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Project: Investigation of markers of fatty acid metabolism and B-oxidation in breast cancer cells

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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 rate. 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 (more stem cell-like), 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 radiotherapy and display a more aggressive and migratory phenotype making them more likely to spread to other parts of the body. The mechanism responsible for this has not been determined but many ideas have been suggested. We think changes in breast cancer cell metabolism may play an important role. Metabolism is important in cancer and allows rapid growth and development of tumours. Adipocytes can release metabolites that are able to be used in both glycolysis and fatty acid metabolism. Previous studies have shown changes in glycolysis and fatty acid metabolism in varying types of cancer.

There is no current literature exploring the changes in metabolism in breast cancer cells grown with adipocytes. Our hypothesis is that breast cancer cells induce adipocyte lipolysis and the release of glycerol and free fatty acids from the adipocytes provides a rich source of metabolites that fuels breast cancer cell invasion and metastasis.

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 changes in cancer cell metabolism that may be contributing to the aggressive phenotype seen in breast tumour cells co-cultured with adipocytes. This will give greater insight into the biology of adipocytes and their interactions with breast tumour cells.

Method:

Adipose tissue samples were collected by the Cancer Society Tissue Bank from patients at Christchurch Hospital undergoing surgery for therapeutic mastectomy, prophylactic mastectomy and breast reductions. Breast tumour cells (MCF7 and MDA-MB-231) were co-cultured with adipocytes isolated from these breast adipose tissue samples. MDA-MB-231 is a triple negative cell line meaning it lacks hormone receptors for estrogen, progesterone and human epidermal growth factor (ER-, PR-, HER2-), whilst MCF7 is an ER+ cell line.

Previous work in this lab has shown changes in breast tumour cell metabolism during adipocyte co-culture. Thus, after co-culture, breast tumour cells were analysed using Western blotting to look at specific proteins in metabolic pathways and invasion assays to look at metastatic potential.

We then compared the differences between breast tumour cells grown with or without adipocytes. Western blotting was used to assess differences in the expression of proteins involved in β-oxidation (fatty acid metabolism) between breast cancer cells grown alone or in co-culture with adipocytes. Carnitine palmitoyltransferase 1 (CPT1A) is a protein involved in translocation of fatty acids into the mitochondrial matrix for β-oxidation. Phosphorylation of the key metabolic enzyme, acetyl-CoA carboxylase (ACC), relieves inhibition of CPT1A to allow fatty acid translocation. AMP-activated protein kinase (AMPK) is activated by phosphorylation and inhibits ACC, increasing CPT1A availability. Invasion assays were used to determine if functional changes occurring in the breast cancer cells may be linked to observed metabolic changes.

Results:

Western blotting found no difference in CPT1A protein level between co-culture with adipocytes, or cells grown alone, for MCF7 or MDA-MB-231. This was unexpected as CPT1A is a key protein in the β-oxidation pathway and thus we expected alterations in concentration during co-culture. ACC2 was significantly lower in adipocyte co-cultured cells compared to cells grown alone for MCF7 but not MDA-MB-231. In line with this, phosphorylated-ACC was significantly higher in cells grown in adipocyte co-culture compared to those grown alone for both MCF7 and MDA-MB-231. This decrease in active ACC means inhibition of CPT1A is reduced and therefore more CPT1A is available for fatty acid translocation. There was no difference in phosphorylated-AMPK levels between adipocyte co-cultured cells or cells grown alone, although, there was a noticeable trend toward higher levels in adipocyte co-culture for MCF7.Invasion assays indicated that MDA-MB-231 had significantly greater invasion after adipocyte co-culture compared to being grown alone. There was however no significant difference in invasion for MCF7 cells grown alone or in adipocyte co-culture. MDA-MB-231 are considered a more aggressive cell line which may be partly due to their ability to take advantage of adipocyte co-culture to increase invasion whilst MCF7 show no change.

Conclusion:

The investigation of proteins involved in β-oxidation has shown that there are alterations in this metabolic pathway during adipocyte co-culture. This suggests that breast cancer cells are changing their metabolism to take advantage of fatty acids released by the adipocytes. The increase in invasion seen in MDA-MB-231 suggests that these metabolic changes may have an influence on breast tumour cell behaviour, allowing a more aggressive phenotype. There is a lot of future work to be done in exploring breast tumour cell metabolism in adipocyte co-culture, but results so far are promising and direct us to further investigate these lines of research.