ATTENUATION OF BREAST CANCER CELL GROWTH BY AN AQUEOUS EXTRACT OF BITTER MELON AND ITS EFFECT ON ANGIOGENIC POTENTIAL

IDEXX RADIL™

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INTRODUCTION

- The role of diet and development of cancer is made clear by numerous studies.
  - High fat and red meat intake increase breast and colorectal cancer incidence.
  - Antioxidants have an anti-cancer effect.
  - Diet combined with therapeutic drugs can have a synergistic, favorable outcome.
  - Dietary nutrients are effective in terms of preventative medicine.
- Metformin, an anti-diabetes medication, has shown anti-cancer function in many tumor cells (1) and in many human cancer patients.
- Bitter melon (BM) *Momordica charanita*, a tropical vegetable, which is widely used in Asia, South America and Africa, has also been shown to have an anti-diabetic effect.
  - Reports indicate that BM is effective in reducing cancer cell proliferation (2, 3).
- Regulation of angiogenesis is another molecular mechanism that has a potential in controlling cancer growth.
  - Angiogenesis is essential for growth and metastatic spread of cancer as well as in cancer-relapse (4, 5).
  - Vascular endothelial growth factor (VEGF) is an important "tumor angiogenesis factor" (TAF) (6) and its role in tumor-angiogenesis and metastatic spread of cancer is firmly established (7-12).
  - Anti-VEGF therapy in conjunction with standard cancer therapies could control tumor-angiogenesis quite effectively but the therapeutic effect is found to be relatively short (13-16).

OBJECTIVE

- In order to understand how BM extract might be involved in reducing breast cancer cells we begun analyzing the cell growth assay.
- To investigate whether BM extract has an anti-angiogenic activity, we have begun investigating its effect on VEGF expression.

RESULTS

Cells treated with BM extract show less growth as compared to untreated cells

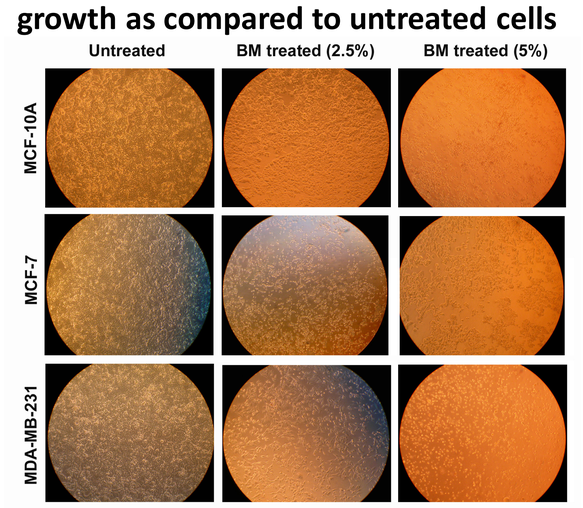

Figure 1. Growth of cells in presence of bitter melon (BM) extract. Cells were grown overnight in DMEM-HG + 7% FCS. In some cultures, bitter melon juice was added at a final concentration of 2.5% and 7.5% (v/v) and the cells were grown for additional 24 h and then photographed under microscope.

Treatment with BM causes decreased proliferation into wounded areas

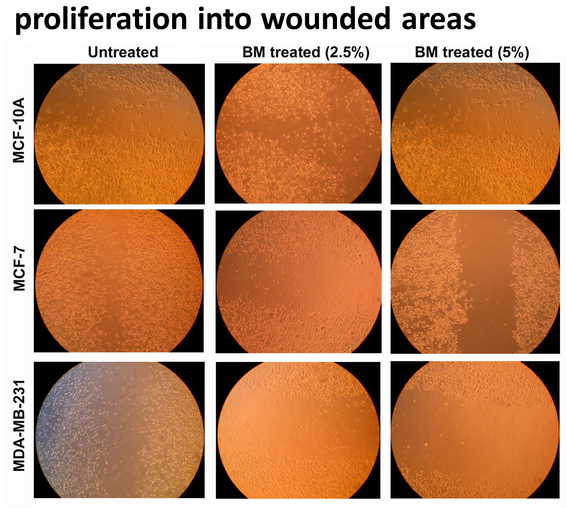

Figure 2. Effect of bitter melon (BM) extract on wound healing assay for cell proliferation. In some cultures, bitter melon juice was added at a final concentration of 2.5% and 7.5% (v/v) and the cells were grown for 2h. Using a pipet tip, the surface of the cultured cells in each well was scratched to remove cells. The cells were allowed to grow for 24 h and then photographed under microscope.

Expression of VEGF is reduced in BM treated breast cancer cells

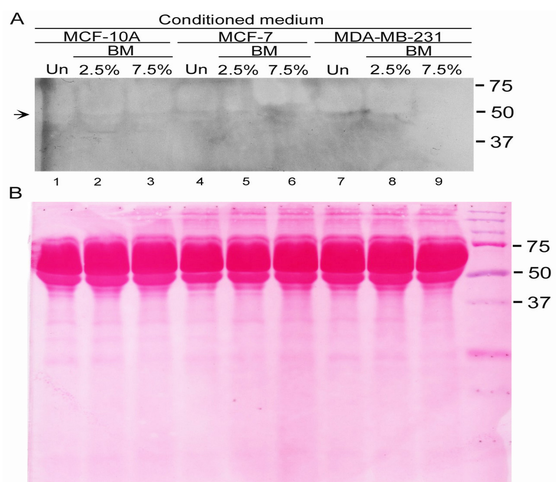

Figure 3. Effect of bitter melon (BM) extract on VEGF expression. *Panel A.* Conditioned medium from cells grown in DMEM-HG + 7% FCS in absence (Un) or in presence of BM extract at a final concentration of 2.5% and 7.5% (v/v). The culture medium was collected, centrifuged to remove any debris and 35 ml samples were fractionated in an 11% SDS-polyacrylamide gel. Following transfer onto a PVDF membrane, protein was detected by using anti-VEGF antibody. Arrow indicates the migration position of VEGF. Molecular weight marker positions are indicated. *Panel B.* Ponceau S stain of the PVDF membrane following transfer of proteins showing equal loading of proteins in each lane.

Decreased proliferation of breast cancer cells is observed in the presence of BM extract in a dose dependent manner as quantitated by MTT assay.

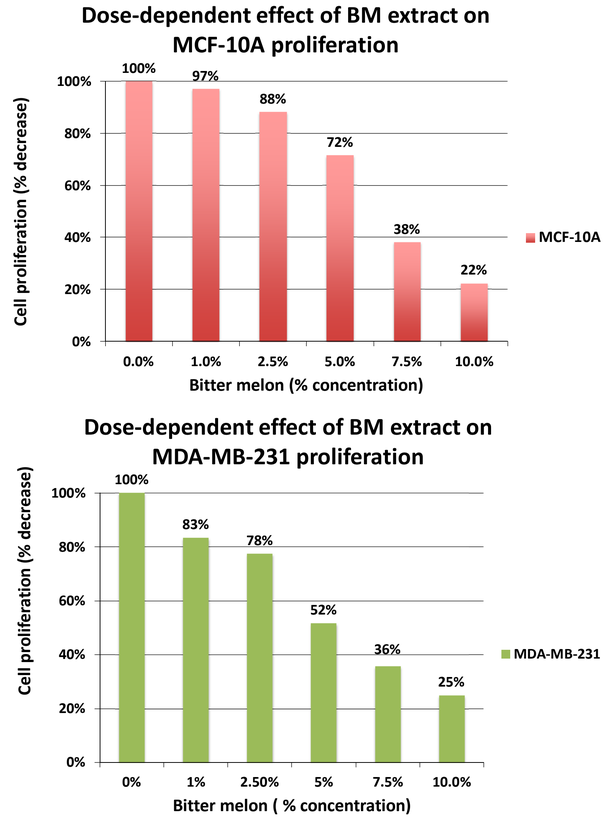

Figure 4. Analysis of cell proliferation by MTT assay. In some cultures, bitter melon juice was added at final concentrations of 1%, 2.5%, 5%, 7.5% and 10% (v/v) and the cells were grown for an additional 24 h. Cell proliferation was measured by using MTT assay kit (Promega Corp) following manufacturer's protocol and the color read at 562 nm. Results represent percent inhibition of cell proliferation.

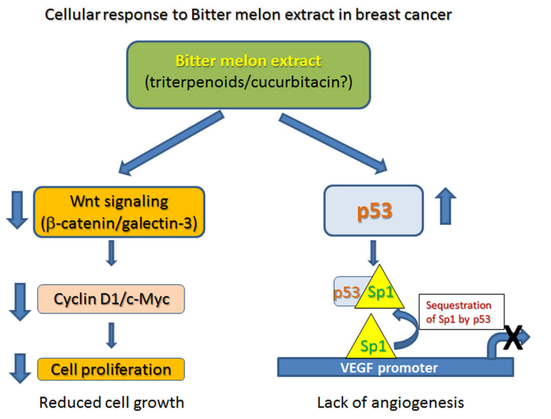

Figure 5. A model depicting a possible mechanism of the role of bitter melon extract in breast cancer cell proliferation and angiogenesis. Some of the active components of bitter melon extract, triterpenoids and cucurbitacin, most likely up-regulate p53 expression while down-regulating wnt signaling pathway. These events lead to the loss of angiogenesis and cell growth arrest, which together might be responsible for inhibition of breast cancer growth and metastasis.

CONCLUSIONS

- Breast cancer cells seem to grow less while in the presence of 2.5% and 5% BM extract.
- In addition, the cells can be observed to proliferate less when treated with BM as seen in the wound healing assay as compared to the untreated control.
- A decline in breast cancer cell proliferation is quantitated by the MTT assay where up to a 78% decrease is seen (MCF-10A cells).
- VEGF expression is higher in breast cancer tissue cells, mainly MDA-MB-231, in untreated cells as compared to lower VEGF expression in BM treated cells.

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REFERENCES

1.Libby, G., Donnelly, L.A., Donnan, P.T., Alessi, D.R., Morris, A.D. and Evans, J.M.M. *Diabetes Care*, **32**, 1620-1625.
2.Ray, R.B., Raychoudhuri, A., Steele, R. and Nerurkar, P. *Cancer Res*, **70**, 1925-1931.
3.Ru, P., Steele, R., Nerurkar, P.V., Phillips, N. and Ray, R.B. *Cancer Prevention Research*, **4**, 2122-2130.
4.Engels, K., Fox, S.B., Whitehouse, R.M., Gatter, K.C. and Harris, A.L. (1997). *Journal of Pathology*, **181**, 207-212.
5.Hanahan, D. and Folkman, J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*, **86**, 353-364.
6.Ferrara, N. (1995) The role of vascular endothelial growth factor in pathological angiogenesis. *Breast Cancer Research & Treatment*, **36**, 127-137.
7.Ferrara, N. (2002) Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. *Seminars in Oncology*, **29**, 10-14.
8.Folkman, J. (1971) Tumor angiogenesis: therapeutic implications. *New England Journal of Medicine*, **285**, 1182-1186.
9.Yoshiji, H., Gomez, D.E., Shibuya, M. and Thorgeirsson, U.P. (1996) *Cancer Research*, **56**, 2013-2016.
10.Brown, L.F., Berse, B., Jackman, R.W., Tognazzi, K., Guidi, A.J., Dvorak, H.F., Senger, D.R., Connolly, J.L. and Schnitt, S.J. (1995). *Human Pathology*, **26**, 86-91.
11.Van der Auwera, I., Van Laere, S.J., Van den Eynden, G.G., Benoy, I., van Dam, P., Colpaert, C.G., Fox, S.B., Turley, H., Harris, A.L., Van Marck, E.A. *et al.* (2004 *Clinical Cancer Research*, **10**, 7965-7971.
12.Jacobs, E.J., Feigelson, H.S., Bain, E.B., Brady, K.A., Rodriguez, C., Stevens, V.L., Patel, A.V., Thun, M.J. and Calle, E.E. (2006). *Breast Cancer Research*, **8**, R22.
13.Giantonio, B.J., Catalano, P.J., Meropol, N.J., O'Dwyer, P.J., Mitchell, E.P., Alberts, S.R., Schwartz, M.A., Benson, A.B., 3rd and Eastern Cooperative Oncology Group Study, E. (2007). *Journal of Clinical Oncology*, **25**, 1539-1544.
14.O'Dwyer, P.J. (2006) The present and future of angiogenesis-directed treatments of colorectal cancer. *Oncologist*, **11**, 992-998.
15.Niethammer, A.G., Xiang, R., Becker, J.C., Wodrich, H., Pertl, U., Karsten, G., Eliceiri, B.P. and Reisfeld, R.A. (2002). *Nature Medicine*, **8**, 1369-1375.
16.Holash, J., Davis, S., Papadopoulos, N., Croll, S.D., Ho, L., Russell, M., Boland, P., Leidich, R., Hylton, D., Burova, E. *et al.* (2002) *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11393-11398.
17.Dakeng, S., Duangmano, S., Jiratchariyakul, W., U-Pratya, Y., Bogler, O., and Patmasiriwat, P. (2012). *Journal of Cellular Biochemistry,* **113**, 49-60.
18.Ghahremani, M.F., Goossens, S., and Haigh, J.J. (2013) The p53 family and VEGF regulation. *Cell Cycle*, **12**, 1331-1332.

REFERENCES