S Liao 廖述宗

The medicinal action of androgens and green tea epigallocatechin gallate

男性荷爾蒙和綠茶成分epigallocatechin gallate的醫療功效

Unorthodox (non-traditional or alternative) medicinal practices have been expanding very rapidly in western countries. Modern physicians, scientists, and non-traditional medicine practitioners now must join forces to promote evidence-based medicine to benefit patients. Green tea extracts are among the most widely used ancient medicinal agents, while androgens are probably the oldest drugs used in a purified form in traditional Chinese medicine. It is now clear that a specific green tea catechin, (-)epigallocatechin-3-gallate, can modulate the production and biological actions of androgens and other hormones. Modulation of androgenic activity and administration of (-)epigallocatechin-3-gallate may be useful for the treatment of various hormone-related abnormalities, such as benign prostatic hyperplasia, baldness, and acne, as well as androgen-dependent and -independent prostate cancers. (-)Epigallocatechin-3-gallate has also been shown to modulate appetite and control obesity in animals.

另類醫療已經在西方國家迅速擴展。現代醫生，科學家和從事非傳統醫療業者必須合作，努力促進對病人有利的實證醫學。綠茶精是古代廣泛使用的藥劑之一，而男性荷爾蒙大概是傳統中藥的純化形式中最古老的藥。我們知道綠茶 catechin ，(-)epigallocatechin-3-gallate ，有調節男性荷爾蒙及其他荷爾蒙的製造及生物功能的作用。調節男性荷爾蒙的活動及服用(-)epigallocatechin-3-gallate 可能有助於治療各種因荷爾蒙失調而引起的病症，如良性前列腺增生、禿頭、暗瘡、及與男性荷爾蒙有關或無關的前列腺癌。(-)epigallocatechin-3-gallate還證實可以在動物中調節食慾及控制肥胖。

Key words:
Catechin;
Medicine, Chinese traditional;
Obesity;
Prostatic neoplasms;
Testosterone 5-alpha-reductase

關鍵詞：
Catechin；
醫學，中國傳統；
肥胖；
前列腺瘤；
Testosterone 5-alpha-reductase

HKMJ 2001;7:369-74

Tang Center for Herbal Medicine Research, Ben May Institute for Cancer Research and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, US
S Liao, PhD

Correspondence to: Prof S Liao

Evidence-based unorthodox medicine

As many as 70% of patients in western countries are now utilising unorthodox, non-traditional medicines developed in China, India, South America, and Africa, and by Native Americans in the US. Up to 80% of patients using alternative medical treatments in North America and Europe are satisfied with their unorthodox health care and its cost. This compares with only approximately 45% satisfaction for patients using western (orthodox, biomedical) therapies based on scientific evidence. Given the popularity of non-traditional medicines, physicians and scientists cannot simply dismiss unorthodox medicine as quackery.

In the US, currently two thirds of medical schools, including Harvard, John Hopkins, Chicago, and Stanford, teach alternative therapies, such as herbal medicine, acupuncture, chiropractic massage, therapeutic touch, homeopathy, and mind-body techniques. Many medical doctors and educators of orthodox medicine, however, are still very sceptical about alternative medicine and refer to this trend as disturbing and harmful.

Many forms of non-traditional medicine have a long history of practice, having been developed many hundreds or thousand years before the modern sciences. They were clearly on the frontier of medicine and biology when they evolved. The strength of old or new medicine, however, is dependent on the constant scrutiny of past practices and willingness to explore new approaches. What seemed appropriate at an earlier time may not be so in the light of new information, requiring revision as we learn more. This realisation is necessary to facilitate the development of evidence-based unorthodox medicine in order to ensure its efficacy and safety.

There are many advantages in utilising the knowledge derived from both traditional and non-traditional medicine. For example, herbal medicine has helped in the development of many new drugs now utilised in traditional western therapies. More than 40% of drugs, including aspirin and taxol, originated from plants or herbs. Scientific research into herbal medicine will undoubtedly lead to the development of many more drug therapies. The concept of using a combination of components for treatment is common in unorthodox medicine and has also been adapted by orthodox medicine, for example, in the formulation of drugs, or in the treatment of diseases, such as AIDS. It is time for both unorthodox and orthodox medical scientists and physicians to join forces to develop one medicine that benefits all.[1]

Two ancient medicines: green tea and androgen

According to Chinese history, emperor Sin-Non declared more than 3000 years ago that a daily cup of tea could dissolve many poisons in the body. It is a widely held belief in oriental cultures that tea has medicinal efficacy in the prevention and treatment of many diseases. Longevity is also often associated with drinking tea.

Scientific and medical evaluation of the benefits of tea, however, has been initiated only recently. These studies have considered the antiviral and antibacterial activities of tea, as well as the treatment of cancer, cardiovascular disease, diabetes, dermatological problems, obesity, and effects on oral health. Many of the benefits claimed are not convincing, while others warrant careful evaluation and further scientific study.[2]

Reports of the ability of green tea and its associated catechins to act as antioxidants and radical scavengers, and to inhibit the growth of cancer cells in culture and the induction of carcinogenesis in experimental animals, raised the possibility that consumption of green tea and its associated catechins might lower cancer risk in humans. Epidemiological studies, however, have not produced conclusive evidence for health benefits of tea consumption in humans. Possible confounding factors contributing to equivocal results include population sampling errors, the diversity of green tea products used and the amounts consumed, interference by tea adulterants, the over-riding effects of life style, and potential selectivity for certain types of cancer. To prove that ingestion of green tea is beneficial to cancer patients, well-controlled evidence-based clinical studies are required.

Another ancient medicine used was the male hormone, androgen. As early as 300 BC, Aristotle described the influence of castration on the modification of sexual characteristics in boys. These findings showed that the testis was related to the sexual characteristics and reproductive abilities of the individual. By circa 200 BC, crystals of apparently androgenic steroids prepared from urine by sublimation, were already being used in China to treat individuals lacking 'maleness activity'.[3] Most Chinese medicine researchers, however, are unaware of this great discovery, described by Joseph Needham, a distinguished biochemist in England, as the single most important pre-biochemical discovery. It is clear that Chinese medicine used pure compounds for medical purposes at a very early stage.

Another important contribution made by traditional Chinese medicine was the development of the concept of Nei-tan (inner elixir) and Wai-tan (outer elixir) during the Tang dynasty (700-1000 AD). For health maintenance, Wai-tan was used to modulate Nei-tan, equivalent to today's pharmacological modification of endocrine systems in medicine. The effect of green tea, (-)epigallocatechin-3-gallate (EGCG) on various endocrine systems will be summarised below.

Control of androgen action by fatty acids and green tea, (-)epigallocatechin-3-gallate

In the early 1960s, we found that androgens can rapidly enhance RNA synthesis in target organs, such as the ventral prostate of rats, suggesting that androgens act by modulating gene expression. Subsequent studies have shown that in target organs, testosterone, the major androgen produced by the testis circulating in the blood, is converted by 5α-reductase to 5α-dihydrotestosterone (DHT) (Fig 1), binding to

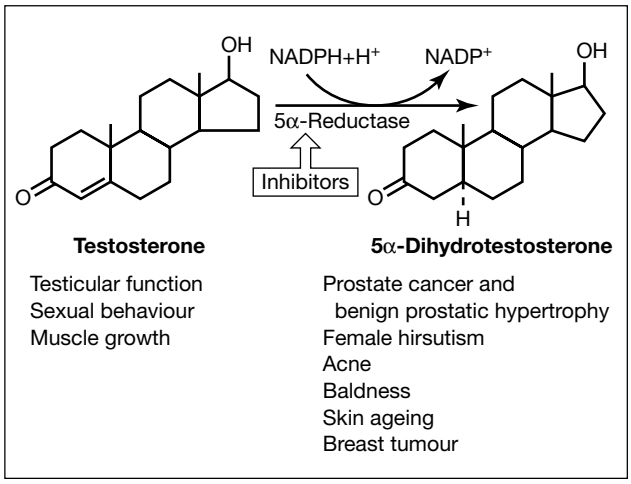

Fig 1. In many organs, testosterone is converted by 5α-reductase to a more active androgen, 5α-dihydrotestosterone, which can promote androgen-dependent abnormalities and diseases
Inhibitors of 5α-reductase can be therapeutically useful, while having the advantage of allowing testosterone to maintain certain normal testicular functions, sexual behaviour, and muscle growth

a specific nuclear androgen receptor (AR). The DHT-AR complex, apparently in conjunction with other chromosomal proteins, then regulates the synthesis of specific RNA and modulates cellular activities and organ functions. This research and that of other investigators also showed that mutations in the genes for 5α-reductase or the AR are responsible for androgen-insensitivity syndromes in humans and animals.[3]

The molecular steps required for androgen action provide two effective methods for control of testosterone-regulated responses: (a) the use of a 5α-reductase inhibitor to suppress DHT production, and (b) the use of anti-androgens to block the interaction of DHT with the AR. Both methods are now being utilised as therapies for androgen-related disorders.[4] Synthetic inhibitors of the reductase have been prepared by pharmaceutical companies. The synthetic 4-aza-steroid, finasteride (Fig 2), is now prescribed as Proscar (Merck & Co., Whitehouse Station, New Jersey, US) for benign prostatic hyperplasia and as Propecia (Merck & Co., Whitehouse Station, New Jersey, US) for male pattern baldness. A number of natural compounds that inhibit 5α-reductase have been found in our laboratory. The first, identified in 1992, was γ-linolenic acid (GLA) (Fig 2), an essential fatty acid present in many plant oils, including evening primrose and borage oil, now being used as health food products. Conjugated fatty acids (glycerides or esters) are not active. γ-Linolenic acid is far more active than many dozens of other fatty acids tested and is active at concentrations lower than 5 μmol/L. We also found that certain green tea catechins (Fig 2) were also active 5α-reductase inhibitors. Catechin gallates, such as EGCG, (-)epicatechin-3-gallate (ECG), and (-)catechin-3-gallate are active at concentrations less than 5 μM. The gallate group is important for inhibitory activity. Non-gallated catechins, such as (-)epicatechin (EC) or EGC are not active. Gallic acid and the methyl ester of

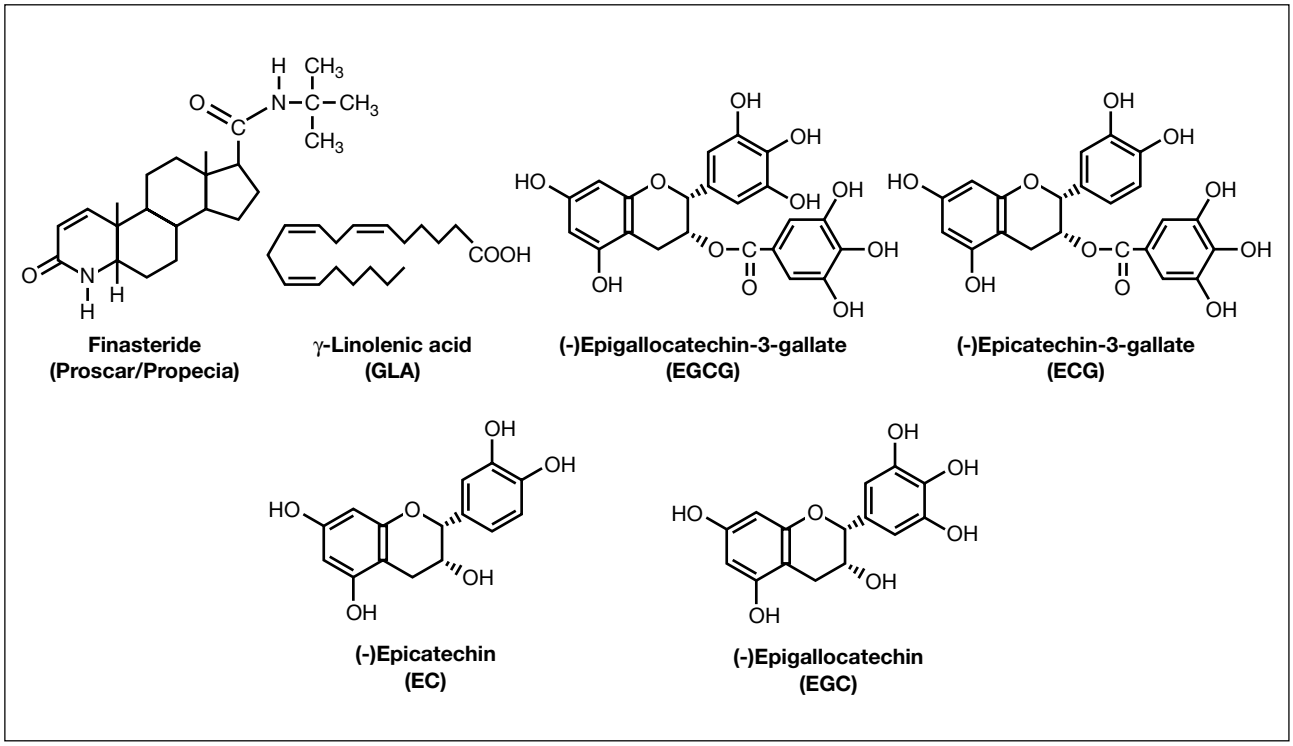

Fig 2. Chemical structures of some 5α-reductase inhibitors (finasteride, γ-linolenic acid, (-)epigallocatechin-3-gallate, and (-)epicatechin-3-gallate) and the inactive catechins, (-)epicatechin and (-)epigallocatechin

gallic acid are not active, suggesting that catechin structure, especially its trihydroxy phenol moiety, is important for inhibitory activity against 5α-reductase.

The biological activity of GLA and EGCG has been tested in vivo using the flank organs of male hamsters as an animal model.[5,6] When hamsters are castrated, flank organ growth is suppressed. Topical application of testosterone on the flank organ stimulates the growth of the organ, but this growth is effectively suppressed by topical application of GLA or EGCG. Sebum production from the male forehead skin has also been shown to be inhibited by topical application of GLA or EGCG.

Two isozymes of 5α-reductase have been identified. The specific roles of the individual isozymes are not well understood. Finasteride is a selective inhibitor of the type 2 isozyme of 5α-reductase. γ-Linolenic acid can inhibit both the type 1 and type 2 isozymes, whereas EGCG and ECG inhibit only the type 1 isozyme of 5α-reductase at concentrations less than 30 mmol/L. The biomedical utility of this difference is not clear at this time. The ability of GLA and EGCG to interfere, in vivo, with DHT-dependent sebum production and flank organ growth suggests that these natural substances, as well as other natural 5α-reductase inhibitors, may be useful for the therapeutic treatment of skin problems, including acne and baldness.

Androgen suppression of prostate tumours

Prostate cancer is androgen-dependent initially. Hormonal therapy, pioneered by Charles Huggins 60 years ago using castration, and more recently, anti-androgens (chemical castration), has been the standard therapy. Although more than 70% of patients benefit from this therapy, prostate cancer recurs in most patients within 1 to 3 years, with the development of tumours that do not need androgen for growth. Many patients die as a result of these androgen-independent cancers, due to a lack of effective therapies.

Umekita et al[7] and Kokontis and Liao[8,9] have established that androgen-independent human prostate cancer cells (LNCaP 104-R) can develop from a clonally derived androgen-dependent cancer cell line (LNCaP 104-S) during long-term (1-3 years) culture in androgen-depleted media. This transition to androgen independence is accompanied by dramatically increased AR expression without gene amplification or new mutations in the AR gene. Surprisingly, the growth of these cells is inhibited by physiological levels of androgens (Fig 3).

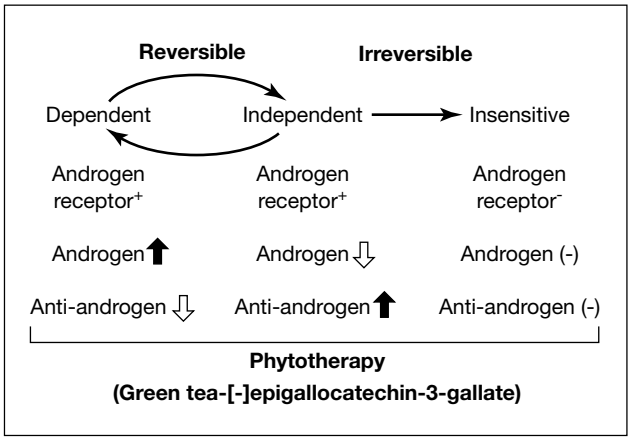

Fig 3. Tumour cell progression and its reversibility in human prostate LNCaP cells in culture and in mice
Androgen-dependent LNCaP 104-S cells can progress to androgen-independent LNCaP 104-R cells, containing increased levels of androgen receptor and proliferatively suppressed by androgen. Testosterone suppression is inhibited by 5α-reductase inhibitors or anti-androgens that, thus, stimulate proliferation of LNCaP-104-R cells. LNCaP 104-R cells can revert back to androgen-dependent LNCaP 104-S cells that can again be proliferatively stimulated by androgen and inhibited by anti-androgen. LNCaP 104-R cells may progress irreversibly to cancer cells that are androgen receptor negative and, hence, are insensitive to 5α-reductase inhibitors, androgens, or anti-androgens. At any stage of prostate cancer progression, (-)epigallocatechin-3-gallate seems to be effective in suppression of prostate cancer

These androgen-independent prostate cancer cells grow well as tumours in castrated athymic mice, but not in normal athymic male mice. Adminstration of androgen to castrated mice prevents tumour growth and suppresses prostate tumours already present in these animals. The 5α-reductase inhibitor, finasteride (Proscar) or anti-androgens, such as Casodex (Astra Zeneca Pharmaceuticals, Wilmington, Delaware, US), block the repressive effect of testosterone on these xenografts and stimulate tumour growth, suggesting that the growth suppression requires conversion of testosterone to DHT and binding of DHT to AR. If testosterone can suppress prostate cancer growth at this stage of progression in patients, the use of these drugs may enhance the growth of prostate cancers. The use of anti-androgenic compounds, either herbal medicine or synthetic drugs, therefore, may be detrimental to patients with androgen-independent prostate cancer.

(-)Epigallocatechin-3-gallate suppression of prostate and breast tumours

To examine the ability of green tea to control cancer growth, we injected purified catechins intraperitoneally (IP) into tumour-bearing mice. Human prostate cancer cell lines, LNCaP 104-S (AR positive and androgen-dependent), LNCaP 104-R (AR positive, androgen-repressive), and PC-3 (AR negative and androgen-

insensitive) inoculated subcutaneously into nude mice, produce prostate tumours. Green tea EGCG, injected IP, significantly inhibited the growth and rapidly (in 1 week) reduced the size of all types of human prostate tumours in athymic mice. Structurally related catechins, such as ECG, that differ from EGCG in only one of the eight hydroxyl groups, were totally inactive. (-)Epicatechin and EGC were similarly inactive.[10]

Since both androgen-dependent and androgen-independent prostate tumours respond to tumour suppression by EGCG (Fig 3), EGCG action does not appear to be related to the modulation of androgen activity. In addition, the growth of human breast tumours in nude mice, produced by the human breast cancer cell line MCF-7 cells, was also inhibited during the first week of IP injection of EGCG. Whether EGCG can control prostate and breast cancers in humans has yet to be studied. The clinical incidence of metastatic prostate and breast cancer varies considerably between countries (low in Japan and high in the US). If consumption of green tea beverage is related to this difference, EGCG may play an important role in preventing the progression or metastasis of cancer cells.[10]

(-)Epigallocatechin-3-gallate modulation of food intake and obesity

Green tea beverage is often considered beneficial for maintaining an appropriate body weight. Clear scientific evidence, however, has not been available until recently. We have found that EGCG, given to rats by IP injection, could reduce body weight by about 20% to 30% within 2 to 7 days. Other structurally related catechins, such as EC, EGC, or ECG are not effective at the same dose. The body weight loss is reversible; when EGCG administration is stopped, animals regain body weight. Reduction of body weight seems to be due to EGCG-induced reduction in food intake. The loss of appetite might involve neuropeptide(s) other than leptin, since EGCG is effective in reducing body weight of lean and obese (leptin-receptor defective) female and male rats.[11,12]

Many hormones, including cholecystokinin, glucagon-like peptide-1, glucagon, substance P, somatostatin, and bombesin have been reported to inhibit food intake. It has also been reported that plasma cholecystokinin levels are elevated in rats given a diet supplemented with tea polyphenols. Further study is required to determine whether the expression of other hypothalamic or gastrointestinal neuropeptide genes controlling appetite are altered by EGCG, possibly explaining the effect of EGCG on food intake.

Orally administered EGCG is not as effective as IP injected EGCG. This is probably due to poor absorption or degradation of EGCG in the intestine and other organs. It is possible, however, that long-term oral use of a moderate amount of green tea beverage or EGCG-containing drinks may mimic the effects of IP injected EGCG. Since EGCG can also selectively reduce body fat accumulation, EGCG may be potentially useful for the treatment of obesity.

(-)Epigallocatechin-3-gallate modulation of endocrine systems

In order to fully investigate the health benefits of tea consumption, it is important to study the effects of green tea catechins on endocrine systems. Recent work has shown that rats injected IP with EGCG demonstrate significant changes in various endocrine parameters. After 7 days of IP treatment with EGCG, circulating levels of testosterone are reduced by approximately 75% in male rats and 17β-estradiol levels by 34% in female rats. In male rats, the weight of androgen-sensitive organs, such as the ventral prostate, seminal vesicles, and the coagulating and preputial glands, are reduced by 50% to 70% after EGCG treatment. In female rats, the weight of oestrogen-sensitive organs, such as the uterus and ovary, is reduced by approximately 50% after EGCG treatment. These changes in the weight of sexual organs are catechin-specific, with EGCG showing the largest effect. The effect of EGCG on prostate and uterine weight loss is due to reduced sex hormone levels, and not a direct effect of EGCG. The organ weight loss is completely reversed with external supply of sex hormones.[11]

With male and female rats treated with EGCG for 7 days, the serum level of luteinising hormone (LH) is significantly reduced (by 40% to 50%), suggesting that low LH production led to the reduced blood levels of sex hormones. In both male and female rats, 7 days of EGCG treatment causes significant reductions in blood levels of leptin, insulin growth factor-1 and insulin. The effect of EGCG on these peptide hormones is not seen with structurally similar catechins, EC, EGC, or ECG, at an equivalent dose.

In EGCG-treated male rats, the serum level of protein, fatty acids, and glycerol are not altered, but significant reductions in serum glucose (-32%), lipids (-15%), triglycerides (-46%), and cholesterol (-20%) are observed. Based on proximate composition analysis, rats treated daily with EGCG for 7 days have no change in percent water and protein content, a moderate decrease in carbohydrate content, but a very

large reduction in fat content, decreasing from 4.1% in controls to 1.4% in the EGCG-treated group. Within 7 to 8 days, EGCG treatment decreases subcutaneous fat by 40% to 70%, and abdominal fat by 20% to 35% in male rats.[11]

Conclusion

Green tea beverage originated many thousands of years ago as a medicinal tonic. Although the historical use of folk remedies does not prove their medical usefulness, recent scientific evidence seems to support the contention that green tea catechins are medically valuable. However, it is important to also consider potential adverse effects accompanying the use of green tea or catechins. For example, any effects on endocrine systems may have serious consequences in pregnant women and young children, for example.

While the effects of IP injection of EGCG on body weight loss, hormone level changes, food intake, and tumour suppression can be observed, these effects are not present when the same amount of EGCG is given orally. This may be due to inefficient absorption of EGCG, or metabolism in the digestive tract. This suggests that the effects of IP administration of EGCG are not due to interaction of EGCG with food, or to EGCG action within the gastrointestinal tract.

Although oral administration of EGCG is less effective, long-term oral consumption of green tea or EGCG-containing extracts may mimic some of the acute EGCG effects seen by IP administration of EGCG in animals, and may be beneficial to health. Based on pharmacokinetic studies of EGCG after oral consumption of green tea, a daily use of green tea beverage equivalent to 20 g or more of dried green tea would be required to show an effect in humans.

Historical accounts suggest androgen may be the first natural medicine used in purified form for therapy. It provides the best example that traditional Chinese medicines are not necessarily effective only in combination. Unfortunately, many Chinese medicine researchers and practitioners are not aware of this great discovery and still support strongly an anti-molecular approach, discouraging isolation of pure substances for treatment. It is time to correct this misconception.

Acknowledgement

The studies in this paper were supported by US NIH Grants AT000850 and CA-58073.

References

1. Liao S. Putting science into herbal medicine. Bio/Pharma Quarterly 1999;5:2-3.
2. Liao S, Kao YH, Hiipakka RA. Green tea: biochemical and biological basis for health benefits. Vitam Horm 2001;62:1-94.
3. Hiipakka RA, Liao S. Molecular mechanism of androgen action. Trends Endocrinol Metab 1998;9:317-24.
4. Liao S. Androgen and control of prostate cancer growth. Clin Endocr 2000;48:5-15.
5. Liang T, Liao S. Growth suppression of hamster flank organs by topical application of gamma-linolenic and other fatty acid inhibitors of 5alpha-reductase. J Invest Dermatol 1997;109: 152-7.
6. Liao S, Lin J, Dang MT, et al. Growth suppression of hamster organs by topical application of catechins, alizarin, curcumin, and myristoleic acid. Arch Dermatol Res 2001;293:200-5.
7. Umekita Y, Hiipakka RA, Kokontis JM, Liao S. Human prostate tumour growth in athymic mice: inhibition by androgens and stimulation by finasteride. Proc Natl Acad Sci USA 1996;93:11802-7.
8. Kokontis JM, Liao S. Molecular action of androgen in the normal and neoplastic prostate. Vitam Horm 1999;55:219-307.
9. Kokontis JM, Hay N, Liao S. Progression of LNCaP prostate tumour cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip 1 in androgen-induced cell cycle arrest. Mol Endocrinol 1998;12:941-53.
10. Liao S, Umekita Y, Guo J, Kokontis JM, Hiipakka RA. Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate. Cancer Lett 1995;96:239-43.
11. Kao YH, Hiipakka RA, Liao S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 2000;141:980-7.
12. Kao YH, Hiipakka RA, Liao S. Modulation of obesity by a green tea catechin. Am J Clin Nutr 2000;72:1232-4.