Anti-cancer properties of Microalgae (T1) Extract in Breast Cancer Cell Lines

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ABSTRACT

Breast cancer (BC), a worldwide health issue, is the most common malignant cancer in females in GCC, including the State of Qatar (Ferlay et al., 2019;Narayan et al., 2020). Unfortunately, malignant tumors has the capability to metastasis, which involves both migration and invasion of cancer cells which are the most threatening aspects of cancer (McSherry et al., 2007). Consequently, researchers have concentrated on Complementary and Alternative medicine (CAM) modalities, as conventional medicine has been facing various challenges such as; poor understanding of the mechanisms with BC proliferation and invasion within various groups of patients, drug resistance, and the failure of current therapies to completely cure the disease. A significant CAM method have been raised which is the treatment with herbs and extracts derived from seeds, leaves, fruits and roots of plants; each of these invariably represents a combination of several bioactive compounds. Our biofuel has provided us with a crude extract of a microalgae coded as T1 that consist of carotenoids, chlorophyll a, and chlorophyll b. Carotenoids is a bioactive molecule that inhibits the proliferation, migration, invasion and induce apoptosis to tumor cells (Zhang X, Zhao WE, et.al.2011) (Koklesova L, Liskova A, et.al. 2020).

OBJECTIVES

Our present study aims to examine the effect of T1 extract on the proliferation, migration and invasion of MDA-MB-231 triple negative metastatic breast tumour cell line compared to the 3T3L normal cell line.

METHODOLOGY

Cell Proliferation Assay

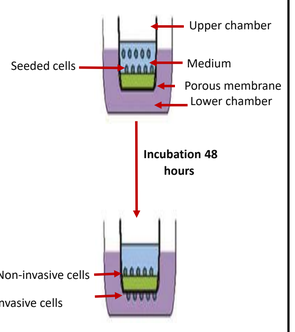
MDA-MB-231, MDA-MB-468 and normal 3T3L cells were seeded in 96 well plate.

Next day, 0.5% of T1 extract were added to cells.

Alamarblue were added to cells after 48 hours.

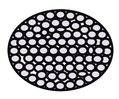
Flourescence measurements were taken using TECAN infinite M200 plate reader.

Cell Invasion Assay

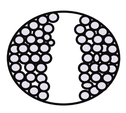


Cells Invasion assay was performed using Corning Matrigel Invasion Chambers. Cells were counted and resuspended in 0.5% Microalgae T1 extract and added onto the upper chamber of Matrigel plate. After 48 hours of incubation, cells were stained with crystal violet and pictures were taken in random areas using inverted microscope and number of cells were quantified using ImageJ software.

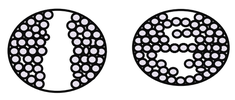
Wound Healing Assay



Cells were treated with 0.5% T1 extract in 6 well plate for 48 hours.



Straight scratch was made using sterile Tips.



The initial width of the scratch was measured at 0,24 and 48 hours

RESULTS

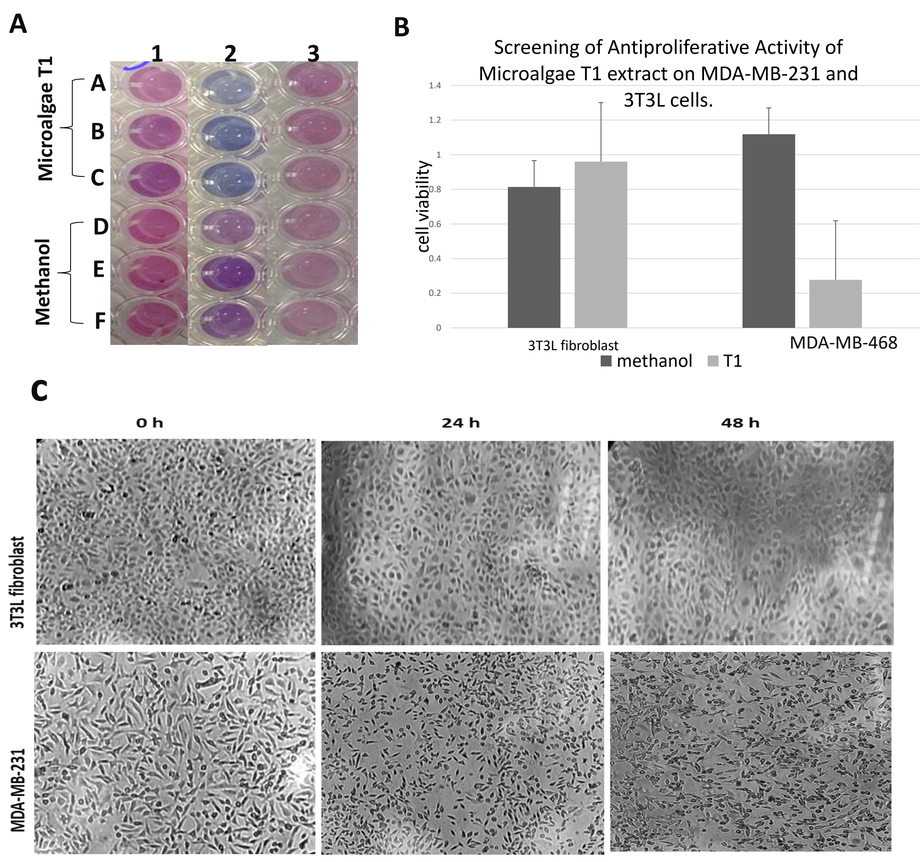


Figure1: **(A)** Alamar blue cell proliferation assay results of 1-MDA-MB-231,2-MDA-MB-468 breast cancer cells compared to normal 3T3L fibroblast after 48 hours of T1 extract treatment. **(B)** Showing the effect of T1 extract on MDA-MB-468 BC cells compared to normal 3T3L cells. **(C)** Showing morphology changes of MDA-MB-231 cells after treatment with microalgae (T1) extract for 24 and 48 hours compared to normal 3T3L fibroblast. Treated cells were rounded up, shrunken in size, and detached from surface of the wells after 48 hours. Number of cells was also found decreased when compared to the control.

RESULTS

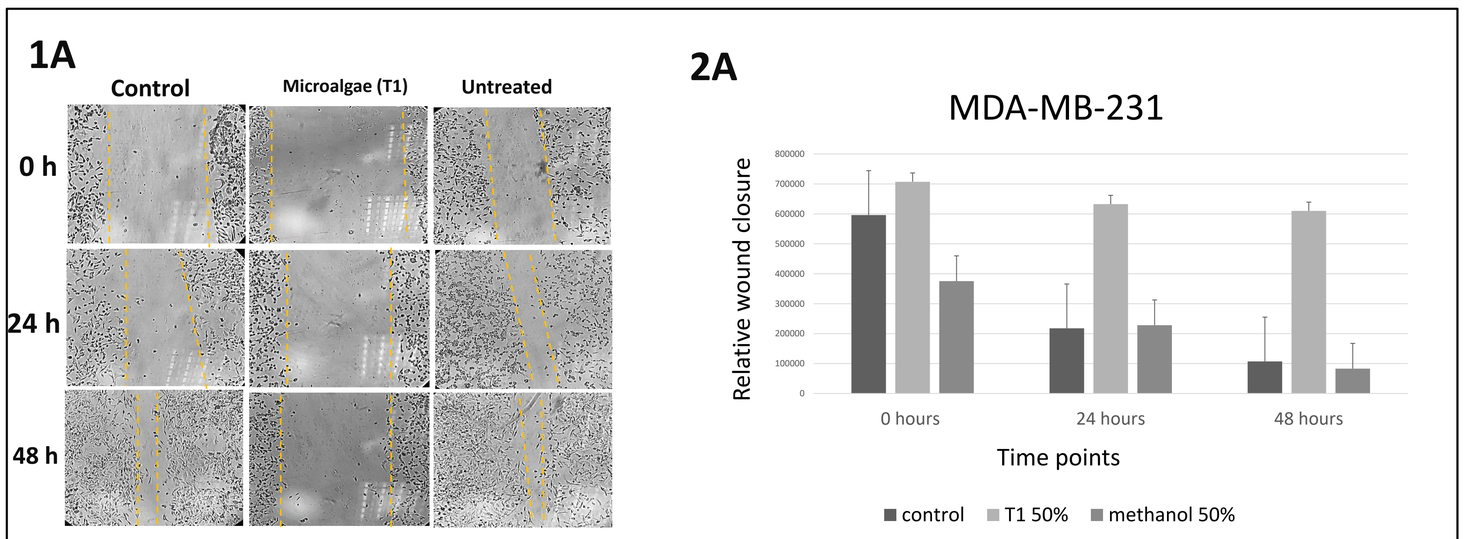


Figure 2: Wound healing assay showing the effect of Microalgae T1 on migration of MDA-MB-231 cell line. **(1A)** Death of MDA-MB-231 cells caused by Microalgae T1 extract decreased cell migration compared to methanol and control. **(2A)** Graphical representation of the relative wound closure after treatment with 0.5% Microalgae T1 extract and 0.5% methanol compared to control at different time points 0,24 and 48 hours.

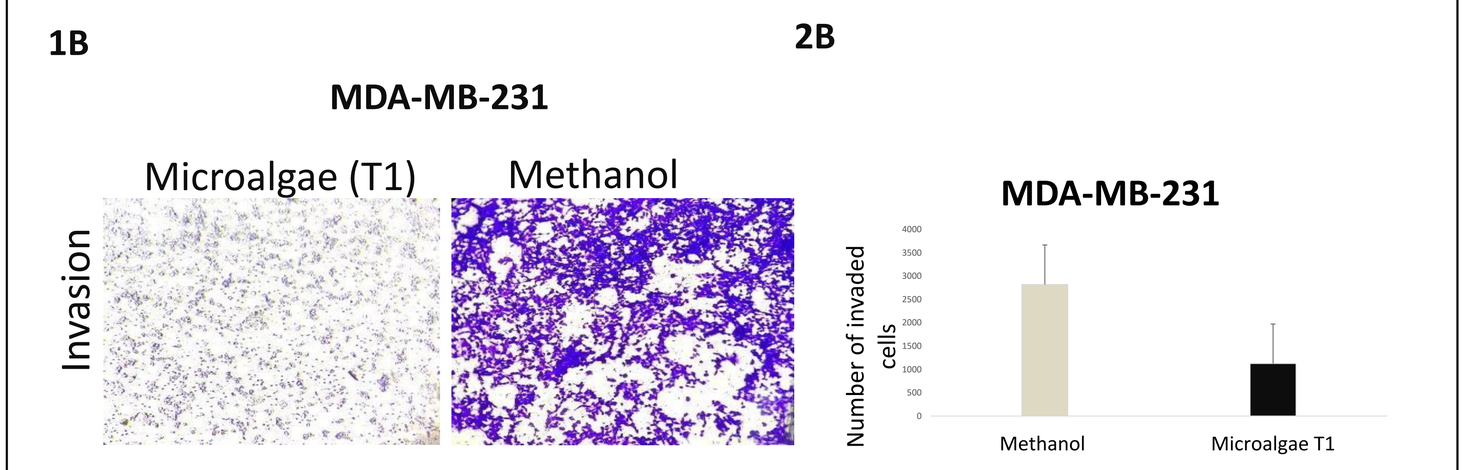


Figure 3: Microalgae extract inhibited the invasion of MDA-MB-231 cells. Boyden chamber assay was performed to assess invasion in MDA-MB-231 cells. **(1B)** Showing crystal violet-stained invaded cells at the lower chamber of transwell membrane after treating MDA-MB-231 cells by Microalgae T1 compared to methanol (control). **(2B)** Data illustrates the relative cell number of treated cells compared to methanol with a minimum of three areas were randomly selected to count cells under an inverted microscope.

Discussion

According to (Zhang X, Zhao WE, et.al.2011) carotenoids inhibit the proliferation of several cancer cell lines including breast, prostate, melanoma, lung, and leukemia, resulting in cycle-cycle arrest (Stivala LA, Savio M, et.al. 2000), inhibition of the malignant transformation of cancer cells (Bertram JS, Pung A, et.al. 1991) and the induction of apoptosis (Muto Y, Fujii J, et.al.1995). Interestingly, carotenoids act as an inhibitor to various invasive and metastatic mechanisms (Koklesova L, Liskova A, et.al. 2020). Carotenoids clearly decreased the expression of MMP-2, MMP-9, N-cadherin, CD44 receptor, and β-catenin. Similarly, it had inhibited the MAPK, NOTCH signaling, PI3K/AKT/NF-κB, Wnt pathway (Koklesova L, Liskova A, et.al. 2020). These data prove the effect of carotenoids on inhibiting proliferation, migration, invasion and inducing apoptosis at molecular level.

Conclusion

In conclusion, the microalgae (T1) extract inhibited cell proliferation and invasion of MDA-MB-231 BC cell line. Ongoing experiments aim to further validate this observation in various other cancer cell lines, and reveal the underlying molecular mechanisms mediating its anticancer properties.

REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A & Bray F 2019 Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International Journal of Cancer* 144 1941-1953.
2. Narayan AK, Al-Naemi H, Aly A, Kharita MH, Khera RD, Hajaj M & Rehani MM 2020 Breast Cancer Detection in Qatar: Evaluation of Mammography Image Quality Using A Standardized Assessment Tool. *Eur J Breast Health* 16 124-128.
3. McSherry EA, Donatello S, Hopkins AM & McDonnell S 2007 Molecular basis of invasion in breast cancer. *Cell Mol Life Sci* 64 3201-3218.
4. Naor D, Nedvetzki S, Golan I, Melnik L & Faitelson Y 2002 CD44 in cancer. *Crit Rev Clin Lab Sci* 39 527-579.
5. Koklesova L, Liskova A, Samec M, Zhai K, Abotaleb M, Ashrafizadeh M, Brockmueller A, Shakibaei M, Biringer K, Bugos O, Najafi M, Golubnitschaja O, Büsselberg D, Kubatka P. Carotenoids in Cancer Metastasis-Status Quo and Outlook. Biomolecules. 2020 Dec 10;10(12):1653. doi: 10.3390/biom10121653. PMID: 33321708; PMCID: PMC7763577.
6. Zhang X, Zhao WE, Hu L, Zhao L, Huang J. Carotenoids inhibit proliferation and regulate expression of peroxisome proliferators-activated receptor gamma (PPARγ) in K562 cancer cells. Arch Biochem Biophys. 2011 Aug 1;512(1):96-106. doi: 10.1016/j.abb.2011.05.004. Epub 2011 May 18. PMID: 21620794.
7. Muto Y, Fujii J, Shidoji Y, Moriwaki H, Kawaguchi T, Noda T. Growth retardation in human cervical dysplasia-derived cell lines by beta-carotene through down-regulation of epidermal growth factor receptor. Am J Clin Nutr. 1995 Dec;62(6 Suppl):1535S-1540S. doi: 10.1093/ajcn/62.6.1535S. PMID: 7495256.