IDENTIFICATION OF THERAPEUTIC TARGETS IN DIABETES-INDUCED BREAST CANCER METASTASIS

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INTRODUCTION

Breast cancer (BC) is the most common cancer in women worldwide. Recent publications report a significant increased risk of breast cancer in women with diabetes. It has been observed that tumors and other developing cells exhibit a drastic increase in glucose uptake. Cellular metabolism is therefore believed to play a critical role in tumor progression. Individuals with diabetes accumulate greater levels of advanced-glycation end-products (AGEs). Some reports suggest that the receptor for AGE (RAGE), a multi-ligand receptor, contributes to generation of an inflammatory microenvironment and thus possibly favors tumorigenesis.

Bone metastasis is a major cause of mortality and morbidity for patients with BC. Predominantly associated with osteolytic bone lesions, the expression of colony-stimulating factor-1 (CSF1) has previously been identified as one of the main drivers of BC osteolytic metastasis and a marker of poor prognosis. *In vitro* studies have suggested that supplementation with omega-3 fatty acids may have a preventive effect in progression of BC metastasis.

The purpose was to investigate whether the high glucose (HG) concentration in growth environment (mimicking diabetic conditions) mediate its detrimental effects in human BC cells through the expressions of RAGE and CSF1. Furthermore, to determine whether the omega-3 fatty acids supplementation can reverse the HG-mediated expressions of RAGE and CSF1.

MATERIALS & METHODS

Breast cancer cells: MDA-P/MDA-MB-231 and ZR-75

Molecular classification of breast carcinoma

Classification	Immunoprofile	Other characteristics	Example cell lines (adapted from [13,22])
Luminal A	ER^+, $PR^{+/-}$, $HER2^-$	Ki67 low, endocrine responsive, often chemotherapy responsive	MCF-7, T47D, SUM185
Luminal B	ER^+, $PR^{+/-}$, $HER2^+$	Ki67 high, usually endocrine responsive, variable to chemotherapy. $HER2^+$ are trastusumab responsive	BT474, ZR-75
Basal	ER^-, PR^-, $HER2^-$	$EGFR^+$ and/or cytokeratin $5/6^+$, Ki67 high, endocrine nonresponsive, often chemotherapy responsive	MDA-MB-468, SUM190
Claudin-low	ER^-, PR^-, $HER2^-$	Ki67, E-cadherin, claudin-3, claudinin-4 and claudinin-7 low. Intermediate response to chemotherapy	BT549, MDA-MB-231, Hs578T, SUM1315
HER2	ER^-, PR^-, $HER2^+$	Ki67 high, trastusumab responsive, chemotherapy responsive	SKBR3, MDA-MB-453

EGFR, epidermal growth factor receptor; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Fig 1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4656721/

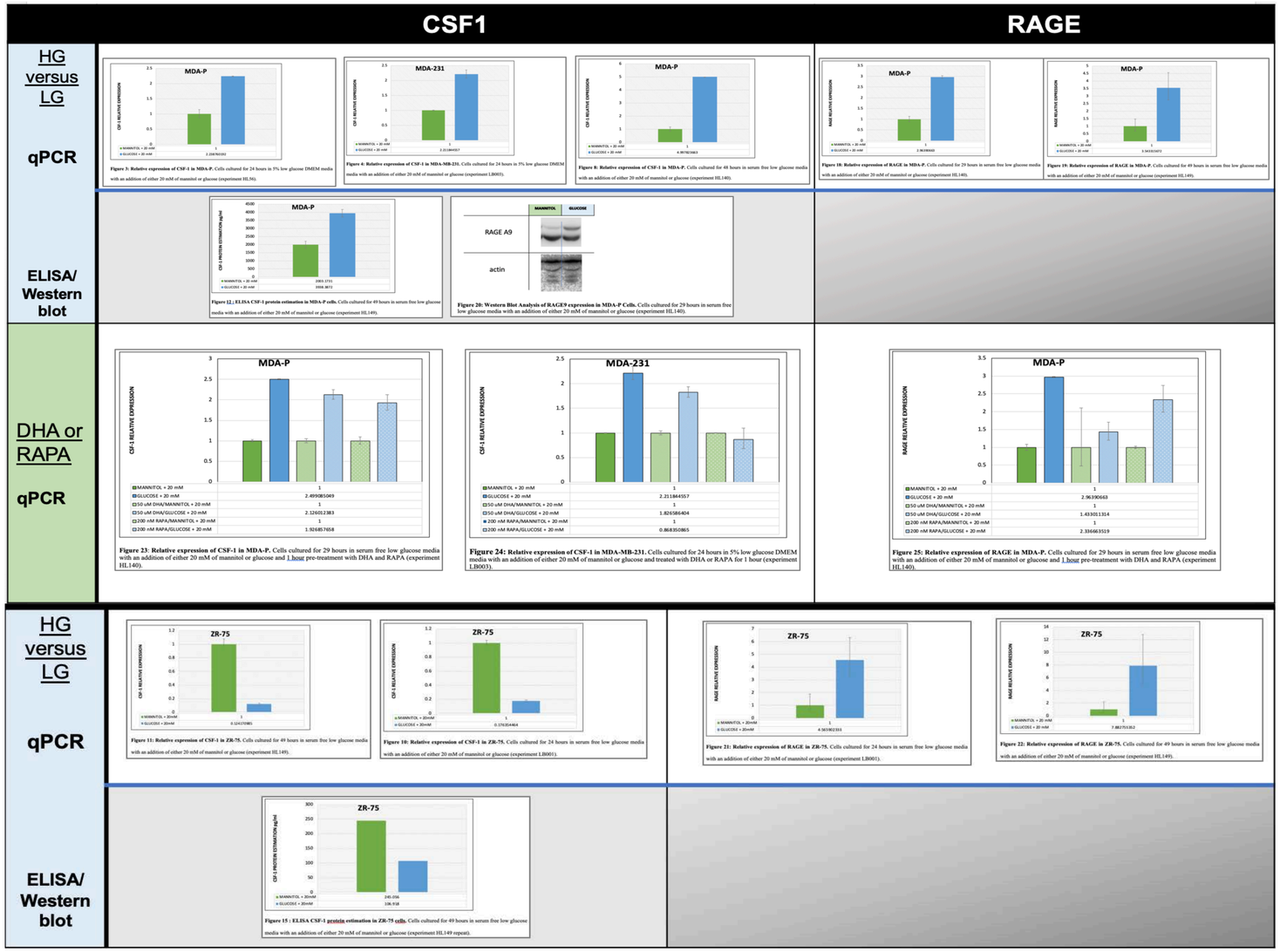
Cell culture and treatment: Cells were cultured in the absence (LG) or presence of high glucose (HG) to mimic diabetic conditions. Cells were later treated with omega-3 fatty acids (namely, docosahexaenoic acid, DHA) or rapamycin (RAPA).

Expression: CSF1 and RAGE protein expression were assessed using quantitative real-time polymerase chain reaction (qRTPCR), Western Blot analysis (for intracellular expression) or enzyme-linked immunosorbent assay (ELISA) (for secreted protein expression secretion).

RESULTS

All experiments were individually assessed. Trends were noted and represented in the results below.

MDA-P/ MDA-231



ZR75

DISCUSSION & CONCLUSION

Based on mild variability in experiments, all were assessed individually and trends were noted. Trends were as follows:

- A consistent approximately 2-fold increase in CSF1 protein expression in MDA-P/MDA-231 cells when cultured in presence of HG based on qPCR data. This trend was also seen in the ELISA data. Treatment with DHA or RAPA was only assessed in MDA-P/MDA-231. DHA and RAPA treatment appeared to be beneficial, reducing slightly such expression. As for CSF1 protein expression in ZR75, based on the qPCR and ELISA data, a consistent increase in expression was seen in HG with variable range between 2 to 4-fold.
- The trend was interestingly reversed in CSF1 protein expression in ZR75 cells when cultured in presence of HG based on qPCR data and ELISA data. Although of variable fold, HG lead to less expression. The trend was preserved in RAGE expression based on the qPCR data with a variable but consistently frank increase in RAGE expression with HG.

In conclusion, HG increased expressions of RAGE and CSF-1, two critical proteins implicated in development of BC metastasis. Highest increase in CSF-1 expression was found in the highly metastatic MDA-MB-231 cells in response to HG compared to the cells with lower metastatic potential or non-metastatic BC cell lines such as ZR-75. Both cell lines demonstrated frank increased expression of RAGE in HG conditions. Addition of DHA or RAPA to HG treatment reduced the expression of these two key proteins emphasized in diabetes-induced BC metastasis, indicating a possible avenue of targeted clinical therapy. This research project has several limitations but highlights areas that could potentially be interesting to focus further investigations.

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