Metastatic breast cancer cells secrete factors that degrade the cerebral endothelial glycocalyx

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Introduction: Metastasis is the formation of a secondary tumour at a distant site from the primary tumour. 10% to 30% of women with metastatic breast cancer suffer brain metastases which severely impact quality of life and life expectancy (median overall survival < 1 year even with treatment). The risk of brain metastasis is usually highest for people with more aggressive subtypes of breast cancer, such as triple-negative breast cancer. The formation of brain metastases from primary breast cancer most commonly occurs via the vasculature since the brain lacks lymphatics. Thus, the metastasising, circulating cancer cells are initially required to adhere to and pass through the blood brain barrier (BBB) which lines the cerebral capillaries. The BBB is composed of a capillary endothelial cell (EC) layer with multiple tight junctions, supporting pericytes, a layer of basement membrane and astrocytic end-feet. The first structure that circulating tumour cells interact with on the BBB is the endothelial surface layer or glycocalyx that covers the luminal surface of all blood vessels. Glycocalyx degradation is associated with a range of disease conditions and its disruption has been reported during tumour progression. However, it is still not fully understood how circulating breast cancer cells can interact with and alter the GCX structure to promote their vascular adhesion and subsequent transmigration into the brain.

Aim: To examine the effect of factors secreted from triple-negative breast cancer cells on the integrity of the glycocalyx of cerebral microvascular ECs.

Methods: hCMEC/D3 cerebral microvascular ECs were cultured to recreate the BBB in vitro and MDA-MB-231 cells were cultured as a model of triple negative breast cancer cells. MDA-MB-231 conditioned medium (CM) was collected from confluent cells cultured for 24 hours in basal EC media. Confluent ECs, in a 96 well plate, were then treated with the cancer cell CM (0, 30, 60 minutes and 24 hours) or neuraminidase as a positive control (30, 60 minutes). Glycocalyx integrity was then assessed using a cell-based fluorescence (CBF) assay. Specifically binding of FITC-WGA to the glycocalyx was measured using a fluorescence plate reader and fluorescence intensity was normalized to protein/well as assessed by BCA assay. Data were expressed as % of untreated control.

Results: Neuraminidase dramatically reduced fluorescence readings after 30- and 60-minutes time points, compared with their controls, confirming the validity of the experimental approach. 30-minutes treatment with CM had no effect on glycocalyx, whereas 60-minutes, 6 hours and 24 hours treatments did reduce glycocalyx as assessed by fluorescence readings. 6 hours CM treatment caused a 10% mean reduction of the hCMEC/D3 GCX (90.3%) in comparison with the control (100%, n=6), while 24 hours treatment reduced WGA-FITC staining of the GCX by 36% (mean 64.0% vs control, 100%, n=3).

Conclusions: MDA-MB-231 CM contains secreted factors that degrade the cerebral microvascular glycocalyx after 6hrs and 24 hrs exposure. This may be a critical initial step in the adhesion of circulating cancer to the brain endothelium and the secreted factors responsible are under investigation.