCA1 mutation carrier (left) and healthy individual (right) stained with anti-Human CD68, followed by DAB chromogen visualization (brown). Nuclei are counterstained with haematoxylin (blue). Image was taken at 200x magnification.

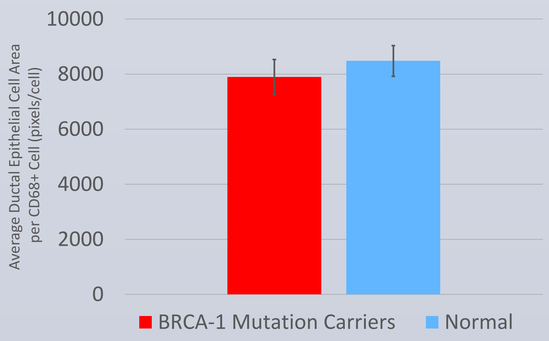

Figure 4. Average luminal epithelial cell area of mammary inter/intralobular ducts in pixels per IHC identified CD68+ cell in BRCA1 mutation carriers (red) and healthy individuals (blue). Data is expressed as the combined mean of each group ± combined standard error (p-value = 0.043).

Conclusions

- In the alveoli/tubules there is a significantly lower (p_{value}=0.007) mean mammary luminal epithelial cell area per CD68+ cell in the BRCA1 mutation carriers than in the healthy individuals. This means that more macrophage infiltration is observed.
- The mean duct epithelial cell area per CD68+ cell is also lower in BRCA1 mutation carriers, it is not as significant (p_{value}=0.043) than in the alveoli, so a definitive conclusion cannot yet be drawn.
- **Our hypothesis was validated by our results, since there was increased macrophage infiltration of tubules/alveoli of mammary glands in BRCA1 mutation carriers, but the results were not as definitive when analyzing the large ducts of the mammary gland.**
- The next step would be to repeat the study and increase the sample size, looking at more mammary gland structures from more patients to reduce variance to more definitively draw a conclusion for the entire mammary gland.
- If immune cell infiltration is higher in pre-cancerous BRCA-1 mutation carriers, then the specific mechanisms in which pre-cancerous BRCA1 mutated mammary cells recruit these immune cells are then of great importance in furthering our understanding of the development of BRCA1-related breast cancer.

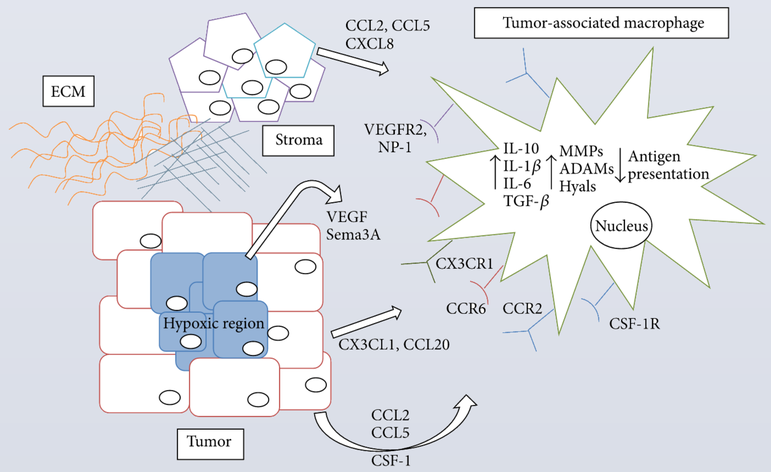

Figure 5. The complex interactions of tumour-associated macrophages and tumour cells (Brady et al. 2016)

References

1) Brady, N. J., Chuntova, P., & Schwertfeger, K. L. (2016). Macrophages: regulators of the inflammatory microenvironment during mammary gland development and breast cancer. *Mediators of inflammation*, *2016*.
2) Buckley, N. E., Hosey, A. M., Gorski, J. J., Purcell, J. W., Mulligan, J. M., Harkin, D. P., & Mullan, P. B. (2007). BRCA1 regulates IFN-γ signaling through a mechanism involving the type I IFNs. *Molecular cancer research*, *5*(3), 261-270.
3) Solinas, G., Germano, G., Mantovani, A., & Allavena, P. (2009). Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *Journal of leukocyte biology*, *86*(5), 1065-1073.
4) Roy, R., Chun, J., & Powell, S. N. (2012). BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nature Reviews Cancer*, *12*(1), 68-78.

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