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Project: The effect of comorbidities on breast cancer

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Introduction:
Breast cancer is one of the most commonly occurring cancers in women, not only in New Zealand but worldwide.

Metabolic syndrome is a cluster of metabolic risk factors that increase the risk of developing conditions such as heart disease and diabetes. These risk factors include abdominal obesity, dyslipidemia, hypertension and hyperglycemia.

In New Zealand, 31% of adults are obese and 60% of obese individuals have metabolic syndrome. It has been found previously that obesity and metabolic syndrome are associated with an increased risk of developing breast cancer as well as with a poorer prognosis. In addition, our group has shown in cell culture that fat cells within and surrounding the tumour promote tumour cell invasion and induce chemotherapy resistance, providing a possible explanation as to why obese women with breast cancer have higher mortality rates and more distant metastases.

Next we wanted to investigate the effects of obesity and metabolic syndrome on breast cancer in a whole organism. To this end, we developed mouse models of breast cancer with these comorbidities.

ApoE mice are obese and exhibit elevated plasma cholesterol and triglyceride levels. The ApoE/ArKO mouse displays all the hallmarks of metabolic syndrome including insulin resistance, progressive hypertension and high fasting glucose levels. A number of molecular factors play important roles in breast cancer progression. HIF-1Î± is a protein stabilised by hypoxia and is known to promote tumour growth and invasion. IL-6 is a pro-inflammatory cytokine involved in cancer growth and metastasis.

Aim:
The aim of this study was to investigate the effect of comorbidities on molecular factors in breast cancer. This will provide a deeper understanding of the underlying mechanisms responsible for the changes in breast cancer progression in the presence of obesity and metabolic syndrome.

Method:
Murine breast cancer cells (EO771) were injected into the mammary fat pad of both mouse models, as well as wild-type (WT) mice as controls. Mice were monitored daily and sacrificed when the tumours reached ethical endpoint. Primary and secondary tumours (metastases), organs and plasma were harvested for analysis.

The HIF-1Î± protein content of tumours was determined by Western Blot analysis. In addition, proliferation of tumour cells, tumour hypoxia, and fat content of tumours were assessed using immunohistochemistry (IHC). Specifically, proliferation was assessed by staining for pHH3, a marker of cell proliferation and fat content by staining for perilipin, a protein expressed on the surface of adipocytes.

Hypoxia was detected by injecting mice with pimonidazole. Pimonidazole perfuses tissues and aggregates in areas of low oxygen and can therefore be used as a hypoxia marker.

Finally, the levels of IL-6 in the plasma and in the tumours were determined by ELISA. The plasma levels of IL-6 were also compared to those of non-tumour-bearing mice.

Results:
Both ApoE and WT mice developed peritoneal metastases in 20-25% of cases. No ApoE/ArKO mice developed metastases. Primary tumours from ApoE/ArKO mice were significantly more proliferative than those from WT mice.

In addition, there was a trend for increased proliferation of primary tumours from ApoE mice compared to WT. Metastases were significantly less proliferative than primary tumours in ApoE mice, with a similar trend seen in WT mice.

There was no difference in proliferation of metastases in ApoE compared to WT mice. The hypoxia level of primary tumours was unchanged across the three groups. However, metastases from ApoE mice were significantly more hypoxic than those from WT mice and significantly more hypoxic than primary tumours from ApoE mice.

Metastases from WT mice did not display a higher degree of hypoxia than primary tumours. HIF-1Î± expression in primary tumours was not significantly different across the three groups and no significant differences were found between primary and secondary tumours, or between secondary tumours from WT and ApoE mice. No difference in adipocyte content of primary or secondary tumours was found between the three mouse genotypes.

In addition, there was no difference in adipocyte content between primary and secondary tumours within a group. All three groups had similar IL-6 concentrations in the plasma.

When tumour-bearing mice were compared with non-tumour-bearing mice, plasma IL-6 tended to be increased in tumour-bearing mice. There was no significant difference in tumour IL-6 concentration between groups, either for primary or secondary tumours. However, the IL-6 concentration in tumours from WT metastases was significantly higher than in primary tumours. This was not observed for ApoE mice.

Conclusion:
Taken together, our results suggest that metabolic syndrome and obesity in mice cause primary tumours to be more proliferative, but have no major impact on hypoxia, fat content or IL-6 concentration.

Furthermore, these comorbidities had different effects in secondary tumours, indicating that obesity and/or elevated plasma triglyceride and cholesterol levels lead to highly hypoxic but less proliferative metastases when compared to primary tumours.

Although this study provides some insight into the mechanisms leading to the poor breast cancer prognosis associated with metabolic syndrome and obesity, further research is necessary to better understand why the presence of comorbidities contributes to more proliferative, more aggressive breast tumours.