

## Modified Arabinoxylan Rice Bran (MGN-3/Biobran) Enhances Yeast-induced Apoptosis in Human Breast Cancer Cells *In Vitro*\*

MAMDOOH GHONEUM<sup>1</sup> and SASTRY GOLLAPUDI<sup>2</sup>

<sup>1</sup>Department of Otolaryngology, Drew University of Medicine and Science,  
1621 E. 120th Street, Los Angeles, CA 90059;

<sup>2</sup>University California, Irvine, Division of Basic and Clinical Immunology, Irvine, CA 92697, U.S.A.

**Abstract.** We have recently reported that phagocytosis of killed *Saccharomyces cerevisiae*, baker's yeast, induced apoptosis in human breast cancer cell lines MCF-7, ZR-75-1 and HCC70. In this study we have evaluated the effect of treatment with MGN-3, a modified arabinoxylan from rice bran, on phagocytosis and yeast-induced apoptosis in breast cancer cells. Cancer cells were cultured with yeast at a ratio of 1:10 in the absence or presence of MGN-3, and the percentages of phagocytic and apoptotic cancer cells were examined by flow cytometry and by cytospin preparations. Cancer cells treated with MGN-3 exhibited increased percentages of attachment (200%) and uptake of yeast (313%) by MCF-7 cells at 0.5 hr, as compared with cells without MGN-3. In addition, treatment with MGN-3 resulted in a 2 fold increase in the percentage of apoptotic MCF-7 cells, 2.5 fold for ZR-75 cells and 1.8 fold for HCC70 cells. MGN-3 effect was dose-dependent and associated with increased activation of caspases 8 and 9 in MCF-7 cells, and caspases 8, 9 and 3 in HCC70 cells. This data demonstrates that MGN-3 accelerates phagocytosis-induced apoptosis of cancer cells, which may represent a novel therapeutic strategy for the treatment of breast cancer.

In the past 30 years researchers have learned a great deal about the diagnosis of cancer, however, its treatment still represents a serious obstacle. In the field of cancer therapeutics, medical oncologists have had to rely mainly on

\*Data have been presented at the American Association for Cancer Research (AACR). Advances in Breast Cancer Research: Genetics, Biology, and Clinical Implications. Huntington Beach, CA. October 8-12, 2003.

Correspondence to: Mamdooh Ghoneum, Department of Otolaryngology, Drew University of Medicine and Science, 1621 E. 120th Street, Los Angeles, CA 90059, U.S.A. Tel: + 1-323-563-5953, Fax: + 1-310-668-4554, e-mail: mghoneum@ucla.edu

Key Words: Breast cancer cells, arabinoxylan rice bran, apoptosis.

surgery, chemotherapy and radiation for cancer treatment. Both chemotherapy and radiation therapy are toxic, immune-suppressive, mutagenic and carcinogenic (1-4). With respect to immunotherapy, many biological response modifiers (BRMs) designed to activate the host immune response have also been shown to produce severe side-effects (5). The need for a new cancer therapy with minimal or no side-effects is greatly warranted. In the present study, we introduced a novel approach to breast cancer therapy using modified arabinoxylan rice bran (MGN-3), to accelerate apoptosis in breast cancer cells (BCCs) post phagocytosis of yeast *in vitro*.

Induction of apoptosis post phagocytosis of microorganisms is a well established phenomenon in professional phagocytic cells. Phagocytosis of *Escherichia coli*, *C. albicans* and *Mycobacterium tuberculosis* induce apoptosis in neutrophils (6-8). Other studies showed that phagocytosis of staphylococcus aureus also induced apoptosis in monocytes (9). Phagocytosis by different types of cancer cells has been reported; these include leukemia, fibrous histiocytoma, dermatofibroma, cervical cancer and lymphatic tumor cells. These cancer cells exhibit phagocytic activities against red blood cells, white blood cells, blood platelets, bacteria, and *Candida albicans* (10-19). BCCs also exhibit phagocytic activity against erythrocytes (20), gelatin matrix (21, 22), and yeast (23). We hypothesized that tumor cells acquire phagocytic properties during the course of malignancy. Interestingly, we recently demonstrated that BCC lines underwent apoptosis post phagocytosis of heat-killed yeast (24). Clearly, factors that accelerate apoptosis in cancer cells post ingestion of microorganisms are in great need of exploration.

MGN-3 is an arabinoxylan from rice bran that has been modified by carbohydrate hydrolyzing enzymes from shiitake mushrooms (25). We have previously reported that MGN-3 enhances NK cell activity (26), increases the production of TNF- $\alpha$  by human peripheral blood lymphocytes (27), and sensitizes human leukemia cells to death receptor [CD95]-induced apoptosis (28). In this study

we tested the ability of MGN-3 to accelerate apoptosis in BCCs after co-culture with heat-killed *S. cerevisiae*. Results show that MGN-3 enhanced both phagocytosis of yeast by BCCs and subsequent apoptosis of cancer cells.

## Materials and Methods

**Cell culture media.** Tumor cells were maintained in our laboratory in the following media: (DMEM) [4.5 mg glucose per ml, 10% fetal calf serum, and 1% antibiotics (100 U penicillin and 100 µg/ml streptomycin)] and complete medium (CM) [RPMI-1640 supplemented with 10% fetal calf serum and 1% antibiotics (as above)].

**Tumor cell lines.** Three human breast cancer cell (BCC) lines were used in the present study. These cell lines were MCF-7, ZR-75-1 and HCC 70. Human macrophage cell line (U937) was used as the control. All cell lines were purchased from ATCC, Manassas VA, USA.

**MGN-3.** MGN-3 is an arabinoxylan extracted from rice bran that is treated enzymatically with an extract from Shiitake mushrooms and which contains polysaccharides (β1, 3-glucan and activated hemicellulose). MGN-3 was freshly prepared by dissolving in distilled H<sub>2</sub>O at a concentration of 30 gm/L. MGN-3 was provided by Daiwa Pharmaceuticals Co. Ltd. Tokyo, Japan.

**Preparation of *S. cerevisiae*.** Commercially available baker's and brewer's yeast, *S. cerevisiae*, was used. Yeast suspensions were washed once with phosphate-buffered saline (PBS) and were incubated for 1 hr at 90°C to kill yeast. Following washing, yeast cells were quantified using a hemocytometer and cell suspensions were adjusted at 1 x 10<sup>7</sup> cells/ml.

**Phagocytic assay.** Phagocytosis was assessed by cytospin preparation and flow cytometry. Phagocytic assay using cytospin preparations was done as previously described with slight modifications (19, 29). In brief, yeast was mixed with tumor cells at a 10:1 ratio (yeast to tumor cell). For this purpose, a 0.5 ml tumor cell suspension in CM containing 1 x 10<sup>6</sup> cells/ml was mixed with 0.5 ml yeast suspension containing 1 x 10<sup>7</sup> organisms/ml. The mixtures were centrifuged in capped plastic tubes (16 x 100 mm; Falcon Plastic, Los Angeles CA, USA) for 5 min at 50 xg, and incubated at 37°C and 5% CO<sub>2</sub>. After 0.5 and 2hr incubation, the mixtures were thoroughly re-suspended to detach loosely attached yeast from tumor cells. Cell suspensions (200 µl) were used to make cytospin preparations (Shandon Southern Instruments, Sewickly, PA, USA). Preparations were fixed in 100% methanol, air-dried, stained with 4% Giemsa for 15 min and were examined using oil immersion and a light microscope fitted with a 100x objective (Nikon, Tokyo, Japan).

Flow cytometric assay for phagocytosis: Tumor cells (1 X 10<sup>6</sup>) were incubated in round-bottomed tubes with propidium iodide-labeled, heat-killed yeast at a cancer cell to yeast ratio of 1:10. After 1 hr incubation at 37°C, samples were analyzed by FACScan flow cytometer. To distinguish between cell-bound and internalized, fluorescent yeast, quenching was performed by the addition of trypan blue dye. Trypan blue quenches the fluorescence of cell-bound but not internalized yeast.

**Assessment of attachment and uptake of yeast by tumor cells.** Assessment of attachment of yeast by tumor cells was calculated as the percentage of 200 tumor cells that attached to one or more yeast, and the attachment index was calculated using the following formula:

$$\text{Attachment index} = \% \text{ Tumor cells attaching to X number yeast} / 100 \text{ cells: } 1000$$

The assessment of uptake of yeast by tumor cells was calculated as the percentage of 200 tumor cells that ingested one or more yeast, and the phagocytic index was calculated using the following formula:

$$\text{Phagocytic index} = \% \text{ Phagocytizing tumor cells X number yeast} / 100 \text{ cells: } 1000$$

**Assessment of tumor cell survival and apoptosis.** Flow cytometry analysis was used to examine the percentage of dead cancer cells. Cancer cells were cultured with yeast cells at a 1:10 ratio and the percentage of dead cancer cells was examined by the propidium iodide (PI) technique using flow cytometry. Briefly, cells (1x10<sup>6</sup>/ml) were incubated with 50 µg/ml of PI for 25 minutes at room temperature. Cells were acquired by FACScan (Becton Dickinson, San Jose, CA) and analyzed by CellQuest software.

Percentage of apoptotic cancer cells was also determined in cytospin preparations that were used for phagocytic assay. Apoptosis is morphologically defined by membrane blebbing and chromatin condensation (30, 31). These criteria were used to identify the apoptotic cancer cells.

To study the effect of MGN-3 on apoptosis, tumor cells were incubated with or without MGN-3 (500 µg/ml) in the presence or absence of yeast for 2 hr and apoptosis was determined as described above.

**Intracellular activity of caspases 8, 9 and 3.** The method is based on carboxyfluorescein-labeled fluoromethyl ketone (FMK)-peptide inhibitors of caspases. These inhibitors are cell permeable and non-toxic. Once inside the cells, these inhibitors bind covalently to the active caspase. Caspase positive (+) cells are distinguished from caspase negative (-) cells with aid of flow cytometry. Briefly, cells undergoing apoptosis were loaded with fluorescein-labeled FMK-peptide inhibitors (FAM-DEVD-FMK for caspase 3, FAM-LETD-FMK for caspase 8, FAM-LEHD-FMK for caspase 9, Intergen Company, NY). After 1 hr incubation, the cells were washed to remove unbound caspase, and cells that contained bound inhibitor were quantified using a FACScan flow cytometer.

**Statistical analyses.** In order to compare the means of treatment 1 (yeast), treatment 2 (MGN-3) and combination of treatments 1 and 2, we used the analysis of variance design.

## Results

**Effect of MGN-3 on attachment of yeast to MCF-7 cells.** Figures 1a & b represent data of 3 experiments. Tumor cells were co-cultured with yeast in the absence or presence of MGN-3 and the percentage of attachment and attachment index were examined at 0.5 hr and 2 hr. At 0.5 hr, the percentage of MCF-7 cells exhibiting binding was 27%. Treatment of MCF-7 cells with MGN-3 significantly increased the level of attachment (54%) representing a 2 fold increase. This

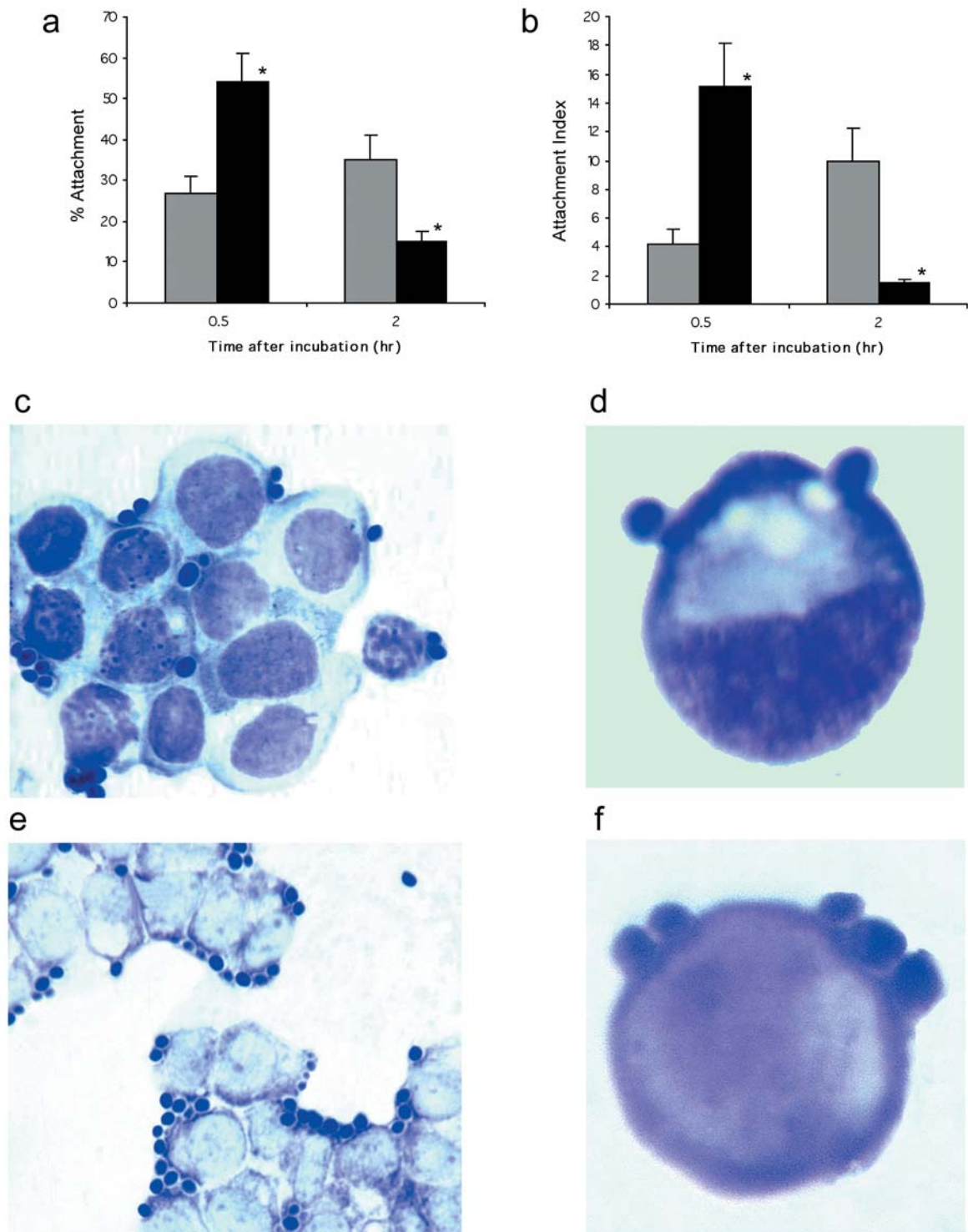


Figure 1. Action of MGN-3 on the attachment of MCF-7 cells to yeast and attachment index. Tumor cells were cultured with yeast at a ratio of 1:10 in the presence (■) or absence (□) of MGN-3. The percentage (%) of attachment (a) and the attachment index (b) were determined at 0.5 and 2 hr. Data represent the mean  $\pm$ SD of 3 different experiments \*,  $p < 0.001$  as compared to MCF-7 cells and yeast. Figs 1c-f Giemsa stained cytopsin preparations showing MCF-7 cells attached to one or two yeast, examined at 10 min. post culture of MCF-7 cells with yeast in the presence of MGN-3(e & f). c&e x 740. d&f x1000.

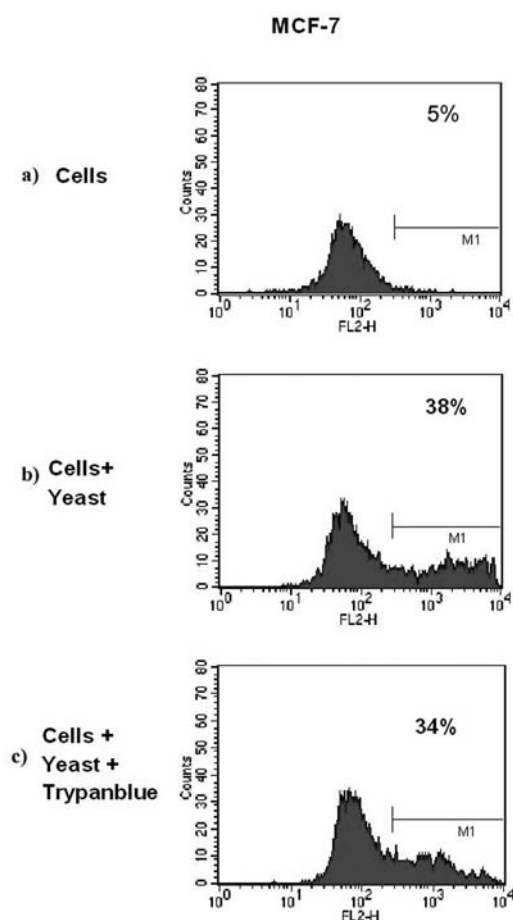


Figure 2. Phagocytosis of yeast by MCF-7 cells. a) Flow cytometric analysis which distinguishes cell-bound and internalized yeast. Tumor cells were incubated with propidium iodide-labeled, heat-killed yeast. Graph is a representative dot blot, showing the percent of MCF-7 cells alone, cells + yeast, and cells + yeast + trypan blue.

proportion was inverted at 2 hr. (Figure 1a). The attachment indices at .5 hr for MCF-7 cells cultured with yeast in the absence or presence of MGN-3 were 4.2 and 15.1, respectively. This represents a 3.6-fold increase associated with MGN-3 treatment. Again this proportion was inverted at 2 hr (Figure 1b). Changes in the level of attachment by MGN-3 treatment are illustrated by cytospin preparations. At 10 minutes many MCF-7 cells attached to 1 or 2 yeast (Figures 1c & d) while MGN-3 treated tumor cells are attached to an increased number of yeast (Figures 1e & f).

*Effect of MGN-3 on phagocytosis of yeast by MCF-7 cells.* Results in Figure 2 show that yeast was phagocytized by MCF-7 cells. This was confirmed by flow cytometric analysis which clearly distinguishes cell-bound and internalized yeast (Figure 2a-c). Cytospin preparations showed MGN-3

treatment for 0.5 hr resulted in a significant increase in the percentage of phagocytosis of yeast by MCF-7 cells (72%), as compared to cells and yeast (23%). At 2 hr, the phagocytic activity of MGN-3 treated MCF-7 cells had declined (3a). The phagocytic indices for MCF-7 cells and MGN-3 treated MCF-7 cells were 5 and 17 respectively, representing a 3.4-fold increase by MGN-3 treatment. At 2 hr, the phagocytic index of MGN-3 treated MCF-7 cells was maintained, while that of MCF-7 cells and yeast rose to 11% (3b). Changes in the phagocytic activity of MCF-7 cells, post treatment with MGN-3, are illustrated by cytospin preparations. At 0.5hr MCF-7 cells showed an increased level of phagocytosis of yeast. Notice the presence of the vacuole surrounding the phagocytosed yeast; this indicates the yeast has been ingested by cancer cells (3c). Also note the presence of several empty vacuoles inside cancer cells (3d) indicating that the yeast has been digested.

*Effect of MGN-3 on apoptosis of MCF-7 cells*

*A. Measurement by cytospin preparations:* The effect of MGN-3 on phagocytosis-induced apoptosis of MCF-7 cells was measured in cytospin preparation. Tumor cells were cultured with yeast at a ratio of 1:10 in the presence or absence of MGN-3. Results depicted in Figure 4 show that the background apoptosis in MCF-7 cells was 5%. At 0.5 hr post phagocytosis, 18.7% of MCF-7 cells underwent apoptosis. Treatment with MGN-3 significantly increased the percentage of apoptosis of MCF-7 cells (32%). At 2 hr the percentages of apoptotic MCF-7 cells continued to rise post treatment with yeast alone (31%) and yeast in the presence of MGN-3 (39.3%).

*B. Morphological examination of apoptic MCF-7 cells:* Giemsa-stained cytospin preparations show apoptotic MGN-3-treated MCF-7 cells, post phagocytizing yeast. At 0.5 hr cancer cells illustrate membrane blebbing and early nuclear condensation (a&b). Preparation also showed cells with severe chromatin condensation. Figure 5 shows several apoptic cells (c) and a separate cell (d) with a very small nucleus, which was situated in an eccentric position. Finally, nuclear fragmentation occurs (notice red-stained fragments in Figure 5e) and these fragments subsequently disappear (5f). Apoptic MCF-7 cells acquire the trypan blue stain (5g). Notice yeast, stained blue, inside the apoptic MCF-7 cells.

*C. Flow cytometry analysis:* Survival of MCF-7 cells was further examined by flow cytometry. Results in Figure 6 show that phagocytosis of yeast caused a significant decrease in MCF-7 cell survival (21.4% dead cells as compared to control 3.4%). MGN-3 treatment further increased tumor cell demise (34.7%). MGN-3 alone caused a substantial increase in cell death (10.9%) as compared to background of MCF-7 alone (3.4%). The effect of MGN-3 on phagocytosis-induced cell death was found to be dose-dependent (Figure 7). MGN-3 at 100 µg/ml showed an increase of MCF-7 apoptic cells

(35.4%), as compared to MCF-7 cells and yeast. At 500 µg/ml, it further increased (40.1%), however, at 1000 µg/ml, it began to decline (33.04%).

*Effect of MGN-3 on apoptosis of other BCC lines.* We examined the accelerative effect of MGN-3 on apoptosis of other BCCs post phagocytosis of yeast. Survival in ZR-75 and HCC70 cell lines, was examined at 0.5 hr post culture with yeast. Results in Figure 8 demonstrated that phagocytosis of yeast resulted in increased percentages of dead cancer cells: ZR 75 (21%) and HCC70 cells (11%). MGN-3 treatment caused a significant increase in the expression of apoptic ZR-75 cells (48%), while the effect was less noticeable in HCC70 cells (20%). MGN-3 alone demonstrated an appreciable increase in percent apoptotic ZR-75 cells (11%) and HCC70 (10%), as compared to background of cancer cells alone (3.5-5%).

#### *Activation of caspases*

*A. Effect of yeast phagocytosis:* To determine whether the decreased survival of tumor cells is due to yeast-induced necrosis or apoptosis, activation of caspases was determined. MCF-7 and HCC70 cells were incubated with yeast for 2 hr and activation of caspases 8, 9 and 3 was determined by flow cytometry. Figure 9 is a representative histogram that illustrates the yeast induced activation of both caspase 8 and caspase 9 in MCF-7 cells, suggesting that death of MCF-7 cells is due to apoptosis. A similar observation was made in HCC70 cells where an increase in caspase 8, 9, and 3 post culture of the cells with yeast was observed.

*B. Effect of MGN-3:* In order to determine the steps in yeast-mediated apoptosis that are affected by MGN-3, we examined the activation of caspase 8 and caspase 9. MCF-7 cells were co-cultured with yeast in the presence or absence of MGN-3 for 2 hr. The proportion of cells with active caspases 8 and 9 was determined with a Caspatag caspase detection kit, using FACScan. The proportions of MCF-7 cells with active caspase 8 and caspase 9 were 3.5% and 4.9%, respectively. Phagocytosis of yeast by MCF-7 cells resulted in an increased proportion of cells exhibiting active caspase 8 (18.9%), and caspase 9 (20%). MGN-3 enhanced phagocytosis-induced activation of caspases 8&9. The action of MGN-3 on induction and enhancement of apoptosis in HCC70 cells was also associated with activation of caspases (Figure 9). MGN-3 has a negligible effect on the activation of caspase 8 in HCC70 cells, but it increased the proportion of cells with active caspase 9 and caspase 3.

*C. Dose-dependant effect of MGN-3:* The effect of MGN-3 on the level of caspases of MCF-7 cells was shown to be dose-dependent. The data demonstrates that the proportion of cells with increased active caspase 8 was higher in MGN-3 (500 µg/ml) treated cells (29.2%), as compared to untreated

control (18.9%). A similar trend of activation with caspase 9 was noted (Figure10).

## **Discussion**

Results of this study reveal that modified arabinoxylan rice bran (MGN-3) accelerates the level of phagocytosis of yeast by MCF-7 cells and significantly enhances apoptosis by MCF-7 cells. Several investigators reported phagocytosis by different types of cancer cells of nonphagocytic origin [10-19] including breast cancer cells (BCCs). These BCCs demonstrated their ability to phagocytize latex beads and fluorescent Matrigel (21, 22). We extended these studies and showed that MCF-7 cells are also able to phagocytize yeast (23), and that phagocytosis subsequently resulted in apoptosis in BCCs (24). Several studies demonstrated that the expression of apoptosis in the human professional phagocytic cells can be modified in response to phagocytosis of microorganisms (6-9). There is a great need to further study the factors that accelerate the expression of apoptosis in BCCs, post ingestion of microorganisms.

Recent studies demonstrate that several natural biological response modifiers (BRMs) induce apoptosis against a variety of cancer cell lines. These include: HL-60 cells by [6]-gingerol and [6]-paradol, (ginger derivatives) (32), erythroleukemia, prostate, and BCC lines by dl-alpha-tocopherol (vitamin E) (33), prostate adenocarcinoma PC 3 line by PC SPES (a Chinese herbal preparation) (34), cervical carcinoma cells by *Coprinus disseminatus* (35), and human endometrial adenocarcinoma cells by a lipoprotein fraction of rice bran (36). Moreover, the growth of human BCCs (MCF-7 and MDA-231) has been inhibited by ergosterol (extract of baker's yeast) (37).

MGN-3 is a polysaccharide and its main chemical structure has been identified as an arabinoxylan with a xylose in its main chain and arabinose polymer in its side chain (25). Earlier studies revealed that MGN-3 enhances human NK cell activity *in vivo* (26), increases T and B cell proliferation (25), and increases TNF- $\alpha$  and IFN- $\gamma$  production (27). In addition, MGN-3 is able to induce cancer cell apoptosis (28). In this study, MGN-3 demonstrated an additional anticancer characteristic that accelerates yeast phagocytosis-induced apoptosis in cancer cells. MGN-3 increases the levels of attachment and phagocytosis as early as 0.5 hr post culture MCF-7 cells with yeast. The mechanism(s) by which MGN-3 induces this effect is not fully understood, but it could be attributed to changes in cancer cell receptors that are involved in attachment/phagocytosis. Whether the receptors found in professional phagocytic cells such as mannose, Fc and C3 receptors (38) are the same ones that govern phagocytosis of yeast by cancer cells is under investigation.

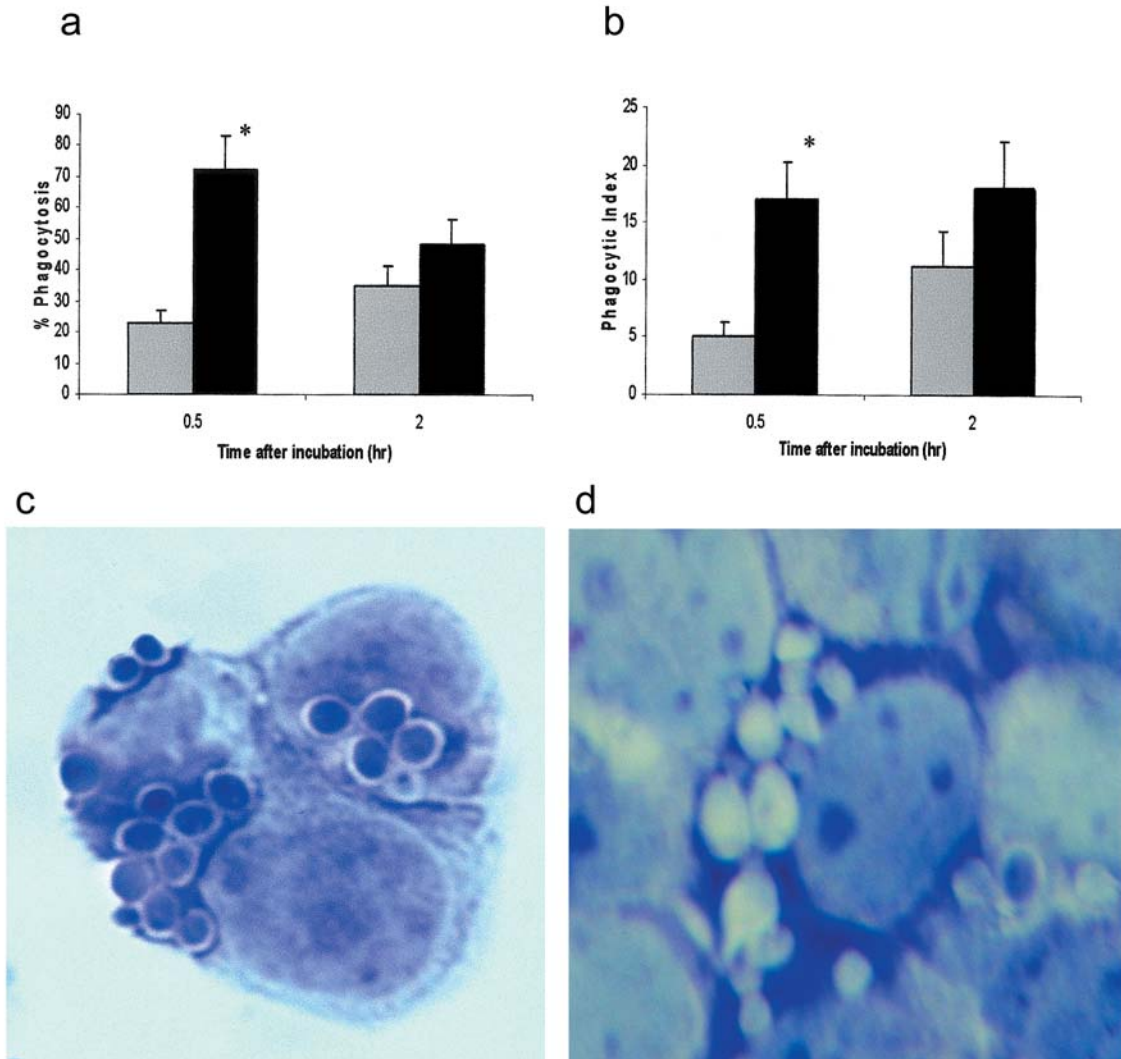


Figure 3. Effect of MGN-3 on the percent of phagocytosis of yeast by MCF-7 cells and phagocytic index. Tumor cells were incubated with yeast at a ratio of 1:10 in the presence (■) or absence of MGN-3 (□). The percentage (%) of phagocytic tumor cells (a) and the phagocytic index (b) were determined at 0.5 and 2 hr. Data represent the mean  $\pm$ SD of 3 different experiments \*,  $p < 0.01$  as compared to MCF-7 cells and yeast. Fig 3c & d Cytospin preparations showing MCF-7 cells treated with MGN-3 phagocytizing several yeast cells examined at 0.5 hr (c). Notice presence of several vacuoles of digested yeast at 1hr. (d). Giemsa x 1000.

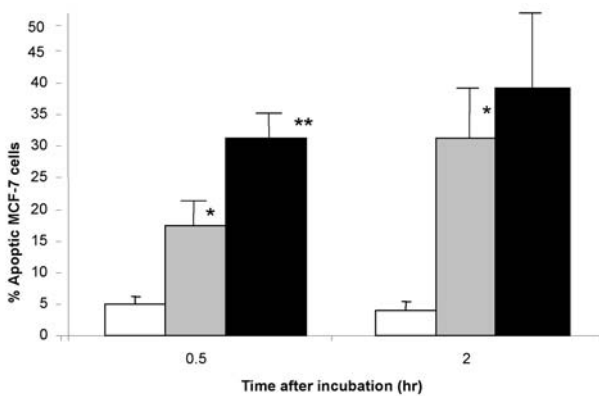


Figure 4. Effect of MGN-3 on apoptosis of MCF-7 as defined in cytospin preparations. Tumor cells were incubated with yeast at a ratio of 1:10 in the presence (■) or absence of MGN-3 (□) and the percentage (%) of apoptotic tumor cells was determined at 0.5 and 2 hr in cytospin preparation. Apoptotic MCF-7 cells alone (□) were also examined. Data represent the mean  $\pm$ SD of 3 different experiments. \* $p < 0.05$ , as compared to control untreated cells. \*\* $p < 0.05$ , as compared to cells + yeast.

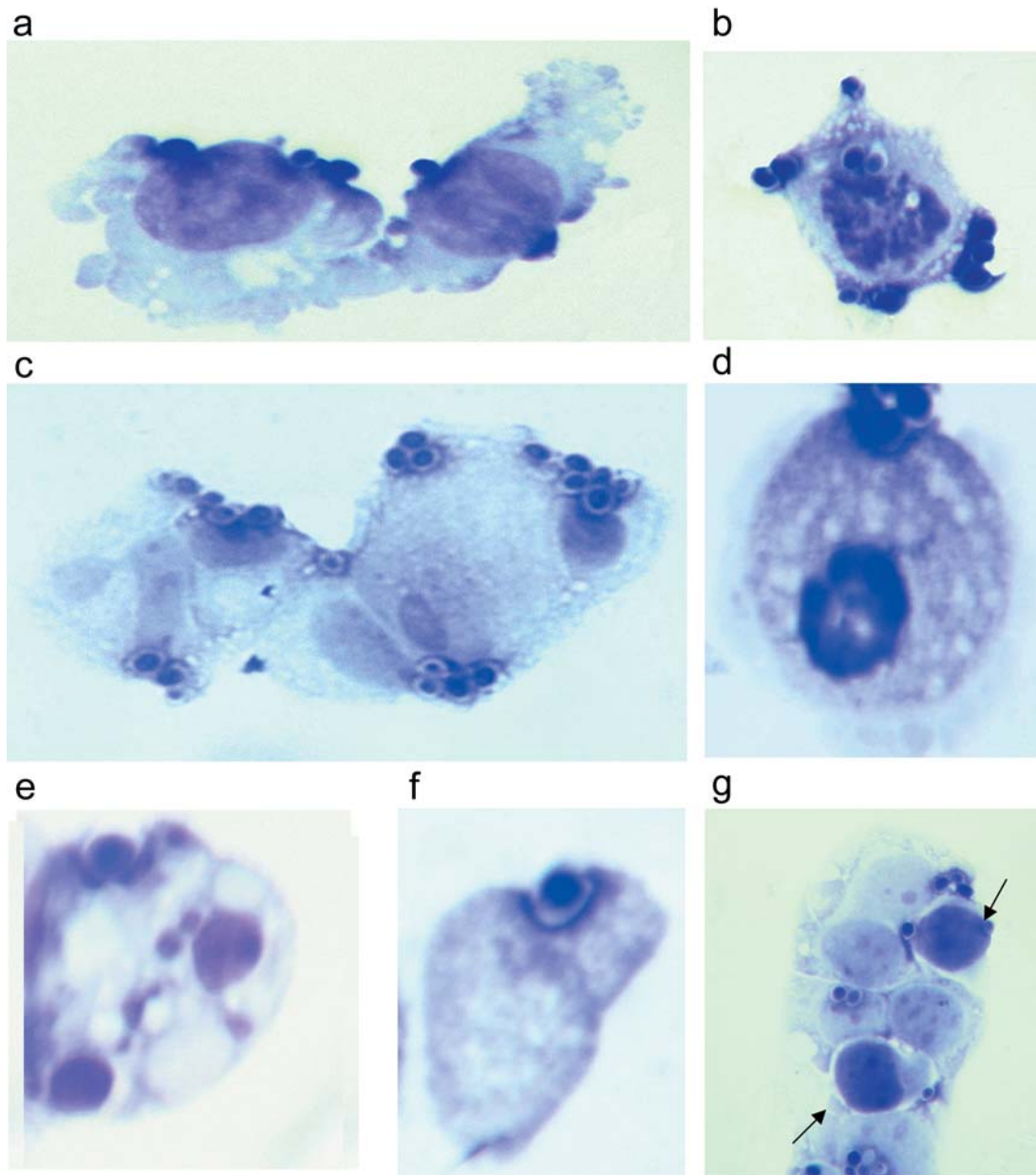


Figure 5. (a – g) Morphological examination of apoptotic MGN-3-treated MCF-7 cells using Giemsa-stained cytospin preparations. Notice tumor cells exhibiting membrane blebbing and early nuclear condensation (a & b). Preparation also showed cells with severe nuclear condensation which was located in an eccentric position (c & d). Finally, nuclear fragmentation occurs (stained red) (e). Nucleus then disappears (f). Notice dead tumor cells stained with trypan blue (g). Also, notice yeast (stained blue) has been ingested inside the tumor cells. Figs 5a, c&g x 400, b, d, e&f x1000

MGN-3 also accelerates the expression of apoptosis in BCCs post phagocytizing yeast. Giemsa-stained cytospin preparations show apoptotic MGN-3-treated MCF-7 cells, post phagocytizing yeast. Cancer cells illustrate membrane blebbing and nuclear condensation. This is followed by nuclear fragmentation that subsequently disappears, and apoptic cells acquire the trypan blue stain. It was noted, in

the three BCC lines used, that the percentage of apoptosis in cancer cells co-cultured with yeast in the presence of MGN-3 was higher than that of either yeast or MGN-3 treatment alone. This could be attributed to a synergistic apoptic effect of yeast alone in addition to the direct apoptic effect of MGN-3. The mechanism by which MGN-3 enhances apoptosis in BCCs after phagocytizing

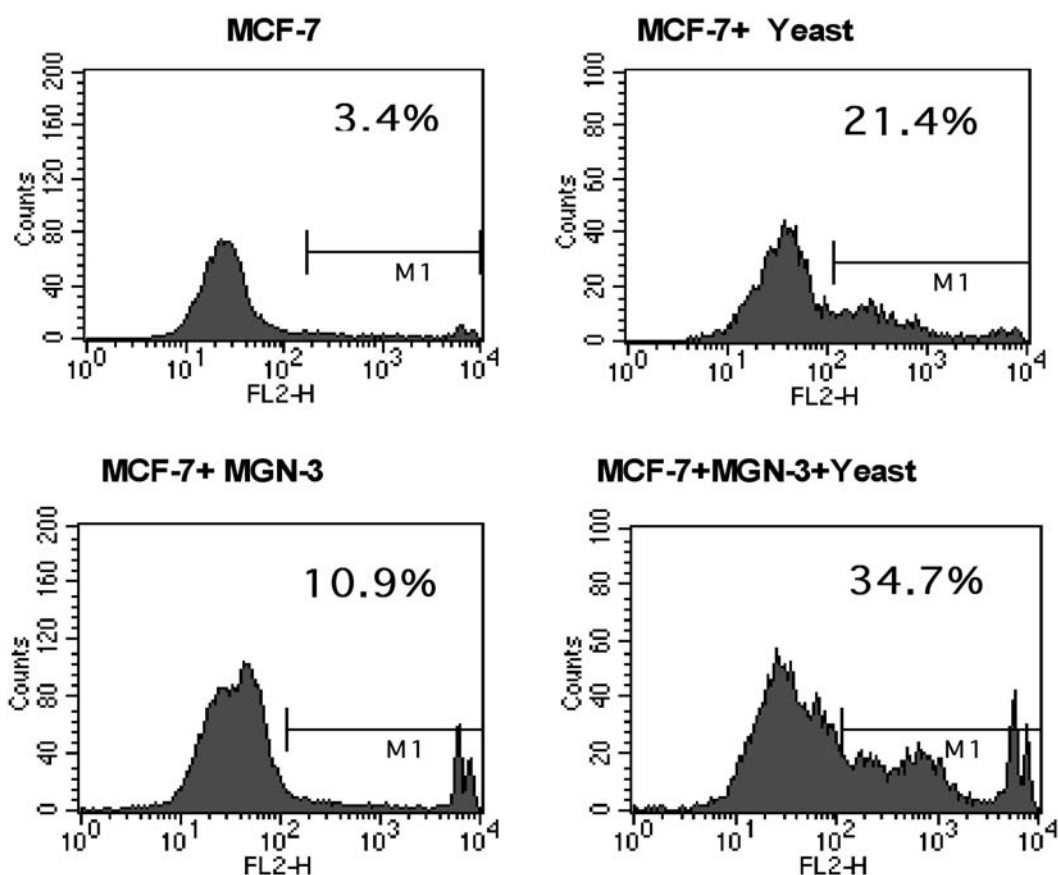


Figure 6. Percent of dead MCF-7 cells as determined by flow cytometry. MCF-7 cells were cultured with yeast at the ratio of 1:10 in the presence or absence of MGN-3 (500  $\mu\text{g/ml}$ ) for 2 hr, and cell survival was determined by flow cytometry using propidium iodide (PI) technique. In this technique dead cells pick up PI and fluorescence. The number in the histograms represents the percent of dead cells.

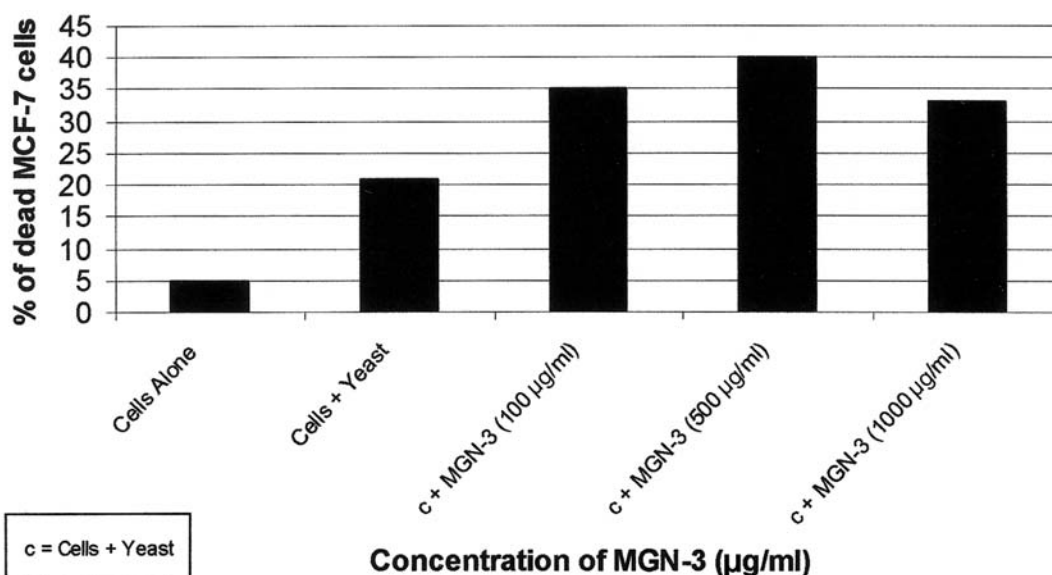


Figure 7. Action of MGN-3 at different concentrations on apoptosis of MCF-7 cells. MCF-7 cells were cultured with yeast at the ratio of 1:10 in the presence of MGN-3 (100-1000  $\mu\text{g/ml}$ ). Cancer cell survival was determined by flow cytometry using propidium iodide (PI) technique. In this technique dead cells pick up PI and fluorescence.



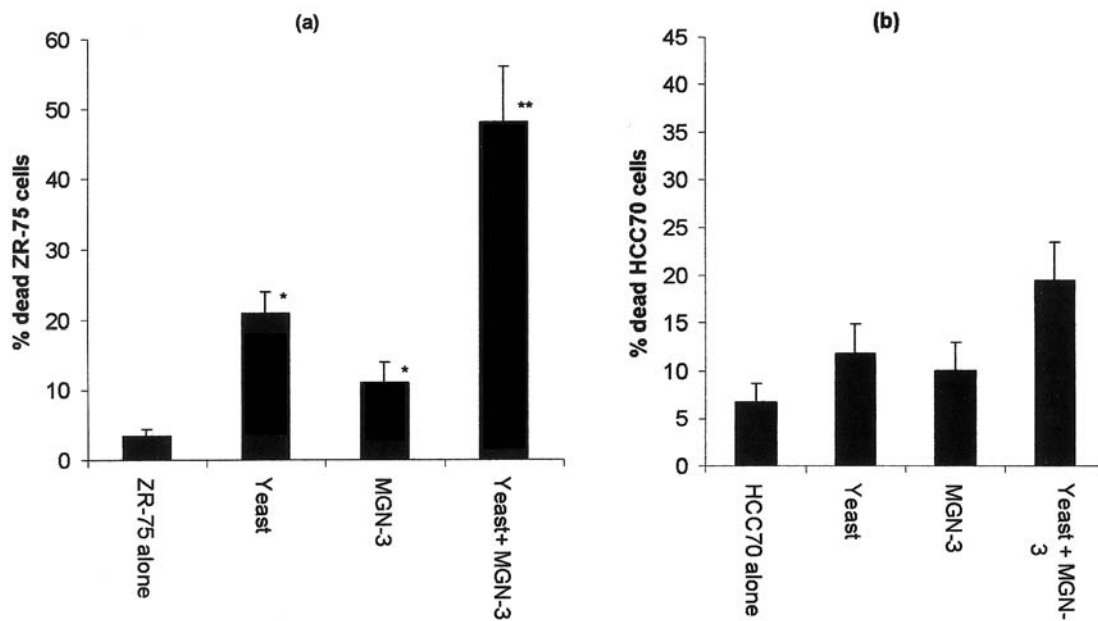


Figure 8. Effect of MGN-3 on phagocytosis-induced apoptosis in ZR-75 and HCC70 cells as determined by flow cytometry. Cancer cells were cultured with yeast at the ratio of 1:10 for 0.5 hr in the presence or absence of MGN-3 (500  $\mu$ g/ml), and survival of ZR-75 cells (a) and HCC70 cells (b) was determined by flow cytometry using the propidium iodide (PI) technique. In this technique dead cells pick up PI and fluoresce. \*  $P < 0.05$  as compared to control untreated cells. \*\* $P < 0.01$  as compared to cells + yeast or cells + MGN-3.

yeast may involve the FAS / FAS Ligand system. The apoptic effect of FasL post phagocytosis of microorganisms has been investigated recently. Baran *et al.* (9) reported release of FasL from monocytes post phagocytosis of *Staphylococcus aureus*, and these FasL induce apoptosis of phagocytic monocytes and, to some extent, the bystander cells. We have recently investigated the effect of MGN-3 on death receptor-induced apoptosis in the human leukemic HUT-78 cell line (28). HUT-78 cells that were pre-treated with MGN-3 and then with the agonistic antibody against death receptor (Fas, CD95) revealed enhanced anti-CD95 induced apoptosis.

Alternatively, MGN-3 may exert its effect through activation of caspases. Data from the present study showed that MGN-3 treatment of MCF-7 cells up regulates the activation of proximal caspases 8 and 9; this occurred in a dose-dependent manner that was maximized at 500  $\mu$ g/ml. The mechanism by which MGN-3 activates caspases may be due to its increased production of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and interferon- $\gamma$  (IFN- $\gamma$ ) (27). Both cytokines are known to induce tumor cell death, or modulate cell death *via* activation of caspases (39-41). MCF-7 cells do not express caspase 3 (42); this suggests that a caspase 3 independent pathway that causes the DNA fragmentation in MCF-7 cells may exist. In addition, studying the mitochondria membrane potential and Bel level may be

helpful in investigating the mechanism by which MGN-3 causes apoptosis of MCF-7 cells.

Data in the present study reveal a differential response among BCC lines towards the augmentory effect of MGN-3 on enhancing apoptosis in cancer cells, post culture with yeast. BCCs, such as MCF-7 and ZR-75, are more responsive than HCC70. The reason for this phenomenon is not known, but it could be attributed to the difference in the mechanisms of apoptosis in these cell lines. MGN-3 increased activation of caspases 8 and 9 in MCF-7 cells, a cell line that lacks caspase 3. On the other hand, HCC70 cells treated with MGN-3 demonstrated an increase in caspases 3 and 9 but not caspase 8. This suggests that treatment with MGN-3 caused apoptosis in MCF-7 cells through both intrinsic and extrinsic pathways. On the other hand, apoptosis in HCC70 cells may occur only through mitochondrial activation. A similar apoptic pathway was noted in mouse macrophages upon ingestion and digestion of *E. coli*, activating caspases 3 and 9, but not caspase 8 (43).

The National Cancer Institute has frequently highlighted the critical need for cancer treatments with greater specificity for cancer cells and less toxicity for normal tissue; this strongly encourages the exploration of such therapies. In the present study, we introduced a novel approach to BCC therapy using *S. cerevisiae*, a heat-killed non-pathogenic yeast, and MGN-3, a safe product made from

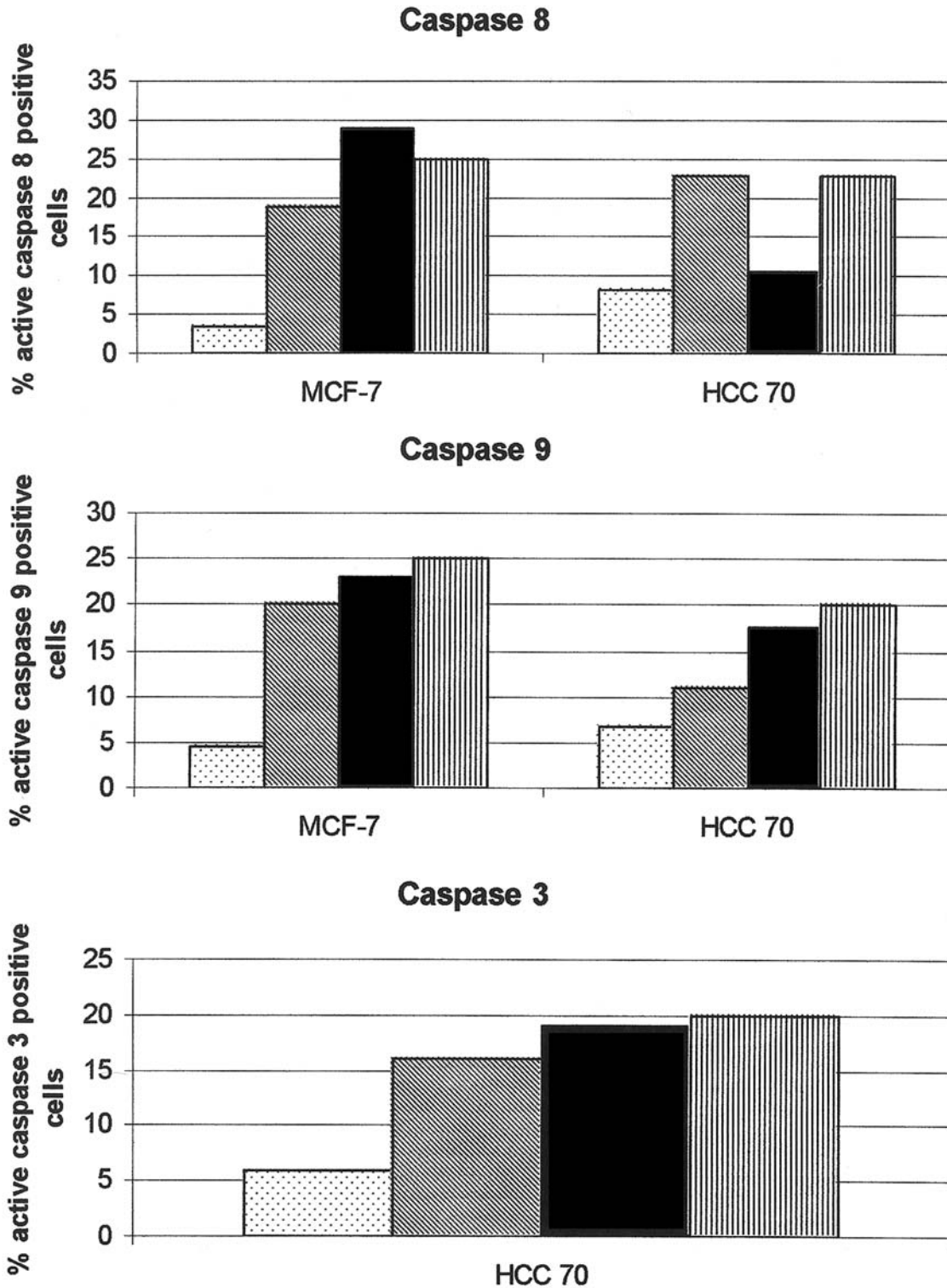


Figure 9. Activation of caspases in MCF-7 and HCC70 cells post treatment with MGN-3. Cancer cells were incubated with yeast at the ratio of 1:10 for 2 hr in the presence and absence of MGN-3 (500 µg/ml). Intracellular active caspase 8, caspase 9 (Figure 9b), and caspase 3 were determined with caspase glow caspases determination kits using FACScan. Activation of caspases was examined in the following groups: cells alone (□), cells + yeast (▨), cells + MGN-3 (■), and cells + yeast + MGN-3 (▩).

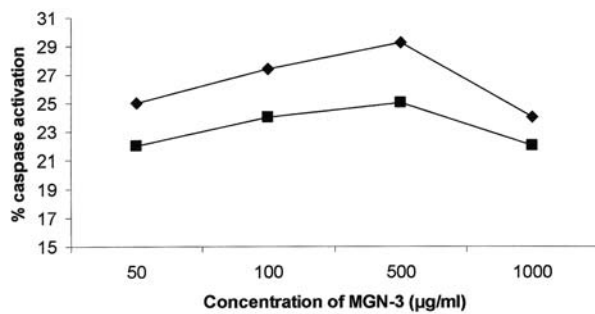


Figure 10. Activation of caspases 8 and 9 of MCF-7 cells post treatment with MGN-3 at different concentrations. MCF-7 cells were incubated with yeast at the ratio of 1:10 and treated with MGN-3 at different concentrations (50-1000 µg/ml). Intracellular active caspase 8 (◆) and caspase 9 (■) were determined with casp glow caspases 8&9 determination kits using FACScan.

arabinoxylan derived from rice bran. We believe that the results of the present study may have implications for the treatment of breast cancer.

### Acknowledgements

We are greatly indebted to Dr. Jamal Abedi, Professor of Statistics, University of California Los Angeles, Department of Education, Social Research Methodology Division, for assistance in statistical analysis. The authors would also like to thank Daiwa Pharmaceutical Co. LTD, Tokyo, Japan for the financial support of this project.

### References

- Marty M, Mignot L, Gisselbrecht G, Morvan F, Gorins A and Boiron M: Teratogenic and mutagenic risks of radiotherapy: when and how to prescribe contraception. *Contracept Fertil Sex* 13: 181-86, 1985.
- Mills KH, Grealley JF, Temperley IJ and Mullins GM: Haematological and immune suppressive effects of total body irradiation in the rat. *Ir J Med Sci* 149: 201-08, 1980.
- Sanderson BJ, Ferguson LR and Denny WA: Mutagenic and carcinogenic properties of platinum-based anticancer drugs. *Mutat Res* 355: 59-70, 1996.
- Santin AD, Hermonat PL, Ravaggi A, Bellone S, Roman J, Pecorelli S, Cannon M and Parham GP: Effects of concurrent cisplatin administration during radiotherapy vs. radiotherapy alone on the immune function of patients with cancer of the uterine cervix. *Int J Radiat Oncol Biol Phys* 48: 997-1006, 2000.
- Vespasiani U, Gentilucci D, Santini A, Picardi B, Vincenzi E, Riva A, Bianchi G and Tonini: Liver cirrhosis after prolonged therapy with IFN-alpha plus interleukin-2 in a metastatic renal cancer long-term survivor. *J Interferon Cytokine Res* 22: 683-88, 2002.
- Watson RW, Redmond HP, Wang JH, Condron C and Bouchier-Hayes D: Neutrophils undergo apoptosis following ingestion of *Escherichia coli*. *J Immunol* 156: 3986-92, 1996.
- Rotstein D, Parodo J, Taneja R and Marshall JC: Phagocytosis of candida albicans induces apoptosis of human neutrophils. *Shock* 14: 278-83, 2000.
- Perskvist N, Long M, Stendahl O and Zheng L: *Mycobacterium tuberculosis* promotes apoptosis in human neutrophils by activating caspase-3 and altering expression of Bax/Bcl-xL via an oxygen-dependent pathway. *J Immunol* 168: 6358-65, 2002.
- Baran J, Weglarczyk K, Mysiak M, Guzik K, Ernst M, Flad HD and Pryjma J: Fas (CD95)-Fas ligand interactions are responsible for monocyte apoptosis occurring as a result of phagocytosis and killing of *Staphylococcus aureus*. *Infect Immun* 69: 1287-97, 2001.
- Ghoneum M and Grewal I: Change in tumour cell-lymphocyte interactions with age. *Hemat Oncol* 7: 71-80, 1990.
- Kadin ME, Kamoun K and Lamberg J: Erythrophagocytic T gamma lymphoma: a clinicopathologist entity resembling malignant histiocytosis. *New Engl J Med* 304: 648-53, 1981.
- Rallet A, Trentesaux C, Jeannesson P, Dufer J, Bouchet P and Jardillier JC: Phagocytosis of the Streptococcal preparation OK-432 (Picibanil) by non-phagocytic human leukemia K 562 cells and its modulation by tumor cell differentiation. *Anticancer Res* 7: 535-40, 1987.
- Palestro CJ, Klein M, Kim CK, Swyer AJ and Goldsmith SJ: Indium-111-labeled leukocyte and technetium-99m-sulfur colloid uptake by a malignant fibrous histiocytoma: phagocytosis by tumor cells? *J Nucl Med* 31: 1548-51, 1990.
- Kiyohara T, Kumakiri M, Kobayashi H, Ohkawara A and Lao LM: Atrophic dermatofibroma. Elastophagocytosis by the tumor cells. *J Cutan Pathol* 27: 312-15, 2000.
- Ghoneum M, Salem F, Shum ST, Perry L and Gill G: *In situ* lymphophagocytosis by nonlymphoreticular neoplasms. *Nat Immun Cell Growth Regul* 6: 77-87, 1987.
- Ghoneum M, Salem F, Allen H and Gill G: Phagocytosis of autologous lymphocytes by cervical preneoplastic and neoplastic cells. *Nat Immun Cell Growth Regul* 7: 239-48, 1988.
- Ghoneum M, Gill G, Stein E, Ansari A and Cooper E: Bacterial phagocytosis by tumor cell *in vitro*, Abstract. Sixth Int Symp Immunol Toronto, Canada. 1986a.
- Ghoneum M, Gill G, Stein E, Salem F and Cooper E: *In vitro* tumor cell phagocytosis of large granular lymphocytes and other leukocytes. *In: Lotzova E and Herberman R (Eds): Natural immunity, cancer and biological response modification*. Karger, Basel, Switzerland. 104-13, 1986b.
- Ghoneum M, Grewal I, Brown J, Osborne R, Elembabi H and Gill G: Phagocytosis of *Candida albicans* by lymphatic tumor cells *in vitro*. *Acta Histochemica*. 105: 127-133, 2003.
- Marin-Padilla M: Erythrophagocytosis by epithelial cells of a breast carcinoma. *Cancer* 39: 1085-89, 1977.
- Montcourrier P, Mangeat PH, Valembois C, Salazar G, Sahuquet A, Duperray C and Rochefort H: Characterization of very acidic phagosomes in breast cancer cells and their association with invasion. *J Cell Sci* 107: 2381-91, 1994.
- Coopman PJ, Do MT, Thompson EW and Mueller SC: Phagocytosis of cross-linked gelatin matrix by human breast carcinoma cells correlates with their invasive capacity. *Clin Cancer Res* 4: 507-15, 1998.
- Ghoneum M and Gollapudi S: Phagocytosis of *Candida albicans* by human metastatic and non-metastatic breast cancer cells *in vitro*. *Cancer Detection & Prevention* 28: 17-26, 2004.

- 24 Ghoneum M and Gollapudi S: Induction of apoptosis in breast cancer cells by *Saccharomyces cerevisiae*, the baker's yeast, *in vitro*. *Anticancer Res* 24: 1455-1463, 2004.
- 25 Ghoneum M: Anti-HIV activity *in vitro* of MGN-3, an activated arabinoxylan from rice bran. *Biochem Bioph Res Comm* 243: 25-29, 1998.
- 26 Ghoneum M: Enhancement of human natural killer cell activity by modified arabinoxylan from rice bran (MGN-3). *Int J Immunother* XIV: 89-99, 1998.
- 27 Ghoneum M and Jewett A: Production of tumor necrosis factor- $\alpha$  and interferon- $\gamma$  from human peripheral blood lymphocytes by MGN-3, a modified arabinoxylan from rice bran, and its synergy with interleukin-2 *in vitro*. *Cancer Detection & Prevention* 24: 314-324, 2000.
- 28 Ghoneum M and Gollapudi S: Modified arabinoxylan rice bran (MGN-3/Biobran) sensitizes human T cell leukemia cells to death receptor (CD95)-induced apoptosis. *Cancer Letters* 20: 41-49, 2003.
- 29 Petrequin PR and Johnson AG: Macrophage activation by adjuvants in aging mice. *J Leukocyte Biol* 35: 251-63, 1984.
- 30 Hockenbery D: Defining apoptosis. *Am J Pathol* 146: 16-19, 1995.
- 31 Wyllie AH, Kerr JFR and Currie AR: Cell death: the significance of apoptosis. *Int Rev Cytol* 68: 251-306, 1980.
- 32 Lee E and Surh Y: Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. *Cancer Letters* 134: 163-8, 1998.
- 33 Sigounas G, Anagnostou A and Steiner M: dl-alpha-Tocopherol induces apoptosis in erythroleukemia, prostate, and breast cancer cells. *Nutr Cancer* 28: 30-35, 1997.
- 34 Halicka D, Ardelt B, Juan G, Mittelman A, Chen S, Traganos F and Darzynkiewics Z: Apoptosis and cell cycle effects induced by extracts of the Chinese herbal preparation PC SPES. *Int J Oncol* 11: 437-48, 1997.
- 35 Han B, Toyomasu T and Shinozawa T: Induction of apoptosis by *Coprinus disseminatus* mycelial culture broth extract in human cervical carcinoma cells. *Cell Struct Funct* 24: 209-15, 1999.
- 36 Fan H, Morioka T and Ito E: Induction of apoptosis and growth inhibition of cultured human endometrial adenocarcinoma cells (Sawano) by an antitumor lipoprotein fraction of rice bran. *Gynecol Oncol* 76: 170-175, 2000.
- 37 Subbiah MT and Abplanalp W: Ergosterol (major sterol of baker's and brewer's yeast extracts) inhibits the growth of human breast cancer cells *in vitro* and the potential role of its oxidation products. *Int J Vitam Nutr Res* 73: 19-23, 2003.
- 38 Zamze S, Martinez-Pomares L, Jones H, Taylor PR, Stillion RJ, Gordon S and Wong SY: Recognition of bacterial capsular polysaccharides and lipopolysaccharides by the macrophage mannose receptor. *J Biol Chem* 277: 41613-23, 2002.
- 39 Aggarwal S, Gollapudi S and Gupta S: Increased TNF-alpha-induced apoptosis in lymphocytes from aged humans: changes in TNF-alpha receptor expression and activation of caspases. *J Immunol* 162: 2154-61, 1999.
- 40 Gupta S: Molecular steps of TNF receptor mediated apoptosis. *Curr Mol Med* 1: 299-306, 2001.
- 41 Varela N, Munoz-Pinedo C, Ruiz-Ruiz C, Robeld G, Pedroso M and Lopez-Rivas A: Interferon-gamma sensitizes human myeloid leukemia cells to death- receptor mediate apoptosis by a pleototropic mechanism. *J Biol Chem* 276: 1779-87, 2001.
- 42 Mooney LM, Al-Sakkaf KA, Brown BL and Dobson PRM: Apoptotic mechanisms in T47D and MCF-7 human breast cancer cells. *British J Cancer* 87: 909-17, 2002.
- 43 Häcker H, Fürmann C, Wagner H and Häcker G: Caspase-9/-3 activation and apoptosis are induced in mouse macrophages upon ingestion and digestion of *Escherichia coli* bacteria. *J Immunol* 169: 3172-9, 2002.

Received May 25, 2004  
Accepted December 28, 2004