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Title: Identification of microRNAs in breast tumour cells after co-culture with adipocytes

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Introduction:

Breast cancer is the most commonly diagnosed cancer in New Zealand women. Poor outcomes for breast cancer patients have been linked with obesity in previous studies. Obese women have more distant metastases at diagnosis and higher mortality rates. For this project, I worked with the Mackenzie Cancer Research Group who developed an experimental co-culture system which grows breast tumour cells with adipocytes (fat cells). This changes how both the adipocytes and the breast tumour cells behave. Adipocytes become dedifferentiated, less lipid rich, and secrete factors which enhance survival and migration of breast tumour cells. The breast tumour cells become more resistant to chemotherapy and display a more aggressive phenotype making them more likely to migrate.

MicroRNAs (miRNAs) are small non-coding lengths of RNA. They have a key role in the regulation of gene expression and are important in healthy individuals. Cancer is a complex genetic disease and so it is no surprise that miRNAs have been identified as having a role in cancer development. It is also thought that miRNAs have a role in the growth and maturity of adipocytes. There is no current literature reporting the identification and function of microRNAs in tumour cells when they are grown with adipocytes.

Aim:

Obesity rates around the world are climbing and the link of obesity to various forms of cancer is becoming a more important topic to understand. The aim of this project was to identify microRNAs that may be contributing to the changes seen in breast tumour cells co-cultured with adipocytes. This will give greater insight into the biology of adipocytes and breast tumour cells and their interactions.

Method:

Breast tumour cells (MCF7) were grown with and without adipocytes. Tumour cell RNA was then extracted and converted to cDNA. miRNAs were identified using a screening kit that allowed detection of 372 individual miRNAs. Data was analysed; based on detection limits only 226 of the miRNAs were included in the data set for further analysis. The differences between miRNAs in breast tumour cells grown with or without adipocytes were compared using one-way ANOVA analysis to identify miRNAs with significant differences in quantity between the conditions. Five candidate miRNAs were chosen for further validation. This was determined using a series of criteria. Candidate miRNAs had to show a significant difference in quantity between conditions, be detected at a reliable quantity, and have a fold change of at least 2.

Results:

The miRNA screening had consistent results across the samples tested. Our results identified 31 miRNAs that showed significantly more or less miRNA in breast tumour cells grown with or without adipocytes. We found that of these 31 miRNAs, 16 increased and 15 decreased significantly when

breast tumour cells were grown with adipocytes. The five candidate miRNAs chosen for further validation were compared to a literature review conducted at the beginning of the study. It was found that all five candidate miRNAs have been implicated in breast cancer in previous studies. Two of the candidate miRNAs, miR-205 and miR-210, are well researched in the field of breast cancer and are promising candidates for further study.

Conclusion:

The discovery that numerous miRNAs vary their quantity between breast tumour cells grown with or without adipocytes is novel. No previous studies have investigated the effect of adipocytes on miRNAs in tumour cells. Evidence from this study suggests that miRNAs are part of the mechanism through which adipocytes promote changes in breast tumour cells. This adds to what is currently known about the interaction of adipocytes with breast cancer cells. This pilot study allows further investigation into this area; starting with validation of results using the candidate miRNAs.

The miRNAs we identified may prove to be important in the interaction between adipocytes and breast tumour cells. Further work is needed to see if they have a role to play the increased invasiveness of breast cancer cells and in worse outcomes for obese breast cancer patients.