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Title: Are Breast Tumour Cells and Adipocytes Co-conspirators in Aggressive Breast Tumours

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Introduction:

Over 600 New Zealand women lose their lives to breast cancer every year. To reduce this figure, the need to further our understanding of breast cancer is paramount. A related medical concern is the rise of obesity in New Zealand, which has been shown to contribute to worse breast cancer outcomes. It is therefore important to investigate cross talk between fat cells (adipocytes) and tumour cells, as well as how one might change the other. It has been reported previously that adipocytes get smaller in the presence of breast cancer. To our knowledge, however, this has never been rigorously tested in patient samples. Furthermore, changes in adipocytes are part of a recent hypothesis into how fat cells may promote breast cancer metastasis. Thus, a rigorous, objective investigation of adipocyte change is needed to assess the validity of this hypothesis. In addition, it has been recently been shown the maturity of the stroma, or supportive tissue, can predict prognosis for colorectal cancer. Whether this is also the case for breast cancer is yet to be tested.

Aims:

Our main aim was to measure the size of adipocytes inside breast cancer (cancer-associated adipocytes or CAAs) and compare them with those in normal breast tissue. We aimed to test whether CAAs produce different proteins to normal fat cells, and also aimed to measure stromal maturity. To gauge whether any changes were meaningful, we aimed to correlate our data with a predictive marker for patient outcome, the Nottingham Prognostic Index (NPI). Doing so would help us address whether changes in fat cells are associated with more aggressive breast tumours.

Methods:

First an understanding of what breast cancer looks like, and the cells involved, was gained to accurately distinguish CAAs from peripheral adipocytes. Immunohistochemistry procedures were optimised, in that the most effective antibody concentration was determined. Specifically, an antibody for the protein perilipin was optimised, which makes fat cells visible by staining the outside of the cell. Optimisation of the antibody for fibroblast specific protein-1, which may be produced by CAAs, was unsuccessful due to insufficient antibody binding.

A tissue microarray, with 90 patient samples, was stained for perilipin. Each sample was taken from the edge of a breast tumour, which was ideal for our purposes because it provided a selection of adipocytes both within and outside tumours. Ten samples were selected that contained both CAAs and peripheral adipocytes, which controlled for individual patient variation in adipocyte size. Adipocyte number and diameter were measured using a set of rules that were used consistently across the samples. Only adipocytes stained for perilipin were measured. Sample size was expanded to 31 by including samples with either CAAs or peripheral adipocytes. Statistical analysis was performed to test whether CAAs were significantly different from peripheral adipocytes. Data was correlated with the NPI, a prognostic tool that incorporates tumour size, grade and whether cancer has spread to the lymph nodes. A NPI higher score indicates worse prognosis.

A scoring system for stromal maturity was created, based on consultation with a pathologist. This measured the stromal density, colour and fibroblast size for each sample. Scores from the three variables were combined to give an overall maturity score, which was correlated with NPI.

Results:

Adipocytes inside breast tumours were significantly smaller than those outside it. This was true for the 10 samples initially selected and the expanded adipocyte size list (n=31).

The strongest correlations came from samples containing both CAAs and peripheral adipocytes (n=9). In particular, significant negative correlations were found between NPI and CAA number, as well as between NPI and the ratio of CAA: peripheral adipocyte number. A negative trend was found between NPI and peripheral adipocyte size. Conversely, a positive trend was found between NPI and the ratio of CAA: peripheral adipocyte size. No correlations were found when sample size was increased, but variation in patients' adipocyte size was not taken into account. No correlations were found between NPI and stromal maturity scores.

Conclusion:

Adipocytes are significantly smaller in breast cancer tumours than those adjacent to the tumour. To our knowledge, this is the first time this effect has been objectively tested and statistically confirmed in patient samples. It remains unclear whether changes in adipocyte size affect the proteins they produce and this should be further explored.

For samples containing both CAAs and peripheral adipocytes, worse prognosis seems to be associated with having fewer, larger CAAs. However, this conclusion is based on a relatively small sample size and further research is needed to assess its validity for the wider population.

Stromal maturity may not be predictive of patient prognosis in breast cancer. However, this may reflect differences in our definition of stromal maturity, compared with the definition applied to colorectal cancer.

Overall, we have clearly demonstrated that adipocytes are altered in the breast cancer process. While we cannot say whether they play an active role, the possibility remains that adipocytes may be co-conspiring with cancer cells to make breast tumours more aggressive.