

# Suppressing Tumor Progression of *In Vitro* Prostate Cancer Cells by Emitted Psychosomatic Power Through Zen Meditation

Tiing Yu

*Department of Applied Chemistry, National Chiao Tung University  
Hsinchu 300, Taiwan*

Hui Ling Tsai

*Department of Life Science, National Tsing Hua University  
Hsinchu 300, Taiwan*

Ming Liang Hwang

*Taiwan Zen Buddhist Association, Taipei 106, Taiwan*

**Abstract:** Human prostate cancer PC3 cells were treated *in vitro* with psychosomatic power emitted by a Buddhist-Zen Master. A significant decrease of growth rate was observed as determined by MTT assay after 48 hours. These cells also had two- to three-fold higher levels of prostatic acid phosphatase (PACp) activity, a prostate tissue-specific differentiation antigen. In addition, the treated cells formed fewer and smaller colonies in soft agar as compared with control cells, which displayed anchorage-independent growth. These observations provide insight into the suppressive effects of healing power through the practice of Buddhist-Zen meditation on tumor progression. The emitted bioenergy may be suggested as an alternative and feasible approach for cancer research and patient treatment.

**Keywords:** Cancer; Complementary and Alternative Medicine; Meditation; Prostate Cancer; Psychosomatic Techniques; Qi; Qigong; Tumor; Zen Meditation.

## Introduction

Prostate cancer is the most frequently diagnosed malignancy affecting adult men in industrialized countries. For example, there will be about 198,100 new cases of prostate cancer in the United States in the year 2001 and about 31,500 men will die of this disease

Correspondence to: Professor Tiing Yu, Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan. Tel: (+886) 3-573-1673, Fax: (+886) 3-572-3764, E-mail: tyu@mail.nctu.edu.tw

according to the statistics of the American Cancer Society. Although a range of therapeutic products are available for androgen-dependent prostate cancer, limited specific intervention modalities can be suggested for androgen-independent prostate cancer. In addition, various multidrug-resistant proteins were expressed during drug treatment and cancerous cells with this phenotype may seriously reduce the effectiveness of chemotherapy. Once prostate cancer gets to an advanced stage, it is difficult to prevent tumor progression and a cure is almost impossible (Miyake *et al.*, 2001; Farhat *et al.*, 2000).

At the same time, there is growing attention to the health benefits of complementary and alternative medicine (CAM), which have been described as additional approaches to care outside of mainstream medical practice. One estimate is that more than US\$13 billion is spent annually on complementary techniques in the United States alone (Schimpff, 1997). A recent report found a significant increase (from 34% in 1990 to 42% in 1997) of CAM use among the general public in the USA (Eisenberg *et al.*, 1998). In particular, mind-body intervention is considered one of the most preferred and helpful complementary choices for cancer patients (Ernst, 2001; Balneaves *et al.*, 1999). Clinical observations suggest dedicated involvement in meditation, or other related psychosomatic (or mind-body) techniques may even prolong the life of some patients with metastatic cancer (Cunningham *et al.*, 2000; Magarcy *et al.*, 1988; Woolley-Hart, 1979). Consequently, some oncologists suggest a need to consider including education about meditation in oncologists' training (Farhat *et al.*, 2000; Newell and Sanson-Fisher, 2000; Brennan and Stevens, 1998).

In the past several decades, scientists have been engaged in exploring the physiological and psychological effects of practicing psychosomatic techniques, such as Yoga, Qigong and Zen meditation. Zen meditation is a mental and physical practice of quieting the practitioner's mind and focusing the attention on the body's chakras (energy points) to transcend from a physical and mental level to a spiritual level. In the spiritual level, there is no thought, only blissful quiet, full of life energy and wisdom power. The three main elements of Zen meditation are: sitting, breathing and concentration. Several studies have provided undeniable evidence for the interconnectedness of psychosomatic power to the individual's cure as well as to animals, cells and materials outside the practitioner's body, including non-living matter. Rigorous experimental procedures were designed and modern instruments were employed to ensure reliable data. It was found that regular practice of meditation is associated with increased physiological levels of melatonin that may enhance the immunity of practitioners and reduce the growth of malignant prostate tumors (Coker, 1999; Massion *et al.*, 1995). In addition to Zen meditation, Qigong exercises are also reported to reduce the drug dosage required for health maintenance and decrease the side effects of chemotherapy (Sancier, 1999). Similar results were found with "emitted psychosomatic power" to experimental animals. Lei *et al.* (1991) demonstrated that Qigong emitted Qi could promote the activity of splenic natural killer (NK) cells, macrophage-mediated tumor cytolysis activity and the production of interleukin-2 (IL-2) in treated mice (Lei *et al.*, 1991). Lee *et al.* (2001) examined *ChunSoo* energy healing on NK cell cytotoxicity *in vitro*. They found that NK activity was significantly increased by emitted Qi treatment. Fukushima *et al.* (2001)

investigated the biological effect on phosphate buffered saline (PBS). They found that the Qi-treated PBS clearly showed stimulating effect on phagocytic activity of human polymorphonuclear leukocytes. The activity of Qi-treated PBS could last for days or even weeks. In an interesting study on green peas and wheat, Haid and Huprikar (2001) illustrated that giving water treated with meditation of either stimulating or inhibiting intents significantly influenced the germination and growth of the plants. Yan *et al.* (1999) clearly showed that the molecular composition of targeted materials (non-living matter) could be significantly affected by external Qi emitted from a Qigong master either on-site or from a long distance.

In the current study, the biological effects of psychosomatic power were investigated on human prostate cancer PC3 cells treated by Mr. Hwang Ming Liang (known as Zen Master Wu Chueh Miao Tien, the 85th patriarch of Zen-Buddhism Sect). The purpose of this paper is not to compare the healing power emitted by Zen meditation and other psychosomatic practitioners. Instead, more evidence is gathered to demonstrate the potential health benefits of practicing different psychosomatic techniques.

## Materials and Methods

### *Treatment of Cancer Cells with Psychosomatic Power*

The objects (the cells in the plates) were treated with the psychosomatic power emitted by Master Wu Chueh Miao Tien. The cell plates were placed on a table in a temperature-controlled room (25°C). The psychosomatic power was emitted from the right hand of the Master. He opened his palm and placed it above the capped plates. The distance between the plates and the hand was around 30 cm. The duration of the emission was 1 minute for all the experiments. The experiments for the three investigations reported herein were repeated on two separate days.

### *Cell Culture and Growth Assay*

Androgen-independent PC3 human prostate cancer cell line was purchased from American Type Culture Collection (ATCC) and maintained in RPMI 1640 culture medium (GibcoBRL, Gaithersburg, MI) containing 10% fetal bovine serum, 100 U/ml penicillin and streptomycin, as the complete culture medium. One day before the experiment,  $5 \times 10^3$  cells per well were seeded in 96-well plates and incubated in a 5% CO<sub>2</sub> incubator at 37°C overnight. The cultures were treated with the psychosomatic power for one minute, while the control cultures were placed at least 3 m away from the control cultures in the same room. Mitochondrial metabolism was measured as a marker for cell growth by adding 50 µl/well 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) (5mg/ml in medium) with a 3-hour incubation at 37°C before adding lysis buffer (1% SDS in 0.1 M HCl). Spectrophotometric analyses were carried out using an enzyme-linked immunosorbent assay (ELISA) reader (Titertek Multiskan) at 570 nm.

### *Differentiation Assay*

The activity of prostatic acid phosphatase (PACp) was measured as a differentiation marker for prostate cells. Briefly, 48 hours after the application of psychosomatic power, the cells were rinsed with sterilized phosphate buffer saline and lysed in acetate buffer (pH 5.5) containing 1% triton-X 100 and various protease inhibitors. After sonication and centrifugation, the protein concentration in the cell lysates was determined by the Bio-Rad dye protein assay using bovine serum albumin as a standard. All the lysates were adjusted to 1 mg/ml. PACp activity was quantified by determining the absorbance of released p-nitrophenol at 410 nm using p-nitrophenyl phosphate as the substrate in 200 µg protein lysate.

### *Anchorage Dependence Assay*

Cells ( $1.5 \times 10^5$ ) were suspended in 15 ml of the complete culture medium containing 0.3% SeaPlaque-agarose. Five milliliters of the suspension was seeded in 60 mm dish plates, and overlaid with 0.5 ml of the same medium after the gel solidified. Eight of the prepared plates (16 plates) were treated with the emitted psychosomatic power for 1 minute while the control plates were kept 3 m away in the same temperature-controlled room. After 14 days, the cell colonies were stained with MTT for visualization.

## **Results**

The PC3 cell line studied in this report belongs to androgen-independent prostate cancer. Due to the low expressed levels of functional androgen receptors (AR) and prostate-specific antigen (PSA), such as PACp, the growth of these cells becomes androgen-unresponsive and refractory to hormonal therapy. In multivariant analysis, post-therapy changes in PSA were shown to be the most significant predictor of survival in patients with androgen-independent prostate cancer (Steineck *et al.*, 1996). Thus, the cell line examined here provides an appropriate model that could be employed to test the effectiveness of psychosomatic power.

The cell growth rate, PACp activity and the anchorage-dependent growth have been carefully investigated by experiments on two separate days. Since uncontrolled cellular proliferation is a fundamental aberration in cellular behavior during carcinogenesis, growth rate measurement is one of the important indicators for cancer treatment. The cellular proliferation and viability was measured based on the reduced activity of MTT by mitochondrial dehydrogenase. A significant growth inhibition ( $p < 0.01$ ) of PC3 cells treated with psychosomatic power was observed (Fig. 1). The growth suppression could be seen as early as 24 hours after treatment, and became even greater as time passed. No cytotoxicity or cell death was found during the experiments.

As can be seen in Fig. 2, PACp activity was almost three-fold higher ( $p = 0.0031$ ) than that of control cells 48 hours after treatment. The increased PACp activity could be sustained and maintained in the treated cells after at least two to three passages (data not shown). The expressed levels of PACp for the cultures treated with the psychosomatic power apparently reflect the suppressive effects on cell growth described above.

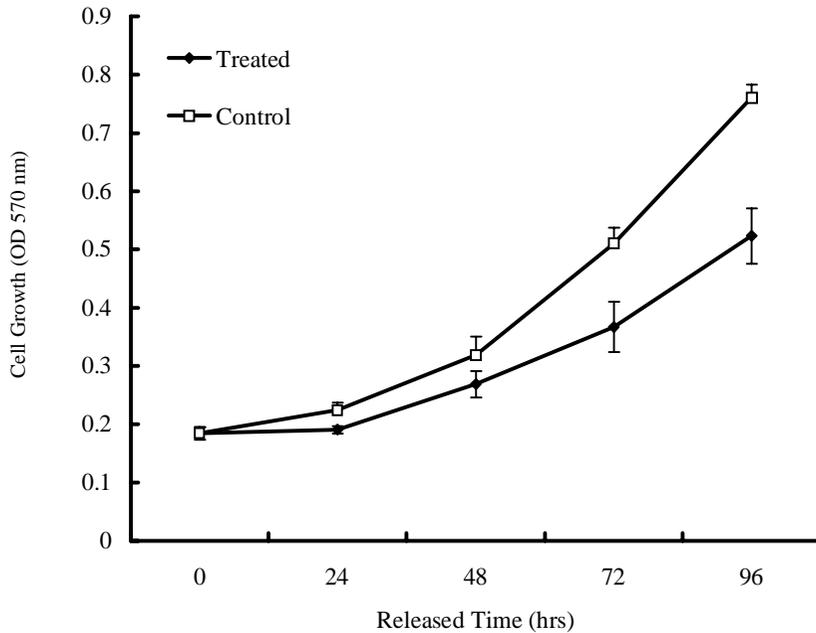


Figure 1. Biological effects of psychosomatic power on cell growth. Androgen-independent PC3 human prostatic cancer cells were seeded in 96-well culture plates one day before the experiment. The cultures were treated with the psychosomatic power for one minute, while the control cultures were placed at least 3 m away from the treated cultures in the same room. Mitochondrial metabolism was measured by MTT assay. Spectrophotometric analyses were carried out at 570 nm and expressed as mean  $\pm$  SD of 16 determinations from both the control and treated samples ( $p < 0.01$ ).

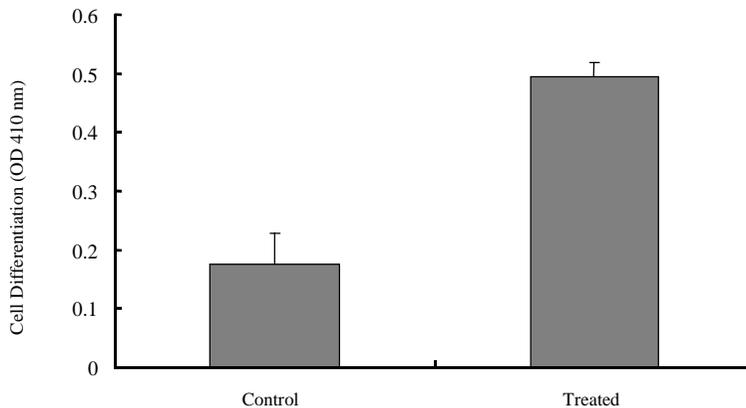


Figure 2. Biological effects of psychosomatic power on PAcP activity. Forty-eight hours after the application of psychosomatic power, cell lysates from both treated and control cells were prepared. PAcP activity was quantified by determining the absorbance of released p-nitrophenol at 410 nm using p-nitrophenyl phosphate as the substrate in 200  $\mu$ g protein lysate. The data were expressed as mean  $\pm$  SD of six determinations from both the control and treated samples ( $p = 0.0031$ ).

Since the acquisition of anchorage-independent growth often correlates with tumorigenicity, we also assayed the treated PC3 cells for their ability to form colonies in soft agar. As shown in Fig. 3, untreated control cells plated in 0.3% soft agar formed numerous cell colonies (plate A in the figure), whereas significant amount of the treated PC3 cells failed to grow and persisted as single cells (plate B in the figure), indicating a down-regulation of tumorigenicity. Furthermore, the positive colonies in the treated plates were much smaller than those in the control plates.

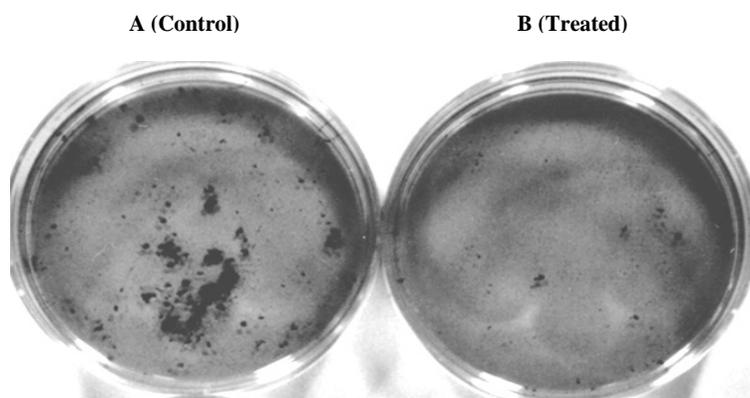


Figure 3. Biological effects of psychosomatic power on anchorage-independent growth. PC3 cells were suspended in the complete culture medium containing 0.3% SeaPlaque-agarose one day before the experiment. Eight plates received emitted psychosomatic power for 1 minute while eight control plates were at least 3 m away in the same temperature-controlled room. After 14 days, all the plates were incubated with MTT to develop color. Typical results are shown in plate A (the control plate) and plate B (the treated plate).

## Discussion

It is interesting to note that the growth of prostate cancer PC3 cells, although not completely terminated, could be reduced through the application of Zen psychosomatic power alone without any drug treatment. Similar findings have been reported in Qigong studies. Feng *et al.* (1982) and Chien *et al.* (1991) all found that the growth of either bacteria or human fibroblast cells in culture could be significantly influenced by the external Qi emitted by a Qigong master. They indicated that certain Qigong masters could emit intentionally two kinds of Qi: “growth” (facilitating) and “termination” (inhibiting) external Qi. These results based on *in vitro* studies support the opinion that certain forms of emitted bioenergy should be recognized as a kind of healing power; not just psychological consolation, like a placebo, to the patients.

As is generally known, the balance among cell growth, cell differentiation and cell death (apoptosis) in signal transduction reflects the cellular activity. The balance, however, is greatly disturbed for cancer cells and results in uncontrolled cell growth. The growth reduction caused by psychosomatic power would reflect a certain shift of the signaling balance in treated cells, and should be echoed by prostate epithelium-specific differentiation antigens.

Among the various identified PSAs, PAcP is a well-known and reliable antigen suggested for clinical evaluation (Steineck *et al.*, 1996; Akimoto *et al.*, 1997; Barichello *et al.*, 1995). It was found that during the progression of prostate carcinoma, the levels of PAcP decreased (Meng and Lin, 1998). More interestingly, Lin *et al.* (1998) demonstrated that over-expression of PAcP in androgen-independent prostate carcinoma, like PC3 cells, could restore the cellular responsiveness to androgen stimulation. In fact, Meng and Lin (1998) provided direct evidence that cellular PAcP could down-regulate prostate cell growth by dephosphorylating Typ(P) on c-ErbB-2 oncoprotein in those cells. Some synthetic vitamin D analogues or dietary natural compounds, such as inositol hexaphosphate (InsP<sub>6</sub>), have also shown the therapeutic potential to inhibit both cell proliferation and induce PAcP secretion in prostate cancer cells (Zhuang and Burnstein, 1998; Hedlund *et al.*, 1997; Abulkalam *et al.*, 1995). It will therefore be of interest to investigate further on whether the de-tumorigenesis of PC3 cells by psychosomatic power shares similar signaling pathways as the above compounds.

Clinical studies showed that progression to androgen independence remains the main obstacle to improving survival for patients with advanced prostate cancer (Farhat *et al.*, 2000). Differentiation therapy may provide an alternative for treatment of cancers that do not respond to hormonal manipulations or cytotoxic chemotherapy. The differentiation-inducing activity of the psychosomatic power may cause the cancerous cells to be more sensitive to hormonal therapy. It was found that treatment of cancerous cells with non-toxic differentiation inducers resulted in dose-dependent inhibition of cell proliferation; with no significant inhibitory effects on normal cells or skin fibroblasts (Samid *et al.*, 1993). Non-toxic differentiation inducers, such as psychosomatic power, used alone or in combination with other antitumor agents, would possibly provide a feasible approach for the treatment of advanced prostate cancer.

Several studies have demonstrated that some adhesion molecules play important roles in cell transformation and tumor progression (Julliano and Varner, 1993; Guan and Shalloway, 1992, Bishop, 1991). The loss of anchorage-independent growth may be due to a different expression profile of adhesion molecules on the surface of treated cells. Accordingly, the involvement of psychosomatic power in regulating the expression of surface antigens or receptors will require further research.

In this study, we examined the effects of emitted psychosomatic power by a Buddhist-Zen Master upon human prostate cancer cells using the well-established PC3 cell model without applying any other medication. These three experimental results strongly suggest that emitted psychosomatic power has antitumor effects on human prostate cancer cells. This is the first investigation using rigorous experimental design to demonstrate the biological effects of psychosomatic power emitted by a Zen master on cancer cells. Although *in vivo* studies are warranted to validate the efficacy, we are observing a great potential to treat human cancers with psychosomatic power. The effectiveness of such bioenergy on cancer cells could be recommended as a supportive medical practice for clinical applications. Using the emitted psychosomatic power alone or combining with traditional medication may develop into a novel means for curing cancers.

## Acknowledgments

The authors deeply enjoy brainstorming with Dr. Jim J.C. Sheu (National Tsinghua University, Taiwan) in generating this research idea. The generous help from Messrs. H.Y. Chen and T.Y. Wang (National Tsinghua University, Taiwan) in the experimental design and manuscript preparation is also gratefully acknowledged. Financial support from the National Science Council of the Republic of China is also gratefully acknowledged.

## References

- Abulkalam, M., A. Shamsuddin and G.Y. Yang. Inositol hexaphosphate inhibits growth and induces differentiation of PC3 human prostate cancer cells. *Carcinogenesis* 16: 1975–1979, 1995.
- Akimoto, S., Y. Furuya, K. Akakura, J. Shimazaki and H. Ito. Relationship between prostate-specific antigen, clinical stage, and degree of bone metastasis in patients with prostate cancer: comparison with prostatic acid phosphatase and alkaline phosphatase. *Int. J. Urol.* 4: 572–575, 1997.
- Balneaves, L.G., L.J. Kristjanson and D. Tataryn. Beyond convention: describing complementary therapy use by woman living with breast cancer. *Patient Educ. Couns.* 38: 143–153, 1999.
- Barichello, M., M. Gion, A. Bonazza, U.S. Delli Ponti, A. Bolgan, G.P. Contemori, P. Barioli, G. Capitano, B. Pecori and S. Omacini. Prostate-specific antigen as a unique routine test in monitoring therapy for inoperable prostate cancer: comparison with radionuclide bone scan and prostatic acid phosphatase. *Eur. Urol.* 27: 295–300, 1995.
- Bishop, J.M. Molecular themes in oncogenesis. *Cell* 64: 235–248, 1991.
- Brennan, C. and J. Stevens. A grounded theory approach towards understanding the self-perceived effects of meditation on people being treated for cancer. *Aust. J. Holist. Nurs.* 5: 20–26, 1998.
- Chien, C.H., J.J. Tsuei, S.C. Lee, Y.C. Huang and Y.H. Wei. Effect of emitted bioenergy on biochemical functions of cells. *Am. J. Chin. Med.* 19: 285–292, 1991.
- Coker, K.H. Meditation and prostate cancer: integrating a mind/body intervention with traditional therapies. *Semin. Urol. Oncol.* 17: 111–118, 1999.
- Cunningham, A.J., C. Phillips, G.A. Lockwood, D.W. Hedley and C.V. Edmonds. Association of involvement in psychological self-regulation with longer survival in patients with metastatic cancer: an exploratory study. *Adv. Mind Body Med.* 16: 276–287, 2000.
- Eisenberg, D.M., R.B. Davis, S.L. Ettner, S. Appel, S. Wilkey, M. van Rompay and R.C. Kessler. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. *J. Am. Med. Assoc.* 280: 1569–1575, 1998.
- Ernst, E. A primer of complementary and alternative medicine commonly used by cancer patients. *Med. J. Aust.* 174: 88–92, 2001.
- Farhat, W.A., A.A. Habbal and R.B. Khauli. A guideline to clinical utility of prostate specific antigen. *Saudi Med. J.* 21: 223–227, 2000.
- Feng, L., J. Qian, S. Li, F.K. Ma and G. Bao. Effect of emitted Qi (external Qi) on gram negative bacilli. *Nature J.* (in Chinese) 5: 163, 1982.
- Fukushima, M., T. Kataoka, C. Hamada and M. Matsumoto. Evidence of Qi-gong energy and its biological effect on the enhancement of the phagocytic activity of human polymorphonuclear leukocytes. *Am. J. Chin. Med.* 29: 1–16, 2001.
- Guan, J.L. and D. Shalloway. Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. *Nature* 358: 690–692, 1992.

- Haid, M. and S. Huprikar, Modulation of germination and growth of plants by meditation. *Am. J. Chin. Med.* 29: 393–401, 2001.
- Hedlund, T.E., K.A. Moffatt, M.R. Uskokovic and G.J. Miller. Three synthetic vitamin D analogues induce prostate-specific acid phosphatase and prostate-specific antigen while inhibiting the growth of human prostate cancer cells in a vitamin D receptor-dependent fashion. *Clin. Cancer Res.* 3: 1331–1338, 1997.
- Julliano, R.L. and J.A. Varner. Adhesion molecules in cancer: the role of integrins. *Curr. Opin. Cell Biol.* 5: 812–818, 1993.
- Lee, M.S., H.J. Huh, H.S. Jang and C.S. Han. Effects of emitted qi on *in vitro* natural killer cell cytotoxic activity. *Am. J. Chin. Med.* 29: 17–22, 2001.
- Lei, X.F., A.H. Bi, Z.X. Zhang and Z.Y. Cheng. The antitumor effects of qigong-emitted external Qi and its influence on the immunologic functions of tumor-bearing mice. *J. Tongji Med. Univ.* 11: 253–256, 1991.
- Lin, M.F., T.C. Meng, P.S. Rao, C. Chang, A.H. Schonthal and F.F. Lin. Expression of human prostatic acid phosphatase correlates with androgen-stimulated cell proliferation in prostate cell lines. *J. Biol. Chem.* 273: 5939–5947, 1998.
- Magarcy, C.J. Aspects of the psychological management of breast cancer. *Med. J. Aust.* 148: 239–242, 1988.
- Massion, A.O., J. Teas, J.R. Hebert, M.D. Wertheimer and J. Kabat-Zinn. Meditation, melatonin and breast/prostate cancer: hypothesis and preliminary data. *Med. Hypotheses* 44: 39–46, 1995.
- Meng, T.C. and M.F. Lin. Tyrosine phosphorylation of c-ErbB-2 is regulated by the cellular form of prostate cancer cells. *J. Biol. Chem.* 273: 22096–22104, 1998.
- Miyake, H., I. Hara, S. Kamidono and M.E. Gleave. Novel therapeutic strategy for advanced prostate cancer using antisense oligodeoxynucleotides targeting anti-apoptotic genes upregulated after androgen withdrawal to delay androgen-independent progression and chemosensitivity. *Int. J. Urol.* 8: 337–349, 2001.
- Newell, S. and R.W. Sanson-Fisher. Australian oncologists' self-reported knowledge and attitudes about non-traditional therapies used by cancer patients. *Med. J. Aust.* 172: 110–113, 2000.
- Samid, D., S. Shack and C.E. Myers. Selective growth arrest and phenotypic reversion of prostate cancer cells *in vitro* by nontoxic pharmacological concentrations of phenylacetate. *J. Clin. Invest.* 91: 2288–2295, 1993.
- Sancier, K.M. Therapeutic benefits of qigong exercises in combination with drugs. *J. Altern. Complement.* 5: 383–389, 1999.
- Schimpff, S.C. Complementary medicine. *Curr. Opin. Oncol.* 9: 327–331, 1997.
- Steineck, G., W.K. Kelly, M. Mazumdar, V. Vlamis, M. Schwartz and H.I. Scher. Acid phosphatase: defining a role in androgen-independent prostate cancer. *Urology* 47: 719–726, 1996.
- Woolley-Hart, A. Meditation and cancer: slowing down the inevitable. *Nurs. Mirror* 149: 36–39, 1979.
- Yan, X., H. Lin, H. Li, A. Traynor-Kaplan, Z.Q. Xia, Y. Fang and M. Dao. Structure and property changes in certain materials influenced by the external qi of qigong. *Mat. Res. Innovat.* 2: 349–359, 1999.
- Zhuang, S.H. and K.L. Burnstein. Antiproliferative effect of 1-alpha,25-dihydroxyvitamin D3 in human prostate cancer cell line LNCaP involves reduction of cyclin-dependent kinase 2 activity and persistent G1 accumulation. *Endocrinology* 139: 1197–1207, 1998.

