

Targeting Multiple Signaling Pathways as a Strategy for Managing Prostate Cancer: Multifocal Signal Modulation Therapy

Mark F. McCarty

The aberrant behavior of cancer reflects upregulation of certain oncogenic signaling pathways that promote proliferation, inhibit apoptosis, and enable the cancer to spread and evoke angiogenesis. Theoretically, it should be feasible to decrease the activity of these pathways—or increase the activity of pathways that oppose them—with noncytotoxic agents. Since multiple pathways are dysfunctional in most cancers, and cancers accumulate new oncogenic mutations as they progress, the greatest and most durable therapeutic benefit will likely be achieved with combination regimens that address several targets. Thus, a multifocal signal modulation therapy (MSMT) of cancer is proposed. This concept has already been documented by researchers who have shown that certain combinations of signal modulators—of limited utility when administered individually—can achieve dramatic suppression of tumor growth in rodent xenograft models. The present essay attempts to guide development of MSMTs for prostate cancer. Androgen ablation is a signal-modulating measure already in standard use in the management of delocalized prostate cancer. The additional molecular targets considered here include the type I insulin-like growth factor receptor, the epidermal growth factor receptor, mammalian target of rapamycin, NF- κ B, hypoxia-inducible factor-1 α , hsp90, cyclooxygenase-2, protein kinase A type I, vascular endothelial growth factor, 5-lipoxygenase, 12-lipoxygenase, angiotensin II receptor type 1, bradykinin receptor type 1, c-Src, interleukin-6, ras, MDM2, bcl-2/bcl-xL, vitamin D receptor, estrogen receptor- β , and PPAR- γ . Various nutrients and phytochemicals suspected to have potential utility in prostate cancer prevention and therapy, but whose key molecular targets are still unknown, might reasonably be incorporated into MSMTs for prostate cancer; these include lycopene, selenium, green tea polyphenols, genistein, and silibinin. MSMTs can be developed systematically by testing various combinations of signal-modulating agents, in concentrations that can feasibly be achieved and maintained clinically, on human prostate cancer cell lines; combinations that appear promising can then be tested in xenograft models and, ultimately, in the clinic. Some signal modulators can increase response to cytotoxic drugs by upregulating effectors of apoptosis. When MSMTs fail to raise the spontaneous apoptosis rate sufficiently to achieve tumor stasis or regression, incorporation of appropriate cy-

totoxic agents into the regimen may improve the clinical outcome.

Keywords: prostate cancer; signal modulation; IGF-I, TOR; hsp 90; cyclooxygenase-2; c-Src; NF- κ B

Signal Modulation Therapy for Cancer Control

Malignant cells are characterized by the upregulation or constitutive activation of multiple signaling pathways that promote proliferation, inhibit apoptosis, and enable the cells to invade and migrate through tissues while evoking angiogenesis. Downregulation or loss of proteins and pathways that oppose these behaviors is also commonly seen. Theoretically, once these upregulated oncogenic pathways have been identified in a cancer, it should prove feasible to achieve partial inhibition of these pathways with tolerable doses of several noncytotoxic pharmaceutical agents, thereby suppressing the malignant behavior of the cancer and tipping the balance of proliferation/apoptosis toward the latter; in some favorable cases, tumor stasis or even regression might be achievable. The likelihood that any single drug would have a “magic bullet”-like impact on a cancer is lessened by the fact that over the course of their evolution, cancers acquire multiple oncogenic mutations that are often functionally redundant; thus, inhibiting any single upregulated pathway may have only a modest impact on tumor behavior. Even when a single drug does initially have striking activity (eg, androgen antagonists in early prostate cancer), it is common for cells in the regressing cancer to acquire additional mutations that render them resistant to the functional impact of the original therapy, leading to tumor recurrence. Thus, a plan of attack

MFM is at NutriGuard Research, Encinitas, California.

Correspondence: Mark F. McCarty, NutriGuard Research, 1051 Hermes Ave., Encinitas, CA 92024. E-mail: mccarty@pantox.com.

DOI: 10.1177/1534735404270757

targeting multiple signaling pathways that are prone to upregulation in a given type of cancer seems to offer the best prospects for achieving long-term control of that cancer.

Although these considerations seem virtually self-evident, there appear to be few studies or reviews in the biomedical literature that enunciate or illustrate this strategy. Most studies focus on a single pharmaceutical agent—drug, nutrient, or phytochemical—that addresses a single target. Such studies are essential, as it is necessary to define dosage schedules that can achieve meaningful inhibition (or activation) of the targeted pathway without entailing unacceptable side effects. Yet clinical studies that evaluate the efficacy of a single agent are apt to achieve rather disappointing results—in most cases, retardations of tumor growth and spread that are inherently difficult to verify, rather than the objective remissions or long-term tumor stasis that are the traditional goals of cytotoxic cancer therapy. This is only to be expected and should not give rise to discouragement. The clinical payoff will come when it becomes feasible to administer several different agents simultaneously, targeting multiple oncogenic pathways in the cancer.

The best illustration of this principle of which I am aware has been provided by Tortora and colleagues.¹ These workers tested the efficacy of an inhibitor of the epidermal growth factor receptor (Iressa), a cox-2 inhibitor, and an orally administrable antisense agent targeting protein kinase A type I, administered separately or in combination, against human breast and colon cancer cells *in vitro* as well as human colon cancer xenografts implanted in nude mice. In the cell culture studies, they found that low concentrations of the agents that, given singly, would suppress cell colony growth rate by no more than 15% to 20% could achieve a virtually complete suppression of colony growth when all 3 agents were administered simultaneously. Analogous findings were reported in the xenograft model. The cox-2 inhibitor did not retard growth of the tumors, and no more than a 15-day delay in tumor growth was achieved with either Iressa or the antisense agent administered singly. In contrast, the triple therapy achieved a profound suppression of tumor growth, with 60% of the animals having no histological evidence of cancer 5 weeks after therapy had been terminated. In tumor biopsies obtained 32 days after tumor transplantation, the triple therapy was associated with an 80% reduction in microvessel density and an absence of detectable vascular endothelial growth factor (VEGF). The therapy appeared to be well tolerated, as no weight loss or other signs of toxicity were noted.

It is worthwhile to quote the concluding paragraph of this insightful article in full:

There is growing consensus that the blockade of key mitogenic and angiogenic molecules may be a successful strategy for both chemoprevention and long-term control of cancer. Agents suitable for these purposes should have mild and differing toxicity patterns and simple administration routes. In agreement with this approach, we have shown that using oral administration of different noncytotoxic selective agents targeting molecules involved in mitogenic signaling and angiogenesis, it is possible to achieve an antitumor and antiangiogenic effect. Because all of the agents used in our study are under clinical development, we believe that this study provides a strong rationale to translate this feasible therapeutic strategy into a clinical setting.^{1(p1571)}

The strategy of treating cancer by addressing key signaling pathways with noncytotoxic agents might reasonably be called “signal modulation therapy.” The strategy of targeting several pathways simultaneously to achieve additive or synergistic efficacy could then be described as multifocal signal modulation therapy (MSMT).

Focus on Prostate Cancer

The intent of this article is to provide a concise review of metabolic pathways in prostate cancer whose upregulation (or downregulation) commonly promotes malignant behavior and that thus might be rationally targeted in multifocal signal modulation therapies of the sort envisioned above. Pharmaceutical agents—or lifestyles—that might feasibly be used to address these pathways are also considered.

Androgen-ablation strategies—while they clearly can be categorized as signal modulation therapies—will not be discussed here, as the utility of this approach, as well as its limitations, are already well established. When local control of prostate cancer fails, androgen antagonism remains the standard therapy. Unfortunately, even though most prostate cancers respond initially, selection for androgen-independent cells almost always leads to cancer recurrence; amplifications or mutations of the androgen receptor gene enable the receptor to be activated by nonandrogenic steroids and/or growth factors, while increased expression of various androgen receptor coactivators can promote adequate androgen receptor activity in a low androgen environment.² Recurrent prostate cancers that have achieved androgen independence are notoriously difficult to treat; cytotoxic therapies, if helpful at all, usually provide no more than palliative benefit. Thus, a new approach to the management of

prostate cancers that have spread beyond the prostate and achieved androgen independence is desperately needed.

It should be noted that some of the better tolerated strategies envisioned below might reasonably be used in early-stage prostate cancer (the “watchful waiting period”) or for primary prevention of this disease.

Insulin-like Growth Factor (IGF) Receptor–Type 1

Excessive activation of tyrosine kinase growth factor receptors, by stimulating the PI3K-Akt-mTOR-p70S6k and Ras-Erk1/2 pathways, works in a variety of complementary ways to promote proliferation, inhibit apoptosis, boost angiogenic capacity, and stimulate migration/invasion.³⁻¹⁶ Furthermore, in prostate cancers that have evolved to androgen independence, growth factor receptors may have the remarkable ability to activate androgen receptors in the absence of androgens.¹⁷ The type I IGF receptor (IGFR1) and the receptor for epidermal growth factor (EGFR) are known to play key roles in the evolution of prostate cancer. IGFR1 is now drawing particular attention in light of prospective epidemiological evidence that relatively high serum levels of IGF-I, and relatively low serum levels of its functional antagonist IGFBP-3, are associated with increased risk for advanced prostate cancer.^{18,19}

Recent studies indicate that expression of IGFR1 in primary prostate cancers tends to be upregulated as compared with benign prostate epithelium.²⁰⁻²⁴ High expression of this receptor tends to be maintained in metastatic lesions, although a subset of these lesions is characterized by a decrease in IGFR1 expression coupled with a loss of phosphatase and tensin homolog (PTEN) activity; presumably, the chronic upregulation of PI3K-Akt-mTOR signaling stemming from the loss of PTEN can compensate for a reduction in IGF signaling.^{22,25} Prostate cancers commonly produce IGF-II (rather than IGF-I), giving rise to an autocrine stimulation loop that helps to sustain growth even when malignant prostate cells are cultured in serum-free medium.^{23,26-30} Conversely, antisense suppression of IGFR1 expression slows proliferation and boosts apoptosis in cultured prostate cancer cells.^{20,24} IGF-II production is typically higher in advanced metastatic lesions and high Gleason score cancers, as opposed to primary cancers and those with low Gleason scores.^{23,28-30}

Benign prostate epithelium, as well as prostate cancers, produce the full range of IGF binding proteins, excepting IGFBP-1.^{27,29} Normal epithelium, however, produces the IGFBP-1-related protein, the expression

of which is usually lost in cancers; this protein is induced by IGF-I in healthy epithelium and thus appears to act as a feedback mechanism for controlling prostate growth.^{31,32} In advanced prostate cancers, IGFBP-2 production is usually upregulated, whereas IGFBP-3 is downregulated.^{28,33,34} Remarkably, IGFBP-2, which suppresses IGF-driven growth in healthy prostate epithelium, acts to accelerate growth in prostate cancer cells; the mechanism of this latter effect is obscure.^{33,34} Systemic levels of IGFBP-2 are usually elevated in advanced prostate cancer and tend to correlate with prostate-specific antigen (PSA) levels and cancer aggressiveness.²⁸ The downregulation of IGFBP-3 in prostate cancers is also of functional significance; not only does this protein oppose IGF-driven growth by binding IGFs, but it also exerts a direct growth-inhibitory effect, likely by activating transforming growth factor (TGF)- β receptors.³⁵⁻³⁸ Although prostate cancers do not produce IGFBP-1, exogenous IGFBP-1, which has high affinity for both IGF-I and IGF-II, suppresses the growth of prostate cancer cells *in vitro* by intervening in the IGF-II/IGFR1 autocrine loop.²⁶

Although most prostate cancers generate their own IGF-II, systemic IGFs have the potential to contribute to IGFR1 activation in prostate cancers. Conversely, serum IGFBP-1 and IGFBP-3 have the potential to inhibit prostate cancer growth. These considerations are of importance in light of the fact that lifestyle measures can influence hepatic production of both IGF-I and IGFBP-1. Barnard and colleagues have recently demonstrated that a very-low-fat whole-food diet, coupled with daily walking exercise—the classical “Pritikin regimen”—can markedly influence these parameters.³⁹ In 14 male volunteers participating in the Pritikin regimen for 11 days, serum IGF-I fell by an average of 20%, whereas IGFBP-1 increased by 53%. In 8 subjects of comparable age who had engaged in this program for an average of 14 years, IGF-I was 55% lower and IGFBP-1 150% higher relative to initial values in the first group. The regimen did not influence serum IGFBP-3 in either group. The researchers then assessed the ability of pre- and postregimen serum from the experimental group, as well as serum from the 8 “veteran” subjects, to support the growth of androgen-sensitive LNCaP human prostate cancer cells *in vitro*. As compared to cells incubated with preregimen serum, those grown with postregimen and veteran serum experienced 30% and 44% reductions in growth rate, respectively. Moreover, apoptosis was markedly upregulated in cells grown with the veteran serum. Adding IGF-I to the postregimen serum reversed the favorable effects of this serum on LNCaP proliferation and apoptosis; conversely, adding

IGFBP-1 to the prerigimen serum suppressed proliferation and boosted apoptosis.⁴⁰

In rodents, dietary protein intakes that are low in quantity and/or quality downregulate hepatic IGF-I production while upregulating that of IGFBP-1.⁴¹⁻⁴³ Allen and colleagues have reported that, as compared to omnivores or ovolactovegetarians, serum IGF-I is about 13% lower in vegans, whereas IGFBP-1 averaged 40% higher.⁴⁴ Since the fat intake of the vegans was about 30% of calories, these findings could not be attributed to the fat restriction that is crucial to the Pritikin regimen but rather reflected avoidance of animal protein and possibly saturated fat. The merit of a very-low-fat (10% fat calories) intake in this regard presumably reflects the insulin-sensitizing impact of such diets on skeletal muscle⁴⁵⁻⁴⁷; the resulting downregulation of insulin secretion would be expected to have a direct influence on hepatic production of both IGF-I and IGFBP-1.^{48,49}

Conflating the findings of the Barnard and Allen studies, it is reasonable to suggest that a very-low-fat, whole-food vegan diet, coupled with regular aerobic training, would be expected to downregulate the IGF-I activity of serum, thus aiding prevention and control of IGF-responsive prostate cancers. Indeed, Ornish is in the process of assessing the efficacy of such a regimen (to which he adds stress-reduction techniques) in patients with early-stage prostate cancer; preliminary findings indicate that PSA values tend to fall modestly in patients embarking on this regimen.⁵⁰

These findings may be highly germane to the very low rates of prostate cancer mortality enjoyed by rural Asian and African societies as long as they adhere to their traditional low-fat quasi-vegan diets.^{51,52}

Drug-mediated reduction of hepatic IGF-I production may also be feasible. In women, the recently approved selective estrogen response modulator raloxifene is reported to decrease serum levels of IGF-I while increasing those of IGFBP-3.⁵³⁻⁵⁶ The impact of raloxifene on systemic IGF-I activity in men has received little study, but in 8 acromegalic men, 60 mg raloxifene twice daily was associated with an average reduction of 16% in serum IGF-I; since growth hormone levels did not decrease, this effect presumably reflects decreased hepatic responsiveness to GH.⁵⁷ Moreover, there is evidence that raloxifene may have a direct impact on prostate cancers; in vitro, raloxifene markedly enhanced apoptosis in androgen-dependent (LNCaP) as well as androgen-independent (PC-3, PC-3M, DU145) prostate cancer cell lines.^{58,59} The mechanism of this latter effect remains obscure but might reflect interaction with β -type estrogen receptors expressed in many prostate cancers.⁶⁰

Epidermal Growth Factor (EGFR)

Most human prostate cancers express the EGFR (erbB1/HER-1) and show a proliferative response to the range of ligands for this receptor, including EGF, tumor necrosis factor (TNF)- α , amphiregulin, and heparin-binding EGF.⁶¹⁻⁶⁸ Furthermore, most advanced, androgen-independent prostate cancers express TGF- α , giving rise to an autocrine loop that promotes growth, survival, and migration.^{62,63,65,69,70} Some, though not all, studies indicate that in androgen-dependent tumors, androgens increase the expression of EGFR^{67,71-74}; however, the growth-promoting effects of androgens may not be mediated solely by EGFR. EGFR expression in androgen-independent prostate cancers is usually higher than in androgen-dependent cancers, suggestive of a compensatory upregulation. High-level expression of EGFR has been associated with poor clinical prognosis.⁷⁵

EGFR may play a central coordinating role in the regulation of prostate cancer mitosis that is not yet understood. Thus, the ability of either IGF-I or protein kinase A to activate Erk2 in the androgen-insensitive DU145 cell line is abrogated by concurrent inhibition of EGFR.⁷⁶ This receptor also seems to play a role in the transmission of prometotic signals generated by angiotensin II, at least in breast cancer.⁷⁷

Pharmaceuticals that target EGFR include antibodies directed against the receptor ectodomain (C-225/Erbitux) and small molecules that are receptor-specific tyrosine kinase inhibitors, most notably ZD1839 (Iressa).⁷⁸⁻⁸⁰ These have already achieved clinical approval for treatment of colorectal cancer and non-small-cell lung cancer, respectively. Iressa is of particular interest for long-term therapy, as it is orally administrable and reasonably well tolerated in clinical doses (controllable diarrhea being the most common side effect). A recent study shows that Iressa inhibited EGF-stimulated proliferation in a wide range of established prostate cancer cell lines as well as in primary cell cultures from prostate tumors; the concentration that inhibited EGF-stimulated proliferation by 50% was usually less than 1 μ M.⁶⁶ The impact of Iressa on cell proliferation in the absence of added EGF was much more modest, suggesting that the TGF- α autocrine loop usually does not have a potent effect, at least in vitro. In vivo, C-225 administration has been shown to slow the progression of androgen-independent human prostate cancers DU145 and PC-3 implanted in nude mice.⁷⁵

Mimeault and colleagues have reported that inhibition of protein kinase A activity (with RP-cAMPs) synergizes with EGFR inhibition in suppressing EGF- or serum-driven proliferation in several human prostate

cancer cell lines in vitro.⁸¹ These findings are reminiscent of the work of Tortora cited above. Conversely, the protein kinase A (PKA) activator forskolin has been reported to potentiate the activating impact of EGF on Erk2, for reasons that remain unclear.⁷⁶

Agents that downregulate EGFR expression might have some role in prevention or therapy of prostate cancer. In the transgenic adenocarcinoma of mouse prostate model, dietary genistein downregulates prostate expression of EGFR.⁸² Whether this finding has any relevance to humans is not clear, although recent clinical studies with supplemental genistein suggest that this agent may somehow slow the increase of PSA in early prostate cancer.^{83,84} Another intriguing recent study demonstrates that anandamide, acting via cannabinoid CB(1) receptors, suppresses expression of EGFR by several human prostate cancer cell lines.⁸⁵ Of related interest is a report that tetrahydrocannabinol induces apoptosis dose dependently in PC-3 cell cultures.⁸⁶

Various phytochemicals, including curcumin, grape seed extract, silibinin, and resveratrol, have been reported to block either the activation or downstream effects of EGFR in human prostate cancer cell lines in vitro.^{87,90} Unfortunately, the concentrations of these agents used in these studies were far beyond those that can be achieved and maintained by oral administration in humans; thus, these results are of dubious clinical relevance.

Mammalian Target of Rapamycin

The kinase mammalian target of rapamycin (mTOR) is a branch point at which the PI3K-Akt signaling pathway diverges, phosphorylating 4E-BP1 and activating p70S6 kinase. These actions upregulate protein synthesis, supporting the cell growth required prior to mitosis, while selectively stimulating the translation of proteins that support cell cycle progression from G1 to S phase (including cyclin D1), survival, and angiogenesis (notably HIF-1 α , a transcription factor that promotes expression of VEGF).^{91,92} p70S6 kinase also suppresses apoptosis by inhibitory phosphorylation of BAD.⁵ mTOR may be the most proximal point at which the PI3K-Akt signaling pathway can be inhibited without compromising insulin function.

mTOR is susceptible to inhibition by the natural product rapamycin (also known as sirolimus), derived from a strain of *Streptomyces* discovered on Easter Island.^{93,94} The properties of rapamycin were explored when this agent proved to have immunosuppressive and cancer-retardant activity in screening studies. Rapamycin and certain derivatives thereof—some of which are orally administrable—are currently in use for prevention of graft rejection; however, their clinical

utility in cancer therapy is also being explored.⁹¹ Rapamycin is relatively well tolerated for an immunosuppressive agent: it does not induce nausea and vomiting, and in standard clinical doses, it does not promote opportunistic infections. Skin toxicity—small papules and folliculitis—may be the most common side effect.

The impact of rapamycin on prostate cancer has so far received little attention. This agent has been shown to inhibit the growth of LNCaP and PC-3 human prostate cancer cell lines in vitro (in concentrations less than 100 nM⁹⁵) and, in nude mice, to reverse the resistance of PC-3 to doxorubicin.⁹⁶ In other types of cancer, cells deficient in the PTEN suppressor gene (a phosphatase that reverses the impact of PI3K on phospholipids) are especially sensitive to the cell cycle-retardant impact of rapamycin, reflecting the fact that mTOR is highly active under these circumstances; since most prostate cancers have lost 1 copy of the PTEN gene, and many advanced cancers have lost both, it is reasonable to predict that a high proportion of human prostate cancers will be rapamycin responsive.⁹⁷⁻⁹⁹

Nuclear Factor- κ B (NF- κ B)

Constitutive activation of the transcription factor NF- κ B has been observed in a high proportion of androgen-independent prostate cancers.¹⁰⁰⁻¹⁰² Presumably, the ability of NF- κ B to promote transcription of the prominent antiapoptotic protein Bcl-2¹⁰³ aids the survival of cells that otherwise would be at risk owing to loss of androgen activity. This constitutive activation reflects increased activity of the I κ B kinase (IKK) complex, but why IKK is activated remains unclear.¹⁰² A report that dominant negative NF- κ B-inducing kinase (NIK) and tyrosine kinase inhibitors suppress the constitutively elevated NF- κ B activity in various prostate cancer cell lines suggests that NIK, possibly downstream from a tyrosine kinase, may mediate the constitutive activation of IKK.¹⁰⁴ Other factors suggested to play a role in the constitutive activation of NF- κ B in prostate cancer include 12-(S)-HETE, Id-1, bombesin, and RhoA.¹⁰⁵⁻¹⁰⁸

In addition to suppressing apoptosis, NF- κ B promotes malignant behavior in other ways: stimulating transcription of cell cycle progression factors (c-myc, cyclin D1), proteolytic enzymes (MMP-9, uPA), and angiogenic factors (VEGF, IL-8).^{102,109} Thus, it is not surprising that nuclear localization of NF- κ B in prostate cancer biopsies has been shown to correlate with poor clinical prognosis.¹¹⁰

A bewildering array of natural products, some of which have putative chemopreventive activity, are reported to inhibit constitutive and/or stimulated NF-

κ B activity in human prostate cancer cell lines in vitro: apigenin,¹¹¹⁻¹¹³ epigallocatechin-3-gallate,¹¹⁴⁻¹¹⁶ curcumin,^{117,118} inositol hexaphosphate,¹¹⁹ PC-SPES,¹²⁰ selenium,¹²¹ zinc,¹²² genistein,^{123,124} silibinin,¹²⁵ and indole-3-carbinol.¹²⁶ Few if any of these studies made an effort to use concentrations of the agent tested that could feasibly be achieved clinically via oral administration; thus, it is unclear whether any of these observations are clinically relevant. (Although the selenium study claims to use a concentration of selenium comparable to serum levels, this ignores the fact that the large majority of serum selenium is incorporated in protein-bound selenoamino acids that have no immediate metabolic availability.) Silibinin, which can suppress NF- κ B activity in DU145 cells in a concentration as low as 10 μ M¹²⁵—an effect reflecting direct inhibition of IKK α activity—may merit further examination in this regard since oral silibinin appears to have therapeutic efficacy in human liver disorders¹²⁷ and can slow the growth of DU145 xenografts in nude mice.^{128,129}

Since the transcriptional activity of NF- κ B hinges on proteasomal degradation of the inhibitory protein I κ B, proteasome inhibitors can be used to suppress NF- κ B activity. Thus, the proteasome inhibitor PS-341, now being developed as a cancer drug, has potential for treatment of prostate cancer and has been shown to induce growth arrest and apoptosis in LNCaP cells.¹³⁰ (Ironically, this effect likely reflects something other than NF- κ B suppression, inasmuch as the NF- κ B activity of LNCaP cells, an androgen-sensitive tumor, is said to be quite low.) Of related interest is a report that in a concentration of 10 μ M, the protease inhibitor AIDS drug saquinavir inhibits proteasome activity in a range of human prostate cancer cell lines in vitro, an effect associated with increased apoptosis.¹³¹

PS-341 is a boronic acid dipeptide with serine protease inhibitory activity.¹³² Boric acid per se can act as a serine protease inhibitor.¹³³ It has recently been reported that relatively modest dietary intakes of boric acid (1.7-9.0 mgB/kg/d) decrease the growth of LNCaP in nude mice and markedly decrease the expression of PSA by the tumor.¹³⁴ The latter observation was also made in the study with PS-341.¹³⁰ Could supranutritional doses of boric acid have proteasome-inhibitory activity? The impact of boron on other prostate cancer cell lines should be studied.

Copper depletion, using the well-tolerated copper-sequestering drug tetrathiomolybdate (TM), is now being studied as an angiostatic treatment for cancer.¹³⁵ There is recent evidence that the angiostatic impact of copper depletion is primarily attributable to suppression of NF- κ B activity in cancer cells and a consequent downregulation of tumor expression of various angiogenic factors.^{136,137} Thus, copper depletion markedly inhibited the growth of SUM149 human breast car-

cinoma in nude mice but did not inhibit the (much slower) growth of the same cancer cells that had been transfected with a dominant negative I κ B before transplantation. Preliminary copper depletion with TM was found to decrease subsequent tumor growth of Dunning rat prostate cancer by more than 50%.¹³⁸ It should be noted that TM, though difficult to obtain, is currently available as it is now the preferred agent for management of Wilson's disease. Theoretically, high-dose zinc could be used to achieve significant copper depletion,¹³⁹ and a previously cited report regarding a downregulatory impact of zinc on NF- κ B activation in vitro is of some interest in this regard.¹²² Moreover, zinc administered by osmotic pump has been shown to suppress growth of PC-3 cells in nude mice.^{140,141} Why copper depletion should influence NF- κ B activation remains mysterious, as the enzymes involved in activation of this transcription factor are not known to be copper dependent.

Hypoxia-Inducible Factor-1 α (HIF-1 α)

HIF-1 α , like NF- κ B, is a transcription factor that plays a key role in promotion of tumor angiogenesis. As its name implies, its activity is evoked by hypoxia, owing to the fact that its stability is substantially enhanced under hypoxic conditions. In cells that are well oxygenated, this protein has a short half-life; oxygen-dependent prolyl hydroxylations of HIF-1 α enable it to be ubiquitinated by the von Hippel Lindau factor (VHL) and quickly degraded in proteasomes.¹⁴² Hypoxia slows these prolyl hydroxylations, thereby prolonging the survival of HIF-1 α . Acting in concert with its constitutively expressed heterodimer partner HIF-1 β , HIF-1 α stimulates transcription of VEGF, matrix proteases, the glucose transporters GLUT1 and GLUT3, as well as a number of glycolytic enzymes; these adaptations aid survival of hypoxic cancer cells by promoting angiogenesis while boosting glycolytic ATP generation.¹⁴³⁻¹⁴⁵ Overexpression of HIF-1 α is common in cancers and may be largely responsible for the phenomenon of "aerobic glycolysis" described long ago by Warburg.¹⁴⁶ Not surprisingly, overexpression of HIF-1 α has been linked to poor prognosis in many types of cancer,¹⁴⁷⁻¹⁵⁷ albeit prostate cancer has not yet been investigated in this regard.

Expression of HIF-1 α protein has been found to be increased in prostate intraepithelial neoplasia and in prostate cancers, relative to the levels seen in healthy prostate epithelium and benign prostatic hyperplasia.¹⁵⁸⁻¹⁶¹ This is readily predictable, owing to the fact that both the PI3K-Akt-mTOR-p70S6 kinase pathway and Erk1/2 enhance the synthesis/activity of HIF-1 α . The former pathway accelerates the translation of HIF-1 α mRNA,^{162,163} whereas MAP kinases increase the

intracellular transport and transactivating activity of HIF-1 α .¹⁶⁴⁻¹⁶⁷ Transcription of the HIF-1 α gene can be stimulated by diacylglycerol-dependent isoforms of protein kinase C.¹⁶⁸ Modulation of these pathways has the expected impact on HIF-1 α in human prostate cancer cells.¹⁶⁹⁻¹⁷³ Gene amplification may contribute to high HIF-1 α expression in some prostate cancers.¹⁵⁹ In addition, mutations leading to altered structure of the HIF-1 α protein domain required for ubiquitinylation have been observed in 2 prostate cancers; the functional significance of these mutations awaits clarification.¹⁷⁴

The ubiquitous heat shock protein Hsp90 binds to HIF-1 α , and there is recent evidence that this interaction stabilizes the latter, preventing its ubiquitinylation and proteasomal degradation by VHL-independent mechanisms.¹⁷⁵ The Hsp90 antagonist geldanamycin, an antibiotic that inhibits Hsp90 function by suppressing its ATPase activity, disrupts the interaction between Hsp90 and HIF-1 α and presumably as a consequence accelerates proteasomal degradation of HIF-1 α under both hypoxic and normoxic circumstances in PC-3 and LNCaP cells *in vitro*.¹⁷⁶ The geldanamycin derivative 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) has been shown to inhibit growth of prostate cancer xenografts in nude mice, in doses that appeared to be well tolerated.¹⁷⁷ This effect may not solely reflect downregulation of HIF-1 α , as 17-AAG was shown to decrease tumor expression of other oncogenic proteins that are stabilized by Hsp90, including HER-2, Akt, and the androgen receptor. (Indeed, Workman notes that Hsp90 inhibitors can “block multiple mission critical oncogenic pathways in the cancer cell”¹⁷⁸; these considerations suggest that 17-AAG or other Hsp90 inhibitors may demonstrate versatile utility in MSMTs.) Accelerated degradation of the androgen receptor—an effect that potentially could aid control of both androgen-dependent and -independent tumors—has also been demonstrated in geldanamycin-treated LNCaP cells.¹⁷⁹ Another recent study reports an additive or synergistic interaction of 17-AAG and x-irradiation with respect to induction of growth delays in human prostate cancer spheroids *in vitro*.¹⁸⁰ 17-AAG is currently in clinical evaluation and is said to have shown an acceptable toxicity profile in phase I studies.¹⁸¹ Hepatotoxicity is dose limiting; this complication does not appear to be intrinsic to Hsp90 inhibition, as other inhibitors of this factor have not been hepatotoxic in rodents.

HIF-1 α can also be downregulated by measures that decrease the activity of either p70S6 kinase or Erk1/2. mTOR inhibitors and strategies that suppress the activity of tyrosine kinase hormone receptors can suppress p70S6 kinase activation. Erk1/2 activity can be

downregulated by decreasing the activity of tyrosine kinase receptors and/or protein kinase A. Cox-2 inhibition should be helpful with regard to the latter strategy (see below), and indeed, nonsteroidal anti-inflammatory drugs are reported to decrease HIF-1 α level and activity in PC-3 and DU145 cells.¹⁸²

Physiological levels of ascorbate and of iron are required for catalysis of the ubiquitinylation reactions that decrease HIF-1 α half-life.¹⁸³ However, there currently is no evidence that supplemental intakes of either of these nutrients can influence HIF-1 α activity in subjects not markedly deficient in them.

Suppression of HIF-1 α activity would appear to be a critical component of successful angiostatic regimens. Whether levels of HIF-1 α are elevated in a relatively normoxic tumor, the tumor hypoxia that will be the inevitable result of inhibiting the angiogenic response to that tumor will upregulate HIF-1 α activity, evoking a compensatory production of angiogenic factors while improving the ability of hypoxic cancer cells to maintain energy homeostasis. Once Hsp90 inhibitors are indeed approved for clinical use (as appears likely based on initial clinical assessments), they may become important adjuvants for angiostatic strategies such as copper depletion. A portion of the angiostatic activity of geldanamycin analogs may be attributable to a direct impact on endothelial cells.¹⁸⁴

Hsp90

Hsp90 merits further consideration here, in light of its versatile impact on oncogenic signaling pathways. In conjunction with other chaperone molecules, Hsp90 forms so-called “closed conformation” complexes with a number of client proteins; these complexes protect the client proteins from proteasomal degradation and in some instances enhance their metabolic activities. Hsp90 inhibitors such as geldanamycin analogs bind to Hsp90 in such a way as to prevent the formation of these closed conformation complexes, thereby destabilizing Hsp90 client proteins. A large number of enzymes are stabilized by Hsp90 complexes, including many that participate in oncogenic signaling.^{178,185} These include enzymes involved in tyrosine kinase signaling (IGFR1, ErbB2, VEGFR2, c-src), the mitogen-activated protein (MAP) kinase pathway (raf-1, B-raf, MEK), the PI3K pathway (PDK1, Akt), and NF- κ B signaling (the I κ B kinases). Other proteins stabilized by Hsp90 complexes include steroid receptors (including androgen and estrogen receptors), Stat3, p53, Apaf-1 (a mediator of mitochondrially triggered apoptosis), MDM2, and of course HIF-1 α . These proteins vary in the degree to which Hsp90 inhibition reduces their half-lives and activities; it should not be assumed that clinically tolerable concentrations of

Hsp90 inhibitors will have a functionally important impact on each of these proteins.

Remarkably, the affinity of Hsp90 for 17-AAG is far higher in cancer cells than in normal cells, apparently because, for reasons that remain obscure, most Hsp90 in transformed cells is associated with various co-chaperones that alter its configuration.^{185,186} (This phenomenon has been demonstrated with a number of rodent and human cancer cell lines, as well as clinical samples from breast and colon carcinomas, albeit prostate cancers were not examined in these studies.) Thus, the impact of geldanamycin analogs may be somewhat tumor selective. Other small-molecule inhibitors of Hsp90 are being developed; it is not yet known whether they will share this property of geldanamycin analogs.¹⁸⁵

The potential of Hsp90 inhibitors to address a range of oncogenic signaling pathways is so striking as to suggest that these agents might be viewed as MSMTs in and of themselves! In the words of Zhang and Burrows, "Targeting Hsp90, a central regulatory node in oncogenic signal transduction, is an exciting and novel approach to pleiotropic intervention in cancer."^{185(p196)}

Cyclooxygenase-2 (Cox-2)

Many, though not all,^{187,188} researchers have concluded that cox-2 is overexpressed in prostate cancers relative to normal prostate epithelium or benign prostatic hyperplasia.¹⁸⁹⁻¹⁹² This is likely a logical consequence of the fact that both NF- κ B and AP-1 (activated by Erk1/2) can bind the cox-2 promoter and boost transcription of the cox-2 gene.¹⁹³⁻¹⁹⁵ Numerous studies conclude that inhibition of cox-2 slows proliferation and/or upregulates apoptosis in both androgen-dependent and -independent human prostate cancer cell cultures¹⁹⁶⁻²⁰⁰; inhibition of prostate cancer xenografts in nude mice with cox-2 inhibitors has also been reported.^{198,201} A portion of the efficacy demonstrated in these studies, however, reflects extraneous effects of specific cox-2 inhibitors, independent of cox-2 inhibition, seen at high dose levels.²⁰²⁻²⁰⁵ Thus, celecoxib has often shown greater tumor-retardant activity than other cox-2 inhibitors that are comparably effective for cox-2 inhibition.

Relatively few studies have addressed the molecular mechanisms responsible for the growth-inhibitory effects of cox-2 inhibition on prostate cancer. Prostaglandin E2 (PGE2) is the predominant cyclooxygenase product of both healthy and malignant prostate tissue.²⁰⁶ The receptors for PGE2 expressed by human prostate cancer cell lines appear to be of the EP2 and EP4 subtypes,²⁰⁷ both of which are linked to stimulation of adenylate cyclase via G α_s .²⁰⁸ Thus, it is

reasonable to presume that downregulation of cAMP mediates the impact of cyclooxygenase inhibition on prostate cancer. Although cAMP inhibits MAP kinase activation in some cell types, it can potentiate growth factor-mediated activation of MAP kinase in malignant or rapidly proliferating cells that typically express the type I isoform of PKA (as discussed below)⁷⁶; thus, upregulation of MAP kinase activity may mediate at least some of the impact of cyclooxygenase activity on prostate cancer cells. PGE2 enhances the nuclear translocation and transcriptional activity of HIF-1 α in PC-3 cells, an effect mediated by activation of the MAP kinase pathway.¹⁶⁵ PGE2 and cAMP promote the transcription of VEGF and other angiogenic factors, likely reflecting the presence of HIF-1 and AP-1 response elements in the VEGF promoter.^{144,209-211}; they also boost expression of the key survival factor Bcl-2.²¹² The cox-2 inhibitor NS398 decreases expression of bcl-2 protein in LNCaP cells¹⁹⁶ and suppresses the hypoxia-induced upregulation of VEGF in a highly metastatic subline of PC-3 cells; this latter effect was abrogated by concurrent treatment with PGE2.²¹³ Downregulation of VEGF expression in PC-3 xenografts with this drug has also been reported.²⁰¹ Cox inhibitors have also been shown to suppress the invasiveness of DU145 and PC-3 cells in vitro by downregulating metalloproteinase expression; once again, concurrent PGE2 reversed this effect.^{214,215}

The first published pilot clinical trial of cox-2 inhibition in prostate cancer has yielded encouraging results.²¹⁶ Twelve patients who had chemical relapse after radiation therapy or radical prostatectomy were treated with celecoxib 200 mg twice daily. After 3 months of treatment, PSA levels had fallen or stabilized in 8 of the patients; 3 of the remaining 4 patients experienced a marked decrease in PSA doubling time. The authors concluded that "cox-2 inhibitors may help to delay or prevent disease progression in these patients, and thereby help extend the time until androgen deprivation therapy."^{216(p275)}

Also worthy of mention is an in vitro study demonstrating that saw palmetto berry extract inhibits the growth of several human prostate cancer cell lines, an effect associated with decreased expression of both cox-2 and bcl-2.²¹⁷

PKA Type I

PGE2 is not the only autocrine/paracrine factor that can activate adenylate cyclase in prostate cancer; vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide, parathyroid hormone-related peptide, calcitonin, and calcitonin gene-related peptide have this potential as well.²¹⁸⁻²²⁴ Moreover, since most prostate cancers express at least modest amounts

of cox-1,^{190,191} complete suppression of cox-2 activity would not wholly suppress autocrine PGE2 production. Thus, cox-2 inhibition could not be expected to eliminate adenylate cyclase/protein kinase A activity in prostate cancer.

Most cancers and other proliferating cells express the type I PKA (PKA-I), whereas type II PKA predominates in quiescent cells.²²⁵ These 2 forms of PKA share a common catalytic subunit but are distinguished by their regulatory subunits: RI and RII, respectively. When cells are in a proliferative mode, growth factor activity somehow selectively boosts expression of RI, such that it outcompetes RII for binding to the catalytic subunit of PKA. Possibly owing to differences in subcellular location—PKA-I is linked to EGFR via its RI subunit²²⁶—PKA-I and PKA-II have distinctive activities. In particular, PKA-I acts as a cogrowth factor, aiding activation of MAP kinase, boosting proliferation, suppressing apoptosis, and promoting production of angiogenic factors as well as the multidrug-resistant protein mdr-1.^{76,225-228} When expression of RI is downregulated (as with antisense agents or 8-Cl-cAMP), there is a compensatory upregulation of the RII subunit, such that PKA-II replaces PKA-I, leading to a more quiescent phenotype. Antisense agents targeting the RI α subunit of PKA-I, configured in ways that render them more stable, better tolerated, and susceptible to oral administration—so-called second-generation mixed backbone oligonucleotides—are now being developed as clinical anticancer agents.²²⁹ The results of phase I studies with one of these, GEM 231, have been described.^{230,231} (This agent was 1 of the 3 employed in the Tortora study cited above.)

As noted previously, Mimeault et al have reported that concurrent inhibition of PKA and EGFR synergistically suppresses proliferation and boosts apoptosis in EGF- and serum-stimulated human prostate cancer cell lines (LNCaP, DU145, and PC-3).⁸¹ A similar phenomenon has been demonstrated by Tortora's group in other human cancer cell lines and xenografts, using antisense agents or 8-Cl-cAMP to antagonize PKA-I activity.²³²⁻²³⁴

Although GEM 231 is partially absorbable after oral administration, initial clinical trials have evaluated intermittent slow continuous infusions of this agent.²³¹ Elevations of transaminases and alterations in coagulation parameters are dose limiting, and a low-grade, reversible fatigue is a common side effect. While the plasma half-life of GEM 231 is about 1 hour, tissue half-life is thought to be far longer. Theoretically, orally absorbable agents that are well tolerated during chronic administration should yield the best results in signal modulation therapy. Nonetheless, it may prove feasible and worthwhile to incorporate intermittent

courses of parenterally administered antisense agents into such regimens, if such courses can trigger a substantial temporary increase in tumor apoptosis that promotes tumor regression or synergize with concurrently administered cytotoxic agents.

Vascular Endothelial Growth Factor (VEGF)

VEGF is widely viewed as the most functionally significant angiogenic factor. Histological studies of human prostate cancers conclude that VEGF expression correlates with microvessel density in these tumors,²³⁵⁻²³⁸ and specific VEGF antagonists slow the growth of human prostate cancer xenografts in nude mice²³⁹⁻²⁴¹—findings consistent with an important role for VEGF in the angiogenic response to prostate cancer. VEGF may also have autocrine growth factor activity for some prostate cancers.

The human VEGF promoter has received considerable study.²¹¹ As noted above, the transcription factors NF- κ B and HIF-1 bind to this promoter and boost VEGF transcription. Response elements for AP-1, Sp1, and STAT3 are also found in this promoter, and these transcription factors likewise promote VEGF transcription.^{209,210,242-247} Although AP-2 can also bind to the VEGF promoter, it appears to have an inhibitory impact on VEGF transcription in prostate cancers, consistent with the observation that expression of AP-2 is typically diminished in advanced prostate cancers.²⁴⁸

These findings imply that an optimally comprehensive strategy for downregulating VEGF production by prostate cancers will entail suppression of the transactivational activities of NF- κ B, HIF-1, AP-1, Sp1, and STAT3. Agents that downregulate the former 2 transcription factors have been discussed above. As is well known, Erk1/2 and c-Jun-N-terminal kinase contribute importantly to AP-1 activity. Thus, measures for downregulating the MAP kinase signaling pathway should be helpful in this regard. These include measures that suppress the activities of tyrosine kinase growth factor receptors (such as IGFR-1 and EGFR), farnesyl:protein transferase (required for ras activity, see below), cox-2, and PKA type 1. Hsp90 inhibitors may also aid control of Erk1/2 activity, as they have the potential to decrease the stabilities of IGFR1, ErbB2, raf-1, B-raf, and MEK.¹⁸⁵ With respect to Sp1, Erk1/2 boosts its transactivational activity via threonine phosphorylations.^{249,250} The PI3K pathway may also contribute in this regard, as PKC ζ , downstream from PI3K/PDK-1, can also induce an activating phosphorylation of Sp1.²⁵¹ Inhibition of c-Src kinase may decrease STAT3 activation, as discussed below.

In brief, therapies that simultaneously downregulate NF- κ B, HIF-1 α , Erk1/2, and STAT3 activities

can be expected to have a substantial impact on VEGF expression. Such measures should also suppress the activities of a number of other transcription factors implicated in prostate cancer angiogenesis, including basic fibroblast growth factor,²³⁵ responsive to the transcription factor early growth response-1 (Egr-1).^{252,253} Like c-fos, Egr-1 is rapidly induced by Erk1/2 activity.

5-Lipoxygenase (5LPO)

There are 2 reports that, as contrasted to benign prostate tissue, 5LPO is markedly upregulated in prostate cancers and prostatic intraepithelial neoplasia.^{254,255} Ghosh and colleagues have demonstrated that 5(S)-HETE, a major product of 5LPO, has growth factor activity for both androgen-dependent and -independent prostate cancer cell lines and that, conversely, selective inhibitors of 5LPO promote apoptosis in these cells, an effect reversed by administration of 5(S)-HETE.²⁵⁶⁻²⁵⁸ Other groups have confirmed that 5LPO inhibition suppresses proliferation and promotes apoptosis in prostate cancer cells *in vitro*^{259,260}; however, one group found no effect of such inhibition on survival of PC-3 cells.²⁶¹ So far, there do not appear to be any published studies examining the impact of 5LPO inhibition on prostate cancer xenografts. Clinical tools for suppressing 5LPO activity are available: boswellic acids, the key ingredients of salai guggal, an ancient Ayurvedic remedy used in inflammatory disorders, have been shown to be potent inhibitors of this enzyme,²⁶² and commercially available extracts rich in boswellic acids have shown clinical efficacy in asthma, presumably reflecting inhibition of 5LPO-dependent leukotriene production.²⁶³ The drug zileuton likewise inhibits 5LPO and is useful in asthma management.²⁶⁴ Studies evaluating the impact of these agents on prostate cancer xenografts are evidently needed.

How does 5(S)-HETE act as a growth and survival factor? The membrane receptor for this compound in prostate cancer (PC-3) appears to be a 7-pass receptor that activates pertussis toxin-sensitive G proteins yet is distinct from that expressed by leukocytes.²⁶⁵ This receptor somehow activates both the PI3K-Akt and MEK-Erk1/2 pathways. In a pancreatic cancer cell line responsive to 5(S)-HETE, pertussis toxin, tyrosine kinase inhibition, and inhibitors of PI3K and of MEK block the growth response to 5(S)-HETE.²⁶⁶ Thus, 5(S)-HETE appears to function like a tyrosine kinase-activating growth factor, even though its receptor is not a tyrosine kinase; presumably, a tyrosine kinase is activated downstream of a G protein.

Ghosh has observed that the ability of selenium to promote apoptosis in human prostate cancer cell lines is abrogated by treatment with 5(S)-HETE.²⁶⁷ Thus, he suggests that the efficacy of selenium for prevention or

treatment of prostate cancer may be amplified by concurrent administration of 5LPO inhibitors.

12-Lipoxygenase (12LPO)

Overexpression of the so-called platelet isoform of 12LPO has been observed in about half of prostate cancers and tends to correlate with aggressive tumor behavior.^{268,269} The chief product of this enzyme, 12(S)-HETE, exerts both autocrine and paracrine effects, presumably mediated by an as-yet uncharacterized receptor. In both PC-3 and DU145 cells, prolonged inhibition of 12LPO—with 25 μ M of either the natural flavone baicalein or the drug BHPP—is associated with a marked reduction of proliferation characterized by accumulation of cells in G1 phase, as well as upregulation of apoptosis; decreased expression of D cyclins as well as of the antiapoptotic proteins Bcl-2 and survivin is observed, and presumably contributes to these effects.²⁷⁰ Co-incubation with 12(S)-HETE prevents these effects of 12LPO inhibition. Overexpression of 12LPO in prostate cancer cell lines leads to constitutional activation of NF- κ B, as well as increased membrane expression of the vitronectin-binding integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$.^{261,271} The latter effect enables 12LPO-transfected PC-3 cells to avoid apoptosis when cultured in serum-free medium; the ligand-bound integrins transmit a survival signal. Increased expression of these integrins also boosts the ability of prostate cancer cells to adhere to substratum, migrate, and invade Matrigel,²⁷² consistent with the more aggressive behavior seen with 12LPO-overexpressing prostate cancers.^{268,273} *In vivo*, 12LPO-transfected PC-3 cells are more invasive and metastatic and evoke a stronger angiogenic response²⁷²; conversely, preincubation of prostate cancer cells with 12LPO inhibitors blunts their metastatic potential when they subsequently are injected into mice.²⁷³

Perhaps of more general interest is the fact that 12LPO is expressed in human endothelial cells and appears to play a key role in angiogenesis.²⁷⁴⁻²⁷⁶ Exposure of endothelial cells to angiogenic factors induces release of arachidonic acid from membrane phospholipids, which in turn promotes synthesis of 12(S)-HETE. The latter induces membrane expression of vitronectin-binding integrins, as it does in prostate cancer cells²⁷⁴; these integrins are crucial to the angiogenic process.²⁷⁷ *In vitro*, 12LPO inhibitors suppress the mitogenic, migratory, and tube-forming response of endothelial cells to VEGF and bFGF.^{276,278} Conversely, 12LPO-transfected endothelial cells are more responsive to angiogenic signals.²⁷⁶ This suggests that the 12(S)-HETE elaborated by cancer cells could promote the angiogenic response, consistent with the greater microvessel density noted in tumors derived

from 12LPO-transfected PC-3 cells.²⁷² In the chorio-allantoic membrane assay, pretreatment with baicalein dose dependently inhibited the angiogenic response to bFGF.²⁷⁸

These considerations suggest that effective 12LPO inhibition, if clinically achievable, would have general therapeutic utility as an angiostatic measure and would be of particular value in prostate cancers that overexpress 12LPO. Unfortunately, no studies of 12LPO inhibition in prostate cancer xenografts have been reported, but the growth of a 12LPO-expressing human pancreatic cancer xenograft was inhibited by about 50% when mice were repeatedly gavaged with baicalein (250 mg/kg/d).²⁷⁹ Whether a sufficiently high (but tolerable) oral intake of baicalein could maintain adequate inhibition of 12LPO in humans remains to be seen; like other flavonoids, baicalein may be rapidly conjugated with glucuronic acid or sulphate in vivo. Intriguingly, baicalein is one of the chief flavonoids in *Scutellaria baicalensis*, an herb featured prominently in certain herbal mixtures used traditionally for cancer therapy in East Asia.²⁷⁸ In any case, development of a clinical regimen for effective 12LPO inhibition should be a high research priority.

Angiotensin II Receptor Type 1

Angiotensin II (Ang II), acting via type 1 receptors (AT-1), has been shown to have an upregulatory impact on tumor-evoked angiogenesis; this reflects direct effects of Ang II on both endothelial cells as well as the cancer.²⁸⁰⁻²⁸⁴ The role of Ang II in support of prostate cancer induction and progression has so far received little study. However, Uemura and colleagues have recently reported that AT-1 expression is upregulated in most human prostate cancers in comparison to healthy prostate tissue.²⁸⁵ They further showed that Ang II promoted proliferation of human prostate cancer cell cultures, an effect associated with increased phosphorylation of MAP kinase and of STAT3. This effect was inhibited by concurrent administration of AT-1 blocker drugs, which remarkably also diminished the proliferative response to EGF. Finally, they showed that AT-1 blockers dose dependently inhibited the growth of prostate cancer xenografts in nude mice, an effect associated with decreased microvessel density in the tumors.

Although few studies have examined the signaling mechanisms evoked by the AT-1 receptor in benign or malignant prostate epithelium, this receptor activates both the PI3K-Akt and MAP kinase pathways in smooth muscle cells, even though AT-1 is a 7-pass G-protein-linked receptor (like that for 5-(S)-HETE).²⁸⁶ Activation of the soluble tyrosine kinase c-Src may mediate the growth factor activity of AT-1 in smooth

muscle.^{287,288} In human prostate stromal cells, which express AT-1, this receptor induces proteolytic cleavage and release of the membrane-bound precursor form of heparin-binding EGF; this in turn activates the EGF receptors ErbB1 and ErbB2.²⁸⁹ EGFR also acts as a mediator of the mitogenic activity of Ang II in human breast cancer cells.⁷⁷

The possibility that expression of angiotensin I converting-enzyme (ACE) influences human prostate cancer risk has been suggested by a recent epidemiological study examining the influence of an insertion/deletion polymorphism of the ACE gene.²⁹⁰ The DD genotype, associated with increased expression of ACE, was found to be associated with a significant increase in risk for advanced prostate cancer (odds ratio = 2.18). A previous study had found a trend toward reduced prostate cancer risk in long-term users of the ACE-inhibitory drug captopril; albeit other types of ACE inhibitors did not appear to influence risk in this study.²⁹¹

Bradykinin Receptor Type 1

The somewhat equivocal impact of ACE inhibition on prostate cancer risk in epidemiological studies to date may reflect the fact that bradykinin, whose catabolism is mediated by ACE, has growth factor activity for some prostate cancers. The type 1 bradykinin receptor, not found in benign prostate tissues, is expressed by prostatic intraepithelial neoplasia as well as more advanced prostate cancers²⁹²; prostate cancers also express tissue kallikrein, enabling local generation of bradykinin.²⁹³ In PC-3 cells, bradykinin promotes proliferation, migration, and invasion via the type 1 receptor.^{292,294} This effect is mediated by activation of Erk1/2, which in turn requires upstream activation of PKC and EGFR.²⁹⁴ An antagonist to the type 1 bradykinin receptor slows the growth of PC-3 xenografts in nude mice.^{295,296}

These considerations suggest that downregulation of angiotensin II's impact on prostate cancer proliferation and angiogenesis may be best achieved with drugs that selectively block the AT-1 receptor, rather than with ACE inhibitors that would be expected to increase bradykinin levels. This conclusion draws further support from evidence that the endothelial AT-2 receptor acts to suppress angiogenesis²⁹⁷; thus, when AT-1 receptors are effectively blocked, Ang II may have net angiostatic activity.

c-Src

The receptors for PGE₂, 5-LPO, and Ang II are members of the large class of G protein-coupled receptors (GPCRs), a number of which are expressed by prostate cancer cells.^{298,299} These include receptors for

bombesin, a prominent autocrine growth factor in the many prostate cancers containing neuroendocrine-differentiated cells.³⁰⁰⁻³⁰³ Intriguingly, the increase in the proliferation of PC-3 cells evoked by exposure to 10% fetal bovine serum is almost completely eliminated by pretreatment with pertussis toxin, implying that Gi-coupled receptors are responsible for the bulk of the mitogenic impact of serum exposure.³⁰⁴ The mechanisms by which GPCRs transmit mitogenic signals are still somewhat obscure, but transactivation of the EGFR, mediated at least in part by the non-receptor tyrosine kinase c-Src, often appears to play a key role in this regard. Indeed, serum-stimulated activation of Erk1/2 in PC-3 cells was decreased by 30% to 40% when the cells were treated with PP2, a relatively specific inhibitor of Src family kinases, and inhibition of EGFR almost completely abrogated this activation.³⁰⁴

Joint overexpression of c-Src and EGFR in cancers is associated with highly aggressive behavior, and this likely reflects a key role for c-Src in full activation of EGFR.³⁰⁵⁻³⁰⁷ Activated c-Src can phosphorylate 2 tyrosine residues in EGFR—Tyr-845 and -1101—not susceptible to autophosphorylation. The functional impact of Tyr-1101 phosphorylation remains unclear. c-Src-mediated phosphorylation of Tyr-845 may be more consequential; although it does not influence EGF-mediated activation of MAP kinases, it greatly potentiates the mitogenic response to EGFR agonists.³⁰⁶ In cells transfected with a Y845F mutant EGFR (ie, in which Tyr-845 is replaced by phenylalanine) or a kinase-inactive c-Src, the proliferative response to both EGF and serum is greatly reduced.^{306,308} There is some evidence that Tyr-845 phosphorylation may be crucial for EGFR-mediated activation of STAT3³⁰⁹; indeed, there are many reports that c-Src inhibition compromises the ability of EGF agonists to activate this transcription factor.^{309,313} STAT3 has the potential to contribute to malignant behavior, as it boosts transcription of cyclin D1, c-myc, VEGF, and the antiapoptotic proteins Bcl-xL and mcl-1^{245-247,314,315}; it also amplifies androgen receptor-mediated transcription.³¹⁶⁻³¹⁹ STAT3 tends to be constitutively activated in androgen-independent prostate cancers,³²⁰⁻³²² and antisense suppression of STAT3 expression, or transfection with dominant negative STAT3, markedly decreases the proliferation of prostate cancer cells while promoting apoptosis.^{320,321,323,324}

The activated EGFR can itself activate c-Src, through binding of the latter to receptor phosphotyrosines via its SH2 domain; thus, EGFR and c-Src are mutually stimulatory. How activated c-Src transactivates EGFR in the absence of EGFR agonists—to enable MAP kinase activation^{304,325-328} remains unclear; presumably, it produces a phosphotyrosine docking

site for Grb2/Sos/Ras, on EGFR or some associated protein such as Shc. (Activated c-Src can produce analogous docking sites in other signaling proteins, including Shc and the focal adhesion kinase Pyk2.)^{329,330} GPCRs also can activate c-Src; G α s and G α i are reported to achieve this by direct association,³³¹ G $\beta\gamma$ may also be active in this regard,^{304,332,333} and PKA—stimulated by G α s—activates c-Src via phosphorylation of Ser-17.^{334,335} (Recall that type I PKA associates with the EGFR; this would enable PKA to activate c-Src in the immediate vicinity of this receptor.) In cells that express B-raf (the status of prostate cancer in this regard appears to be unknown), activated c-Src can stimulate the MAP kinase pathway by activating Rap1, which in turn activates B-raf.³³⁵ c-Src also promotes cell migration through its interactions with integrins and focal adhesion kinase³³⁶⁻³³⁸; thus, inhibition of c-Src may have potential for combating the metastatic spread of cancer as well as tumor-evoked angiogenesis.³³⁹

Remarkably, c-Src knockout mice are fairly healthy; their chief problem is that their osteoclast function is impaired, leading to osteopetrosis.³⁴⁰ Two conclusions have been drawn from this: first, that a sufficiently potent and specific inhibitor of c-Src might be reasonably well tolerated as a therapeutic agent; second, that such inhibitors might have utility for treatment of osteoporosis. The most selective inhibitors of c-Src yet developed are the pyrrolopyrimidines CGP77675 and CGP76030^{341,342}; these are now being employed in rodent studies³⁴³ but have not yet been studied clinically. PP2, an inhibitor of the family of Src kinases, has been administered to nude mice bearing human colon cancer xenografts; this treatment inhibited growth of the primary tumors by about 50%, while almost totally preventing hepatic metastases, and was not associated with any evident toxicity.³⁴⁴

Two published studies have examined the impact of c-Src inhibitors on human prostate cancer cell lines; the influence of c-Src inhibition on prostate cancer xenografts has not yet been assessed. CGP77675 and CGP76030 both dose dependently decrease the proliferation rate of PC-3 cells; they also decrease the ability of these cells to adhere and spread on Matrigel and to invade this substrate.³⁴² A previous study had likewise shown that PP2 slows the migration of both PC-3 and DU145 cells.³³⁷ With respect to the contemplated clinical use of c-Src inhibitors in prostate cancer, it has been suggested that the antiosteoclastic impact of such a therapy might lessen the tendency of prostate cancer to metastasize to bone.³⁴²

Owing to its role in transmitting mitogenic signals from GPCRs, its ability to amplify the oncogenic activity of EGFR, and its key role in cellular migration, c-SRC may be an attractive target for signal modu-

lation therapy of cancer.^{340,345} However, realization of this prospect must await the clinical development of highly specific c-Src inhibitors.

Interleukin-6 (IL-6)

Overactivation of c-Src is not the sole reason for up-regulated activation of STAT3 in prostate cancer; autocrine or paracrine IL-6 can also promote STAT3 activation. Autocrine production of IL-6 has been noted in androgen-independent human prostate cancer cell lines^{346,347}—though not androgen-dependent LNCaP cells^{346,348}—as well as in human prostate cancer biopsies.^{348,349} IL-6 protein was reported to average 18-fold higher in clinically localized prostate cancers as compared to healthy prostate tissue.³⁴⁸ Moreover, human prostate cancers and cancer cell lines almost uniformly express the IL-6 receptor,^{347,349,350} giving rise to an IL-6 autocrine loop. Although the impact of IL-6 on LNCaP cells has been inconsistent,^{346,351,352} IL-6 has been found to promote proliferation and suppress apoptosis in androgen-independent prostate cancer cell lines,^{346,347} reflecting IL-6-mediated activation of STAT3 and MAP kinase signaling pathways.³⁵³ Upregulation of Bcl-xL is primarily responsible for the antiapoptotic effect, and this upregulation in turn is primarily attributable to increased STAT3 activity.^{354,355} IL-6's ability to boost the transcriptional activity of androgen receptors, even when androgen levels are very low or absent,^{352,356-358} is also largely mediated by STAT3.³⁵⁷ Thus, androgen deprivation may select for prostate cancer cells that overexpress IL-6. When nude mice bearing PC-3 xenografts were treated with monoclonal antibody targeting IL-6, growth of the tumor was inhibited by approximately 60%³⁵⁹; such antibodies also slow proliferation of prostate cancer cell lines *in vitro* and sensitize them to chemotherapeutic agents.^{346,360} Serum levels of IL-6 and of IL-6 receptors are typically elevated in patients with advanced prostate cancer, particularly bone metastases, and correlate with poor prognosis.^{347,361,362}

In the PC-3 and DU145 cell lines, increased activity of the IL-6 promoter has been traced to constitutive upregulation of NF-κB and AP-1 transcription factors.³⁶³ Thus, the constitutive activation of NF-κB typical of androgen-independent prostate cancers may promote androgen independence via upregulated IL-6 and STAT3. Conversely, therapeutic measures that suppress NF-κB activation in prostate cancer could be expected to decrease tumor production of IL-6 and thus suppress the IL-6 autocrine loop. In this regard, copper deprivation with TM (reported to decrease NF-κB activity in cancer) decreased IL-6 production by a human breast cancer xenograft in nude mice.¹³⁶ Thus, TM therapy, as well as other measures that

downregulate NF-κB, may help to control IL-6/STAT3 overactivity in prostate cancer.

There is suggestive evidence that an autocrine loop involving IL-11, likewise an NF-κB-inducible cytokine,³⁶⁴ may also contribute to STAT3 overactivation in prostate cancer.^{365,366}

Ras

Although oncogenic constitutively active mutants of Ras are commonly encountered in some types of cancer, they appear to be relatively rare in human prostate cancers, with the possible exception of Japanese patients.³⁶⁷⁻³⁶⁹ Statin therapy, which can downregulate the activity of Ras by opposing its isoprenylation, has not been associated with decreased risk for prostate cancer.^{370,371} Nonetheless, a drug that inhibits farnesyl:protein transferase, causing the cytoplasmic accumulation of Ha-Ras, has been shown to have growth-inhibitory effects on a range of human prostate cancer cell lines.³⁷² This effect was seen in lines that lacked a mutant Ras, but the drug literally reversed the neoplastic phenotype in TSU-Pr1 cells, which express an oncogenic Ha-Ras mutant. Thus, it may be worthwhile to devote further study to farnesyl:protein transferase inhibitors (or other agents that interfere with isoprenylation) as potential components of multifocal signal modulation therapies for prostate cancer; several of these inhibitors have already been evaluated in phase I and phase II clinical studies.^{373,374}

Apoptosis Suppressors: MDM2 and Bcl-2/Bcl-xL

The excessive growth and resistance to cytotoxic chemotherapy that characterize many cancers often reflect increased expression or activation of proteins that downregulate apoptosis. These proteins can be targeted with antisense agents.

p53 is activated by signals that detect unrepaired DNA damage or excessive growth factor stimulation; depending on the circumstances, p53 either slows the cell cycle to prevent entry into S phase (via p21) or triggers apoptosis. The activity of p53 is opposed by MDM2, which binds to p53 and ubiquitylates it, routing it to proteasomal destruction. MDM2 suppresses apoptosis in other ways, independent of p53, that are still poorly understood; there is recent evidence that MDM2 promotes proteasomal degradation of p21.³⁷⁵ In a series of 118 localized prostate cancers obtained by prostatectomy, 40% were found to overexpress MDM2, and these tumors tended to be larger than the others.³⁷⁶

A mixed-backbone oligonucleotide antisense agent targeting MDM2 has been studied in human prostate cancer cells and xenografts.³⁷⁷⁻³⁸² This agent

was found to decrease proliferation and increase apoptosis in LNCaP (expressing wild-type p53), DU145 (expressing mutant p53), and PC-3 (not expressing p53); thus, the efficacy of MDM2 suppression appears to be independent of p53 status.³⁷⁸ This agent also sensitized these cells to the cytotoxicity of a taxane and a topoisomerase inhibitor and slowed the growth of LNCaP and PC-3 xenografts in nude mice. Other studies have established that MDM2 antisense can boost the apoptotic response of prostate cancer cells to androgen deprivation and sensitize these cells to radiotherapy.³⁷⁹⁻³⁸¹ Finally, Tortora and colleagues have shown that MDM2 antisense potentiates response to the EGFR inhibitor Iressa in human prostate cancer cell lines and xenografts.³⁸²

Bcl-2 and its close relative Bcl-xL, which oppose mitochondrially triggered apoptosis, have also been targeted with a mixed backbone oligonucleotide antisense agent that can suppress synthesis of both of these proteins.^{383,384} This agent complements the tumor-retardant activity of PKA-RI antisense in nude mice implanted with human colon cancer xenografts.³⁸³ Histological studies have found overexpression of bcl-2 in about 30% of prostate cancers, and, not surprisingly, this overexpression tends to correlate with malignant behavior and poorer prognosis.³⁸⁵⁻³⁸⁸ Thus, there may be a role for bcl-2/bcl-xL antisense in the management of selected prostate cancers.

A review of antisense strategies potentially of use in prostate cancer treatment has appeared recently.³⁸⁹

Vitamin D Receptor

Normal prostate epithelium expresses vitamin D receptors, and calcitriol, the natural agonist for these receptors, exerts a growth-inhibitory effect.³⁹⁰⁻³⁹² These cells also express 1- α -hydroxylase activity and thus can generate their own calcitriol from circulating 25-hydroxycholecalciferol.^{391,393,394} Since the serum level of 25-hydroxycholecalciferol is determined largely by exposure of skin to ultraviolet light, these findings have encouraged the speculation that good vitamin D status might reduce prostate cancer risk. Although epidemiological studies correlating assessed sunlight exposure with subsequent prostate cancer risk are reasonably supportive of this thesis,³⁹⁵⁻⁴⁰¹ prospective studies examining serum levels of calcitriol or 25-hydroxyvitamin D have been much less so.⁴⁰²⁻⁴⁰⁷ Thus, the role of vitamin D status in prostate cancer induction remains unclear. Since supraphysiological concentrations of calcitriol have been employed in most in vitro studies, it is conceivable that the growth-inhibitory impact of this hormone on prostate epithelium is pharmacological rather than physiological.

Calcitriol, and less calcemic synthetic agonists for the vitamin D receptor, likewise have a growth-inhibitory effect on most human prostate cancer cell lines and on prostate cancer xenografts in nude mice.⁴⁰⁸⁻⁴¹⁶ Calcitriol's efficacy in this regard varies considerably depending on the cell line studied; variations in vitamin D receptor expression often do not account for differences in response. Androgen-dependent cell lines tend to be more responsive than those that are androgen independent, for reasons that remain obscure.^{408,417} Owing to the fact that 1- α -hydroxylase is nearly inactive in prostate cancer, 25-hydroxycholecalciferol does not influence proliferation in these cells.⁴¹⁸

The growth-retardant impact of calcitriol on prostate cancers and normal epithelium appears to be mediated primarily by an increase in IGFBP-3 synthesis,^{412,419-421} reflecting the presence of a functional vitamin D response element in the IGFBP-3 promoter.⁴²² While IGFBP-3 can influence prostate function by limiting the availability of IGFs, it also exerts a direct effect on prostate epithelium and cancer cells that upregulates expression of the cyclin-dependent kinase inhibitor p21.^{412,421,423} Recent evidence suggests that IGFBP-3 activates epithelial TGF- β receptors³⁵⁻³⁸; as is well known, the antiproliferative effects of TGF- β are mediated by induction of p21 and other cyclin-dependent kinase inhibitors.^{424,425} Calcitriol may also increase production of TGF- β .⁴²⁶ The effects of calcitriol on cell growth appear to be exerted primarily at the level of proliferation, rather than apoptosis.⁴²⁷ However, calcitriol-mediated downregulation of certain antiapoptotic proteins, including bcl-2 and bcl-xL, has been reported in some studies.⁴¹¹ Calcitriol has also been observed to reduce the invasiveness of certain prostate cancer cell lines⁴⁰⁹ and can decrease production of parathyroid hormone-related peptide,^{428,429} which, as noted above, is an autocrine growth factor for prostate cancer and also has osteolytic activity.

IGFBP-3 is produced constitutively by healthy prostate epithelium; its production is substantially downregulated in most prostate cancers.⁴³⁰⁻⁴³³ Thus, upregulating production of this protective factor with calcitriol (or analogs thereof) may be viewed as a compensatory measure, reversing a shift in prostate cancer differentiation that promotes malignancy.

The fact that prostate cancers have minimal if any 1- α -hydroxylase activity implies that sunlight or vitamin D supplementation are unlikely to influence prostate cancer (unless toxic doses of vitamin D are used). Pilot studies have evaluated the impact of calcitriol or its precursor 1- α -hydroxycholecalciferol in prostate cancer patients.⁴³⁴⁻⁴³⁸ Doses must be adjusted carefully and dietary calcium minimized to avoid induction of excessive hypercalcemia. A simple regimen that has

proved to be well tolerated and that raises serum calcitriol levels to the range that is therapeutic *in vitro* (~1 nM) is 0.5 mg/kg once weekly. A minority of patients achieve a reduction in PSA with such regimens; the more common response has been a worthwhile reduction in PSA doubling time. Since calcitriol has relatively little impact on apoptosis, it is likely to be only palliative unless used in conjunction with cytotoxic or signal-modulating agents that upregulate apoptosis. In this regard, calcitriol has been reported to potentiate response to the cytotoxic agent docetaxel, as well as to radiotherapy.^{439,440}

Estrogen Receptor- β

Estrogens have been used in prostate cancer treatment as a strategy for indirectly suppressing gonadal testosterone production. However, normal prostate epithelium and, to a varying extent, prostate cancers express the β isoform of the estrogen receptor (ER- β). Thus, it is conceivable that estrogens or estrogen antagonists could have some direct impact on the induction or progression of prostate cancer.^{441,442} ER- β expression tends to be decreased in androgen-independent prostate cancer cell lines, owing to methylation of the ER- β promoter.^{443,444} Histological studies also report reduced ER- β expression in many recurrent prostate cancers.^{444,445} However, ER- β expression has been noted in most metastatic lesions.⁴⁴⁶ The tendency of ER- β expression to diminish as prostate cancers mature has prompted the suggestion that this receptor may have a suppressor function in the prostate.⁴⁴⁷ Indeed, prostate hyperplasia is commonly seen in aging ER- β -knockout mice.^{448,449} Transfection of DU-145 cells with the ER- β gene reduces the proliferation and invasiveness of these cells while increasing apoptosis; remarkably, these effects are seen in the absence of exogenous ligand.⁴⁴⁷ ER- β transfection likewise slows proliferation in a human breast cancer cell line⁴⁵⁰; the reported effects on expression of cyclins, cdk inhibitors, and c-myc are opposite to those of NF- κ B. (In some cells, estrogen receptors and NF- κ B have mutually antagonistic activity owing to competition for scarce coactivators^{451,452}: could this be true in prostate epithelium as well? If so, then loss of ER- β expression could contribute to the NF- κ B upregulation typical of androgen-independent prostate cancer.)

Tamoxifen and the pure estrogen antagonist ICI-182,780 reduce the growth of both PC-3 and DU145 cells *in vitro*; coadministration of antisense oligodeoxynucleotides targeting ER- β reverses this effect.⁴⁵³ The selective estrogen response modulator raloxifene, an antagonist for ER- β , has been shown to induce apoptosis in LNCaP, PC-3, PC-3M, and DU145 cell lines.^{454,455} Surprisingly, there do not appear to be

any published studies examining the impact of these estrogen agonists/antagonists on growth of human prostate cancer xenografts in animals.

Genistein, suspected to be a chemopreventive agent for prostate cancer, has estrogen agonist activity that is relatively selective for ER- β .⁴⁵⁶ In concentrations as low as 100 nM—sufficient to activate ER- β —free genistein achieves a modest but statistically significant reduction in the expression of androgen receptors in LNCaP cells, an effect inhibited by coadministration of a pure estrogen antagonist.⁴⁵⁷ As anticipated, a corresponding reduction in PSA secretion was noted in genistein-treated cells. Since plasma concentrations of free genistein as high as 170 nM can be achieved when supranutritional doses (eg, 8 mg/kg) of genistein are administered orally to humans,⁴⁵⁸ these observations may be relevant to the therapeutic and preventive potential of high-dose soy isoflavones. Although genistein has been shown to exert a wide range of antiproliferative effects on prostate cancer cells *in vitro*, including tyrosine kinase inhibition, grossly supraphysiological concentrations of free genistein in the micromolar range are usually required to elicit these; in contrast, the impact of genistein on ER- β function may be of physiological interest and conceivably relevant to the favorable effects of oral genistein reported in prostate cancer xenograft models as well as in initial clinical trials.⁴⁵⁹⁻⁴⁶²

Peroxisome Proliferator-Activated Receptor- γ

Another nuclear receptor that may influence prostate cancer growth is peroxisome proliferator-activated receptor- γ (PPAR- γ). Activation of this receptor with various ligands has promoted apoptosis in several types of cancer. Recent studies show that most prostate cancers express this receptor.⁴⁶³⁻⁴⁶⁶ Ligands for this receptor have been reported to induce cell death or slow proliferation in the LNCaP, DU145, and PC-3 human prostate cancer cell lines^{463,464}; the molecular biology responsible for this effect has not been defined. No published studies to date have examined the impact of pharmaceutical PPAR- γ agonists (such as thiazolidinediones) on prostate cancer xenografts *in vivo*. Nonetheless, the possibility that PPAR- γ agonists might have a clinical impact on prostate cancer is intriguing and merits further examination.

Potential Therapies in Search of a Target

Epidemiology, and/or rodent studies, and in a few instances pilot clinical trials, suggest that various nutrients and phytochemicals may have the potential to aid prevention or promote control of prostate cancer. These agents include lycopene,⁴⁶⁷⁻⁴⁷⁶ selenium,⁴⁷⁷⁻⁴⁸⁷

green tea polyphenols,⁴⁸⁸⁻⁴⁹⁴ grape seed extract,⁴⁹⁵ genistein,^{459-462,496-503} silibinin,^{128,129,504} boron,¹³⁴ inositol hexaphosphate,⁵⁰⁵ and omega-3-rich fish oils. Although putative biochemical targets for many of these agents have been defined in cell culture studies (most of these compounds have the potential to downregulate NF-κB, for example), the tendency of researchers to use supraphysiological concentrations in such studies renders their conclusions questionable. Nonetheless, inasmuch as these agents clearly are not cytotoxic—at least in the moderate concentrations that could be achieved *in vivo*—any genuine cancerostatic activity they possess is likely to reflect signal modulation. Thus, while it would be illuminating and potentially helpful to know the key targets of these agents in physiological concentrations, it could still be appropriate to include these agents in multifocal signaling modulation regimens even if their targets remain obscure.

Several recent epidemiological studies—though not all^{506,507}—suggest that people who consume seafood regularly are at decidedly lower risk for prostate cancers than are those who consume seafood infrequently.⁵⁰⁸⁻⁵¹¹ Furthermore, a diet high in fish oil (and relatively low though adequate in omega-6 oils) slows the growth of DU145 xenografts in nude mice.⁵¹² In the context of a low-fat diet, 3 months of fish oil supplementation has been found to achieve significant increases of omega-3/omega-6 ratio in the plasma and gluteal fat of untreated prostate cancer patients; prostate cox-2 expression declined in the majority of these subjects.⁵¹³ By competing with arachidonic acid for incorporation into membrane phospholipids or at the active sites of cyclooxygenases and lipoxygenases, EPA/DHA have the potential to decrease production of certain arachidonate products (eg, PGE2 and 5-(S)-HETE) that encourage induction and growth of prostate cancer. Hopefully, future research will explore these possibilities. Fish oil omega-3s may also exert an angiostatic action on vascular endothelium⁵¹⁴⁻⁵¹⁸; in particular, EPA and DHA are reported to decrease endothelial expression of the VEGF receptor flk-1 (KDR).^{516,518} Perhaps this plays some role in the quite general cancer-retardant activity of fish oil-rich diets in rodent studies.⁵¹⁹

Toward Development of MSMTs

A rational and straightforward strategy for developing multifocal signal modulation therapies for prostate cancer can be proposed:

1. Identify a group of agents to be tested.
2. Assess the plasma concentrations of these agents that can be achieved through tolerable and feasible oral dosing schedules in humans. It is crucial to determine the concentration of the free, unmetabolized

agent. For example, the bulk of circulating plasma polyphenols are present as soluble conjugates that have limited intracellular penetrance; curcumin is rapidly hydrogenated to a less active form as it transits the human intestinal mucosa.⁵²⁰ For some potential agents, such as antisense oligonucleotides, intravenous infusion during episodic treatment sessions may be the preferred mode of administration.

3. Examine the impact of combinations of these agents, in physiologically realistic concentrations, on proliferation, apoptosis, and proangiogenic capacity of several human prostate cancer cell lines *in vitro*. Presumably, combinations that address multiple pro-oncogenic signaling pathways will be more effective than those that focus on a single pathway.
4. After identifying combinations that have an additive or synergistic impact on several cell lines *in vitro*, test these combinations in human prostate cancer xenografts implanted in nude mice.
5. Once regimens have been established that are effective and seemingly well tolerated in xenograft models, and it has become ethically and legally appropriate to administer the component agents to patients, pilot clinical studies with these regimens can commence.

Concluding Thoughts

Ideally, multifocal signal modulation therapies that are optimally planned and executed should be able to lower the cancer proliferation rate and raise the spontaneous apoptosis rate, to such an extent that the latter equals or exceeds the former, resulting in tumor lysis or even objective remission. Realistically, though, the best that such strategies will achieve in some cases will be a slowing of tumor growth and spread. In such cases, combining signal modulation therapies with cytotoxic measures—drugs or radiotherapy—may enable more definitive therapeutic benefit to be achieved. It is reasonable to expect that signal-modulating measures that upregulate the machinery of apoptosis will potentiate the therapeutic response to certain cytotoxic measures. On the other hand, the downregulatory impact of signal modulation on proliferation rate may influence response to cytotoxins whose efficacy is cell-cycle specific. It is also crucial to consider the impact of signal modulation on the dose-limiting side effects of cytotoxic therapy. Thus, the net impact of signal modulation on response to cytotoxic therapy may be complex and must be evaluated empirically.

Tortora and colleagues have recently summarized evidence that signal-modulation therapies can often potentiate response to cytotoxic measures.³⁷⁴ They have also conducted a series of studies evaluating the interaction between Iressa and a wide range of cytotoxic agents in human ovarian, breast, and colon

cancer cell lines and in a colon cancer xenograft model.⁵²¹ The cell lines chosen for these studies all expressed EGFR as well as autocrine TGF- α (like most advanced prostate cancers) and thus experienced EGFR-driven proliferation. Despite the fact that a diverse range of chemotherapeutic drugs was tested (platinum-based agents, topoisomerase inhibitors, taxanes, and a thymidylate synthetase inhibitor), in virtually every study, Iressa was found to greatly potentiate the apoptotic response to cytotoxins. In the colon cancer xenograft model, concurrent administration of Iressa markedly prolonged survival of mice treated with topotecan, raltitrexed, or paclitaxel. Evidently, these findings bode well for the future of mixed cytotoxic/signal-modulating therapies. On the other hand, there is a report that antisense antagonism of EGFR can decrease the responsiveness of a human breast cancer cell line to cisplatin.⁵²² The timing of administration of the various agents might have an impact on therapeutic outcome.⁵²³ Broad generalizations regarding the interaction of cytotoxic and signal-modulating therapies appear to be unwarranted at this time. Nonetheless, it is reasonable to anticipate that preclinical and clinical experience will eventually identify specific regimens of this type that will prove to be highly efficacious.

Suppression of tumor-evoked angiogenesis is now receiving prominent research attention as a strategy for cancer control. Many if not most of the signal-modulating measures discussed above have the potential to downregulate tumor production of various angiogenic factors. However, angiostatic therapies can also directly target endothelial function. Thus, it should be noted that several of the measures described here can decrease the responsiveness of endothelial cells to angiogenic factors¹³⁹; these include IGF-I downregulation,⁵²⁴⁻⁵²⁷ copper depletion,^{528,529} Hsp90 inhibition,¹⁸⁴ 12LPO inhibition,^{276,278} AT-1 blockers,⁵³⁰⁻⁵³² c-Src inhibition,^{247,339,533} green tea polyphenols,⁵³⁴⁻⁵³⁹ and fish oil.^{516,518,540} Such measures are of particular interest in that their cancer-retardant efficacies may, at least in part, be independent of the metabolic peculiarities of the tumor.

The key point of this discussion is that significant, perhaps substantial progress in clinical treatment of prostate cancer need not be contingent on future “magic bullet” breakthroughs that may or may not ever arrive; the tools may be available now for therapies that are reasonably well tolerated and that can achieve a very worthwhile extension of functional life span in patients whose prostate cancer has escaped local control. What is needed are diligent and focused research efforts by laboratories and clinics that are more motivated to develop effective therapies than to generate profits from a specific patented drug.

Doubtless there are additional molecular targets in prostate cancer, not touched on here, that could be profitably addressed; there can be no pretense that this discussion has been comprehensive. The chief purpose of this article has been to set forth the concept of “multifocal signal modulation therapy”—a concept that has emerged previously in the work of other creative researchers—and to provide some provisional insights and suggestions into how this strategy might be implemented with respect to clinical prostate cancer. Evidently, analogous analyses could be made for other prominent cancers whose molecular biology has already received considerable study.

References

1. Tortora G, Caputo R, Damiano V, et al. Combination of a selective cyclooxygenase-2 inhibitor with epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 and protein kinase A antisense causes cooperative antitumor and antiangiogenic effect. *Clin Cancer Res.* 2003;9:1566-1572.
2. Taplin ME, Balk SP. Androgen receptor: a key molecule in the progression of prostate cancer to hormone independence. *J Cell Biochem.* 2004;91:483-490.
3. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. *Mol Pathol.* 2001;54:133-137.
4. Datta SR, Ranger AM, Lin MZ, et al. Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. *Dev Cell.* 2002;3:631-643.
5. Harada H, Andersen JS, Mann M, Terada N, Korsmeyer SJ. p70S6 kinase signals cell survival as well as growth, inactivating the pro-apoptotic molecule BAD. *Proc Natl Acad Sci U S A.* 2001;98:9666-9670.
6. Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. *J Biol Chem.* 2002;277:38205-38211.
7. Baserga R, Peruzzi F, Reiss K. The IGF-1 receptor in cancer biology. *Int J Cancer.* 2003;107:873-877.
8. Brader S, Eccles SA. Phosphoinositide 3-kinase signalling pathways in tumor progression, invasion and angiogenesis. *Tumori.* 2004;90:2-8.
9. Berra E, Milanini J, Richard DE, et al. Signaling angiogenesis via p42/p44 MAP kinase and hypoxia. *Biochem Pharmacol.* 2000;60:1171-1178.
10. Eisenmann KM, VanBrocklin MW, Staffend NA, Kitchen SM, Koo HM. Mitogen-activated protein kinase pathway-dependent tumor-specific survival signaling in melanoma cells through inactivation of the proapoptotic protein bad. *Cancer Res.* 2003;63:8330-8337.
11. Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW, Sonenberg N. eIF4E: from translation to transformation. *Oncogene.* 2004;23:3172-3179.
12. Reddy KB, Nabha SM, Atanaskova N. Role of MAP kinase in tumor progression and invasion. *Cancer Metastasis Rev.* 2003;22:395-403.
13. Zhang D, Brodt P. Type 1 insulin-like growth factor regulates MT1-MMP synthesis and tumor invasion via PI 3-kinase/Akt signaling. *Oncogene.* 2003;22:974-982.
14. Majumder PK, Febbo PG, Bikoff R, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med.* 2004;10:594-601.

15. Mitsiades CS, Mitsiades N, Koutsilieris M. The Akt pathway: molecular targets for anti-cancer drug development. *Curr Cancer Drug Targets*. 2004;4:235-256.
16. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2:489-501.
17. Culig Z, Hobisch A, Cronauer MV, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res.* 1994;54:5474-5478.
18. Shi R, Berkel HJ, Yu H. Insulin-like growth factor-I and prostate cancer: a meta-analysis. *Br J Cancer*. 2001;85:991-996.
19. Chan JM, Stampfer MJ, Ma J, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J Natl Cancer Inst*. 2002;94:1099-1106.
20. Pietrzkowski Z, Mulholland G, Gomella L, Jameson BA, Wernicke D, Baserga R. Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor I. *Cancer Res*. 1993;53:1102-1106.
21. Nickerson T, Chang F, Lorimer D, Smeekens SP, Sawyers CL, Pollak M. In vivo progression of LAPC-9 and LNCaP prostate cancer models to androgen independence is associated with increased expression of insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR). *Cancer Res*. 2001;61:6276-6280.
22. Hellawell GO, Turner GD, Davies DR, Poulosom R, Brewster SF, Macaulay VM. Expression of the type I insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res*. 2002;62:2942-2950.
23. Cardillo MR, Monti S, Di Silverio F, Gentile V, Sciarra F, Toscano V. Insulin-like growth factor (IGF)-I, IGF-II and IGF type I receptor (IGFR-I) expression in prostatic cancer. *Anticancer Res*. 2003;23:3825-3835.
24. Grzmil M, Hemmerlein B, Thelen P, Schweyer S, Burfeind P. Blockade of the type I IGF receptor expression in human prostate cancer cells inhibits proliferation and invasion, up-regulates IGF binding protein-3, and suppresses MMP-2 expression. *J Pathol*. 2004;202:50-59.
25. Reiss K, Wang JY, Romano G, et al. IGF-I receptor signaling in a prostatic cancer cell line with a PTEN mutation. *Oncogene*. 2000;19:2687-2694.
26. Figueroa JA, Lee AV, Jackson JG, Yee D. Proliferation of cultured human prostate cancer cells is inhibited by insulin-like growth factor (IGF) binding protein-1: evidence for an IGF-II autocrine growth loop. *J Clin Endocrinol Metab*. 1995;80:3476-3482.
27. Kimura G, Kasuya J, Giannini S, et al. Insulin-like growth factor (IGF) system components in human prostatic cancer cell-lines: LNCaP, DU145, and PC-3 cells. *Int J Urol*. 1996;3:39-46.
28. Mita K, Nakahara M, Usui T. Expression of the insulin-like growth factor system and cancer progression in hormone-treated prostate cancer patients. *Int J Urol*. 2000;7:321-329.
29. Mita K, Nakahara M, Usui T. Expression of the insulin-like growth factor system and cancer progression in hormone-treated prostate cancer patients. *Int J Urol*. 2000;7:321-329.
30. Figueroa JA, De Raad S, Speights VO, Rinehart JJ. Gene expression of insulin-like growth factors and receptors in neoplastic prostate tissues: correlation with clinico-pathological parameters. *Cancer Invest*. 2001;19:28-34.
31. Hwa V, Tomasini-Sprenger C, Bermejo AL, Rosenfeld RG, Plymate SR. Characterization of insulin-like growth factor-binding protein-related protein-1 in prostate cells. *J Clin Endocrinol Metab*. 1998;83:4355-4362.
32. Mutaguchi K, Yasumoto H, Mita K, et al. Restoration of insulin-like growth factor binding protein-related protein 1 has a tumor-suppressive activity through induction of apoptosis in human prostate cancer. *Cancer Res*. 2003;63:7717-7723.
33. Moore MG, Wetterau LA, Francis MJ, Peehl DM, Cohen P. Novel stimulatory role for insulin-like growth factor binding protein-2 in prostate cancer cells. *Int J Cancer*. 2003;105:14-19.
34. Kiyama S, Morrison K, Zellweger T, et al. Castration-induced increases in insulin-like growth factor-binding protein 2 promotes proliferation of androgen-independent human prostate LNCaP tumors. *Cancer Res*. 2003;63:3575-3584.
35. Leal SM, Liu Q, Huang SS, Huang JS. The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor. *J Biol Chem*. 1997;272:20572-20576.
36. Leal SM, Huang SS, Huang JS. Interactions of high affinity insulin-like growth factor-binding proteins with the type V transforming growth factor-beta receptor in mink lung epithelial cells. *J Biol Chem*. 1999;274:6711-6717.
37. Fanayan S, Firth SM, Butt AJ, Baxter RC. Growth inhibition by insulin-like growth factor-binding protein-3 in T47D breast cancer cells requires transforming growth factor-beta (TGF-beta) and the type II TGF-beta receptor. *J Biol Chem*. 2000;275:39146-39151.
38. Huang SS, Ling TY, Tseng WF, et al. Cellular growth inhibition by IGFBP-3 and TGF-beta1 requires LRP-1. *FASEB J*. 2003;17:2068-2081.
39. Ngo TH, Barnard RJ, Tymchuk CN, Cohen P, Aronson WJ. Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). *Cancer Causes Control*. 2002;13:929-935.
40. Ngo TH, Barnard RJ, Leung PS, Cohen P, Aronson WJ. Insulin-like growth factor I (IGF-I) and IGF binding protein-1 modulate prostate cancer cell growth and apoptosis: possible mediators for the effects of diet and exercise on cancer cell survival. *Endocrinology*. 2003;144:2319-2324.
41. Fliesen T, Maiter D, Gerard G, Underwood LE, Maes M, Ketelslegers JM. Reduction of serum insulin-like growth factor-I by dietary protein restriction is age dependent. *Pediatr Res*. 1989;26:415-419.
42. Miura Y, Kato H, Noguchi T. Effect of dietary proteins on insulin-like growth factor-I (IGF-1) messenger ribonucleic acid content in rat liver. *Br J Nutr*. 1992;67:257-265.
43. Filho JC, Hazel SJ, Anderstam B, Bergstrom J, Lewitt M, Hall K. Effect of protein intake on plasma and erythrocyte free amino acids and serum IGF-I and IGFBP-1 levels in rats. *Am J Physiol*. 1999;277:E693-E701.
44. Allen NE, Appleby PN, Davey GK, Kaaks R, Rinaldi S, Key TJ. The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev*. 2002;11:1441-1448.
45. Kolterman OG, Greenfield M, Reaven GM, Saekow M, Olefsky JM. Effect of a high carbohydrate diet on insulin binding to adipocytes and on insulin action in vivo in man. *Diabetes*. 1979;28:731-736.
46. Fukagawa NK, Anderson JW, Hageman G, Young VR, Minaker KL. High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. *Am J Clin Nutr*. 1990;52:524-528.
47. Barnard RJ, Ugianskis EJ, Martin DA, Inkeles SB. Role of diet and exercise in the management of hyperinsulinemia and associated atherosclerotic risk factors. *Am J Cardiol*. 1992;69:440-444.
48. Phillips LS, Goldstein S, Pao CI. Nutrition and somatomedin: XXVI. Molecular regulation of IGF-I by insulin in cultured rat hepatocytes. *Diabetes*. 1991;40:1525-1530.
49. Lee PD, Conover CA, Powell DR. Regulation and function of insulin-like growth factor-binding protein-1. *Proc Soc Exp Biol Med*. 1993;204:4-29.

50. Ornish DM, Lee KL, Fair WR, Pettengill EB, Carroll PR. Dietary trial in prostate cancer: early experience and implications for clinical trial design. *Urology*. 2001;57:200-201.
51. Walker AR. Prostate cancer: some aspects of epidemiology, risk factors, treatment and survival. *S Afr Med J*. 1986;69:44-47.
52. Hebert JR, Hurley TG, Olendzki BC, Teas J, Ma Y, Hampl JS. Nutritional and socioeconomic factors in relation to prostate cancer mortality: a cross-national study. *J Natl Cancer Inst*. 1998;90:1637-1647.
53. Torrisi R, Baglietto L, Johansson H, et al. Effect of raloxifene on IGF-I and IGFBP-3 in postmenopausal women with breast cancer. *Br J Cancer*. 2001;85:1838-1841.
54. Andersson B, Johansson G, Holm G, et al. Raloxifene does not affect insulin sensitivity or glycemic control in postmenopausal women with type 2 diabetes mellitus: a randomized clinical trial. *J Clin Endocrinol Metab*. 2002;87:122-128.
55. Attanasio R, Barausse M, Cozzi R. Raloxifene lowers IGF-I levels in acromegalic women. *Eur J Endocrinol*. 2003;148:443-448.
56. Eng-Wong J, Hursting SD, Venzon D, Perkins SN, Zujewski JA. Effect of raloxifene on insulin-like growth factor-I, insulin-like growth factor binding protein-3, and leptin in premenopausal women at high risk for developing breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2003;12:1468-1473.
57. Dimaraki EV, Symons KV, Barkan AL. Raloxifene decreases serum IGF-I in male patients with active acromegaly. *Eur J Endocrinol*. 2004;150:481-487.
58. Kim IY, Seong do H, Kim BC, et al. Raloxifene, a selective estrogen receptor modulator, induces apoptosis in androgen-responsive human prostate cancer cell line LNCaP through an androgen-independent pathway. *Cancer Res*. 2002;62:3649-3653.
59. Kim IY, Kim BC, Seong do H, et al. Raloxifene, a mixed estrogen agonist/antagonist, induces apoptosis in androgen-independent human prostate cancer cell lines. *Cancer Res*. 2002;62:5365-5369.
60. Ho SM. Estrogens and anti-estrogens: key mediators of prostate carcinogenesis and new therapeutic candidates. *J Cell Biochem*. 2004;91:491-503.
61. Morris GL, Dodd JG. Epidermal growth factor receptor mRNA levels in human prostatic tumors and cell lines. *J Urol*. 1990;143:1272-1274.
62. Sherwood ER, Lee C. Epidermal growth factor-related peptides and the epidermal growth factor receptor in normal and malignant prostate. *World J Urol*. 1995;13:290-296.
63. Glynne-Jones E, Goddard L, Harper ME. Comparative analysis of mRNA and protein expression for epidermal growth factor receptor and ligands relative to the proliferative index in human prostate tissue. *Hum Pathol*. 1996;27:688-694.
64. Sherwood ER, Van Dongen JL, Wood CG, Liao S, Kozlowski JM, Lee C. Epidermal growth factor receptor activation in androgen-independent but not androgen-stimulated growth of human prostatic carcinoma cells. *Br J Cancer*. 1998;77:855-861.
65. De Miguel P, Royuela, Bethencourt R, Ruiz A, Fraile B, Paniagua R. Immunohistochemical comparative analysis of transforming growth factor alpha, epidermal growth factor, and epidermal growth factor receptor in normal, hyperplastic and neoplastic human prostates. *Cytokine*. 1999;11:722-727.
66. Vicentini C, Festuccia C, Gravina GL, Angelucci A, Marronaro A, Bologna M. Prostate cancer cell proliferation is strongly reduced by the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 in vitro on human cell lines and primary cultures. *J Cancer Res Clin Oncol*. 2003;129:165-174.
67. Topping N, Dagnaes-Hansen F, Sorensen BS, Nexø E, Hynes NE. ErbB1 and prostate cancer: ErbB1 activity is essential for androgen-induced proliferation and protection from the apoptotic effects of LY294002. *Prostate*. 2003;56:142-149.
68. Mimeault M, Pommery N, Henichart JP. New advances on prostate carcinogenesis and therapies: involvement of EGF-EGFR transduction system. *Growth Factors*. 2003;21:1-14.
69. Myers RB, Kudlow JE, Grizzle WE. Expression of transforming growth factor-alpha, epidermal growth factor and the epidermal growth factor receptor in adenocarcinoma of the prostate and benign prostatic hyperplasia. *Mod Pathol*. 1993;6:733-737.
70. Seth D, Shaw K, Jazayeri J, Leedman PJ. Complex post-transcriptional regulation of EGF-receptor expression by EGF and TGF-alpha in human prostate cancer cells. *Br J Cancer*. 1999;80:657-669.
71. Brass AL, Barnard J, Patai BL, Salvi D, Rukstalis DB. Androgen up-regulates epidermal growth factor receptor expression and binding affinity in PC3 cell lines expressing the human androgen receptor. *Cancer Res*. 1995;55:3197-3203.
72. Myers RB, Oelschlager D, Manne U, Coan PN, Weiss H, Grizzle WE. Androgenic regulation of growth factor and growth factor receptor expression in the CWR22 model of prostatic adenocarcinoma. *Int J Cancer*. 1999;82:424-429.
73. Ye D, Mendelsohn J, Fan Z. Androgen and epidermal growth factor down-regulate cyclin-dependent kinase inhibitor p27Kip1 and costimulate proliferation of MDA PCa 2a and MDA PCa 2b prostate cancer cells. *Clin Cancer Res*. 1999;5:2171-2177.
74. Hobisch A, Fiechtl M, Sandahl-Sorensen B, et al. Prostate cancer cells generated during intermittent androgen ablation acquire a growth advantage and exhibit changes in epidermal growth factor receptor expression. *Prostate*. 2004;59:401-408.
75. Prewett M, Rockwell P, Rockwell RF, et al. The biologic effects of C225, a chimeric monoclonal antibody to the EGFR, on human prostate carcinoma. *J Immunother Emphasis Tumor Immunol*. 1996;19:419-427.
76. Putz T, Culig Z, Eder IE, et al. Epidermal growth factor (EGF) receptor blockade inhibits the action of EGF, insulin-like growth factor I, and a protein kinase A activator on the mitogen-activated protein kinase pathway in prostate cancer cell lines. *Cancer Res*. 1999;59:227-233.
77. Greco S, Muscella A, Elia MG, et al. Angiotensin II activates extracellular signal regulated kinases via protein kinase C and epidermal growth factor receptor in breast cancer cells. *J Cell Physiol*. 2003;196:370-377.
78. Barton J, Blackledge G, Wakeling A. Growth factors and their receptors: new targets for prostate cancer therapy. *Urology*. 2001;58:114-122.
79. Blackledge G, Averbuch S, Kay A, Barton J. Anti-EGF receptor therapy. *Prostate Cancer Prostatic Dis*. 2000;3:296-302.
80. Ciardiello F, De Vita F, Orditura M, De Placido S, Tortora G. Epidermal growth factor receptor tyrosine kinase inhibitors in late stage clinical trials. *Expert Opin Emerg Drugs*. 2003;8:501-514.
81. Mimeault M, Pommery N, Henichart JP. Synergistic antiproliferative and apoptotic effects induced by epidermal growth factor receptor and protein kinase A inhibitors in human prostatic cancer cell lines. *Int J Cancer*. 2003;106:116-124.
82. Wang J, Eltoum IE, Lamartiniere CA. Genistein alters growth factor signaling in transgenic prostate model (TRAMP). *Mol Cell Endocrinol*. 2004;219:171-180.
83. Hussain M, Banerjee M, Sarkar FH, et al. Soy isoflavones in the treatment of prostate cancer. *Nutr Cancer*. 2003;47:111-117.
84. deVere White RW, Hackman RM, Soares SE, Beckett LA, Li Y, Sun B. Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. *Urology*. 2004;63:259-263.
85. Mimeault M, Pommery N, Watez N, Bailly C, Henichart JP. Anti-proliferative and apoptotic effects of anandamide in

- human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. *Prostate*. 2003;56:1-12.
86. Ruiz L, Miguel A, Diaz-Laviada I. Delta9-tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism. *FEBS Lett*. 1999;458:400-404.
 87. Dorai T, Gehani N, Katz A. Therapeutic potential of curcumin in human prostate cancer: II. Curcumin inhibits tyrosine kinase activity of epidermal growth factor receptor and depletes the protein. *Mol Urol*. 2000;4:1-6.
 88. Tyagi A, Agarwal R, Agarwal C. Grape seed extract inhibits EGF-induced and constitutively active mitogenic signaling but activates JNK in human prostate carcinoma DU145 cells: possible role in antiproliferation and apoptosis. *Oncogene*. 2003;22:1302-1316.
 89. Sharma Y, Agarwal C, Singh AK, Agarwal R. Inhibitory effect of silibinin on ligand binding to erbB1 and associated mitogenic signaling, growth, and DNA synthesis in advanced human prostate carcinoma cells. *Mol Carcinog*. 2001;30:224-236.
 90. Stewart JR, O'Brian CA. Resveratrol antagonizes EGFR-dependent Erk1/2 activation in human androgen-independent prostate cancer cells with associated isozyme-selective PKC-alpha inhibition. *Invest New Drugs*. 2004;22:107-117.
 91. Mita MM, Mita A, Rowinsky EK. The molecular target of rapamycin (mTOR) as a therapeutic target against cancer. *Cancer Biol Ther*. 2003;2:S169-S177.
 92. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene*. 2004;23:3151-3171.
 93. Sehgal SN. Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant Proc*. 2003;35:7S-14S.
 94. Dutcher JP. Mammalian target of rapamycin (mTOR) Inhibitors. *Curr Oncol Rep*. 2004;6:111-115.
 95. van der Poel HG, Hanrahan C, Zhong H, Simons JW. Rapamycin induces Smad activity in prostate cancer cell lines. *Urol Res*. 2003;30:380-386.
 96. Grunwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB, Hidalgo M. Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. *Cancer Res*. 2002;62:6141-6145.
 97. Deocampo ND, Huang H, Tindall DJ. The role of PTEN in the progression and survival of prostate cancer. *Minerva Endocrinol*. 2003;28:145-153.
 98. Trotman LC, Niki M, Dotan ZA, et al. PTEN dose dictates cancer progression in the prostate. *PLoS Biol*. 2003;1:E59.
 99. Tolcher AW. Novel therapeutic molecular targets for prostate cancer: the mTOR signaling pathway and epidermal growth factor receptor. *J Urol*. 2004;171:S41-S43.
 100. Gasparian AV, Yao YJ, Kowalczyk D, et al. The role of IKK in constitutive activation of NF-kappaB transcription factor in prostate carcinoma cells. *J Cell Sci*. 2002;115:141-151.
 101. Ismail HA, Lessard L, Mes-Masson AM, Saad F. Expression of NF-kappaB in prostate cancer lymph node metastases. *Prostate*. 2004;58:308-313.
 102. Suh J, Rabson AB. NF-kappaB activation in human prostate cancer: important mediator or epiphenomenon? *J Cell Biochem*. 2004;91:100-117.
 103. Catz SD, Johnson JL. Transcriptional regulation of bcl-2 by nuclear factor kappa B and its significance in prostate cancer. *Oncogene*. 2001;20:7342-7351.
 104. Suh J, Payvandi F, Edelstein LC, et al. Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. *Prostate*. 2002;52:183-200.
 105. Kandouz M, Nie D, Pidgeon GP, Krishnamoorthy S, Maddipati KR, Honn KV. Platelet-type 12-lipoxygenase activates NF-kappaB in prostate cancer cells. *Prostaglandins Other Lipid Mediat*. 2003;71:189-204.
 106. Ling MT, Wang X, Ouyang XS, Xu K, Tsao SW, Wong YC. Id-1 expression promotes cell survival through activation of NF-kappaB signalling pathway in prostate cancer cells. *Oncogene*. 2003;22:4498-4508.
 107. Levine L, Lucci JA III, Pazdrak B, et al. Bombesin stimulates nuclear factor kappa B activation and expression of proangiogenic factors in prostate cancer cells. *Cancer Res*. 2003;63:3495-3502.
 108. Hodge JC, Bub J, Kaul S, Kajdacsy-Balla A, Lindholm PF. Requirement of RhoA activity for increased nuclear factor kappaB activity and PC-3 human prostate cancer cell invasion. *Cancer Res*. 2003;63:1359-1364.
 109. Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. *Oncogene*. 2001;20:4188-4197.
 110. Lessard L, Mes-Masson AM, Lamarre L, Wall L, Lattouf JB, Saad F. NF-kappa B nuclear localization and its prognostic significance in prostate cancer. *BJU Int*. 2003;91:417-420.
 111. Gupta S, Afaq F, Mukhtar H. Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. *Oncogene*. 2002;21:3727-3738.
 112. Shukla S, Gupta S. Molecular mechanisms for apigenin-induced cell-cycle arrest and apoptosis of hormone refractory human prostate carcinoma DU145 cells. *Mol Carcinog*. 2004;39:114-126.
 113. Shukla S, Gupta S. Suppression of constitutive and tumor necrosis factor alpha-induced nuclear factor (NF)-kappaB activation and induction of apoptosis by apigenin in human prostate carcinoma PC-3 cells: correlation with down-regulation of NF-kappaB-responsive genes. *Clin Cancer Res*. 2004;10:3169-3178.
 114. Hastak K, Gupta S, Ahmad N, Agarwal MK, Agarwal ML, Mukhtar H. Role of p53 and NF-kappaB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. *Oncogene*. 2003;22:4851-4859.
 115. Gupta S, Hastak K, Afaq F, Ahmad N, Mukhtar H. Essential role of caspases in epigallocatechin-3-gallate-mediated inhibition of nuclear factor kappa B and induction of apoptosis. *Oncogene*. 2004;23:2507-2522.
 116. Vayalil PK, Katiyar SK. Treatment of epigallocatechin-3-gallate inhibits matrix metalloproteinases-2 and -9 via inhibition of activation of mitogen-activated protein kinases, c-jun and NF-kappaB in human prostate carcinoma DU-145 cells. *Prostate*. 2004;59:33-42.
 117. Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene*. 2001;20:7597-7609.
 118. Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. *Oncogene*. 2004;23:1599-1607.
 119. Agarwal C, Dhanalakshmi S, Singh RP, Agarwal R. Inositol hexaphosphate inhibits constitutive activation of NF-kappa B in androgen-independent human prostate carcinoma DU145 cells. *Anticancer Res*. 2003;23:3855-3861.
 120. Ikezoe T, Yang Y, Heber D, Taguchi H, Koeffler HP. PC-SPES: a potent inhibitor of nuclear factor-kappa B rescues mice from lipopolysaccharide-induced septic shock. *Mol Pharmacol*. 2003;64:1521-1529.

121. Gasparian AV, Yao YJ, Lu J, et al. Selenium compounds inhibit I kappa B kinase (IKK) and nuclear factor-kappa B (NF-kappa B) in prostate cancer cells. *Mol Cancer Ther*. 2002;1:1079-1087.
122. Uzzo RG, Leavis P, Hatch W, et al. Zinc inhibits nuclear factor-kappa B activation and sensitizes prostate cancer cells to cytotoxic agents. *Clin Cancer Res*. 2002;8:3579-3583.
123. Davis JN, Kucuk O, Sarkar FH. Genistein inhibits NF-kappa B activation in prostate cancer cells. *Nutr Cancer*. 1999;35:167-174.
124. Li Y, Sarkar FH. Inhibition of nuclear factor kappaB activation in PC3 cells by genistein is mediated via Akt signaling pathway. *Clin Cancer Res*. 2002;8:2369-2377.
125. Dhanalakshmi S, Singh RP, Agarwal C, Agarwal R. Silibinin inhibits constitutive and TNFalpha-induced activation of NF-kappaB and sensitizes human prostate carcinoma DU145 cells to TNFalpha-induced apoptosis. *Oncogene*. 2002;21:1759-1767.
126. Chinni SR, Li Y, Upadhyay S, Koppolu PK, Sarkar FH. Indole-3-carbinol (I3C) induced cell growth inhibition, G1 cell cycle arrest and apoptosis in prostate cancer cells. *Oncogene*. 2001;20:2927-2936.
127. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs*. 2001;61:2035-2063.
128. Singh RP, Sharma G, Dhanalakshmi S, Agarwal C, Agarwal R. Suppression of advanced human prostate tumor growth in athymic mice by silibinin feeding is associated with reduced cell proliferation, increased apoptosis, and inhibition of angiogenesis. *Cancer Epidemiol Biomarkers Prev*. 2003;12:933-939.
129. Singh RP, Dhanalakshmi S, Tyagi AK, Chan DC, Agarwal C, Agarwal R. Dietary feeding of silibinin inhibits advance human prostate carcinoma growth in athymic nude mice and increases plasma insulin-like growth factor-binding protein-3 levels. *Cancer Res*. 2002;62:3063-3069.
130. Ikezoe T, Yang Y, Saito T, Koeffler HP, Taguchi H. Proteasome inhibitor PS-341 down-regulates prostate-specific antigen (PSA) and induces growth arrest and apoptosis of androgen-dependent human prostate cancer LNCaP cells. *Cancer Sci*. 2004;95:271-275.
131. Pajonk F, Himmelsbach J, Riess K, Sommer A, McBride WH. The human immunodeficiency virus (HIV)-1 protease inhibitor saquinavir inhibits proteasome function and causes apoptosis and radiosensitization in non-HIV-associated human cancer cells. *Cancer Res*. 2002;62:5230-5235.
132. Adams J, Kauffman M. Development of the proteasome inhibitor Velcade (bortezomib). *Cancer Invest*. 2004;22:304-311.
133. Gallardo-Williams MT, Maronpot RR, Wine RN, Brunssen SH, Chapin RE. Inhibition of the enzymatic activity of prostate-specific antigen by boric acid and 3-nitrophenyl boronic acid. *Prostate*. 2003;54:44-49.
134. Gallardo-Williams MT, Chapin RE, King PE, et al. Boron supplementation inhibits the growth and local expression of IGF-1 in human prostate adenocarcinoma (LNCaP) tumors in nude mice. *Toxicol Pathol*. 2004;32:73-78.
135. Brewer GJ, Merajver SD. Cancer therapy with tetrathiomolybdate: antiangiogenesis by lowering body copper—a review. *Integr Cancer Ther*. 2002;1:327-337.
136. Pan Q, Bao LW, Merajver SD. Tetrathiomolybdate inhibits angiogenesis and metastasis through suppression of the NFkappaB signaling cascade. *Mol Cancer Res*. 2003;1:701-706.
137. Pan Q, Bao LW, Merajver SD. Tetrathiomolybdate inhibits angiogenesis and metastasis through suppression of the NFkappaB signaling cascade. *Mol Cancer Res*. 2003;1:701-706.
138. van Golen KL, Bao L, Brewer GJ, et al. Suppression of tumor recurrence and metastasis by a combination of the PHSCN sequence and the antiangiogenic compound tetrathiomolybdate in prostate carcinoma. *Neoplasia*. 2002;4:373-379.
139. McCarty MF. A wholly nutritional “multifocal angiostatic therapy” for control of disseminated cancer. *Med Hypotheses*. 2003;61:1-15.
140. Feng P, Li TL, Guan ZX, Franklin RB, Costello LC. Effect of zinc on prostatic tumorigenicity in nude mice. *Ann NY Acad Sci*. 2003;1010:316-320.
141. Costello LC, Feng P, Milon B, Tan M, Franklin RB. Role of zinc in the pathogenesis and treatment of prostate cancer: critical issues to resolve. *Prostate Cancer Prostatic Dis*. 2004;7:111-117.
142. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *EMBO J*. 2003;22:4082-4090.
143. Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol Med*. 2002;8:S62-S67.
144. Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol*. 1996;16:4604-4613.
145. Krishnamachary B, Berg-Dixon S, Kelly B, et al. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res*. 2003;63:1138-1143.
146. Dang CV, Semenza GL. Oncogenic alterations of metabolism. *Trends Biochem Sci*. 1999;24:68-72.
147. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. *Cancer Res*. 2000;60:4693-4696.
148. Birner P, Gatterbauer B, Oberhuber G, et al. Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. *Cancer*. 2001;92:165-171.
149. Giatromanolaki A, Koukourakis MI, Sivridis E, et al. Relation of hypoxia inducible factor 1 alpha and 2 alpha in operable non-small cell lung cancer to angiogenic/ molecular profile of tumours and survival. *Br J Cancer*. 2001;85:881-890.
150. Schindl M, Schoppmann SF, Samonigg H, et al. Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer. *Clin Cancer Res*. 2002;8:1831-1837.
151. Sivridis E, Giatromanolaki A, Gatter KC, Harris AL, Koukourakis MI. Association of hypoxia-inducible factors 1alpha and 2alpha with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. *Cancer*. 2002;95:1055-1063.
152. Bos R, van der GP, Greijer AE, et al. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. *Cancer*. 2003;97:1573-1581.
153. Kurokawa T, Miyamoto M, Kato K, et al. Overexpression of hypoxia-inducible-factor 1alpha (HIF-1alpha) in oesophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. *Br J Cancer*. 2003;89:1042-1047.
154. Giatromanolaki A, Sivridis E, Kouskoukis C, Gatter KC, Harris AL, Koukourakis MI. Hypoxia-inducible factors 1alpha and 2alpha are related to vascular endothelial growth factor expression and a poorer prognosis in nodular malignant melanomas of the skin. *Melanoma Res*. 2003;13:493-501.
155. Theodoropoulos VE, Lazaris AC, Sofras F, et al. Hypoxia-inducible factor 1alpha expression correlates with angiogenesis and unfavorable prognosis in bladder cancer. *Eur Urol*. 2004;46:200-208.
156. Schindl M, Schoppmann SF, Samonigg H, et al. Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer. *Clin Cancer Res*. 2002;8:1831-1837.

157. Sivridis E, Giatromanolaki A, Gatter KC, Harris AL, Koukourakis MI. Association of hypoxia-inducible factors 1alpha and 2alpha with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. *Cancer*. 2002;95:1055-1063.
158. Zhong H, Agani F, Baccala AA, et al. Increased expression of hypoxia inducible factor-1alpha in rat and human prostate cancer. *Cancer Res*. 1998;58:5280-5284.
159. Saramaki OR, Savinainen KJ, Nupponen NN, Bratt O, Visakorpi T. Amplification of hypoxia-inducible factor 1alpha gene in prostate cancer. *Cancer Genet Cytogenet*. 2001;128:31-34.
160. Du Z, Fujiyama C, Chen Y, Masaki Z. Expression of hypoxia-inducible factor 1alpha in human normal, benign, and malignant prostate tissue. *Chin Med J (Engl)*. 2003;116:1936-1939.
161. Zhong H, Semenza GL, Simons JW, De Marzo AM. Up-regulation of hypoxia-inducible factor 1alpha is an early event in prostate carcinogenesis. *Cancer Detect Prev*. 2004;28:88-93.
162. Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. *J Biol Chem*. 2002;277:27975-27981.
163. Skinner HD, Zhong XS, Gao N, Shi X, Jiang BH. Arsenite induces p70S6K1 activation and HIF-1alpha expression in prostate cancer cells. *Mol Cell Biochem*. 2004;255:19-23.
164. Lee E, Yim S, Lee SK, Park H. Two transactivation domains of hypoxia-inducible factor-1alpha regulated by the MEK-1/p42/p44 MAPK pathway. *Mol Cells*. 2002;14:9-15.
165. Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line. *J Biol Chem*. 2002;277:50081-50086.
166. Mottet D, Michel G, Renard P, Ninane N, Raes M, Michiels C. Role of ERK and calcium in the hypoxia-induced activation of HIF-1. *J Cell Physiol*. 2003;194:30-44.
167. Sang N, Stiehl DP, Bohensky J, Leshchinsky I, Srinivas V, Caro J. MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300. *J Biol Chem*. 2003;278:14013-14019.
168. Page EL, Robitaille GA, Pouyssegur J, Richard DE. Induction of hypoxia-inducible factor-1alpha by transcriptional and translational mechanisms. *J Biol Chem*. 2002;277:48403-48409.
169. Zhong H, Chiles K, Feldser D, et al. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res*. 2000;60:1541-1545.
170. Hudson CC, Liu M, Chiang GG, et al. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol*. 2002;22:7004-7014.
171. Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line. *J Biol Chem*. 2002;277:50081-50086.
172. Mabjeesh NJ, Willard MT, Frederickson CE, Zhong H, Simons JW. Androgens stimulate hypoxia-inducible factor 1 activation via autocrine loop of tyrosine kinase receptor/phosphatidylinositol 3-kinase/protein kinase B in prostate cancer cells. *Clin Cancer Res*. 2003;9:2416-2425.
173. Kruger EA, Blagosklonny MV, Dixon SC, Figg WD. UCN-01, a protein kinase C inhibitor, inhibits endothelial cell proliferation and angiogenic hypoxic response. *Invasion Metastasis*. 1998;18:209-218.
174. Anastasiadis AG, Ghafar MA, Salomon L, et al. Human hormone-refractory prostate cancers can harbor mutations in the O(2)-dependent degradation domain of hypoxia inducible factor-1alpha (HIF-1alpha). *J Cancer Res Clin Oncol*. 2002;128:358-362.
175. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *J Biol Chem*. 2002;277:29936-29944.
176. Mabjeesh NJ, Post DE, Willard MT, et al. Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteasome pathway in prostate cancer cells. *Cancer Res*. 2002;62:2478-2482.
177. Solit DB, Zheng FF, Drobnjak M, et al. 17-allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. *Clin Cancer Res*. 2002;8:986-993.
178. Workman P. Overview: translating Hsp90 biology into Hsp90 drugs. *Curr Cancer Drug Targets*. 2003;3:297-300.
179. Vanaja DK, Mitchell SH, Toft DO, Young CY. Effect of geldanamycin on androgen receptor function and stability. *Cell Stress Chaperones*. 2002;7:55-64.
180. Enmon R, Yang WH, Ballangrud AM, et al. Combination treatment with 17-N-allylamino-17-demethoxy geldanamycin and acute irradiation produces supra-additive growth suppression in human prostate carcinoma spheroids. *Cancer Res*. 2003;63:8393-8399.
181. Goetz MP, Toft DO, Ames MM, Erlichman C. The Hsp90 chaperone complex as a novel target for cancer therapy. *Ann Oncol*. 2003;14:1169-1176.
182. Palayoor ST, Tofilon PJ, Coleman CN. Ibuprofen-mediated reduction of hypoxia-inducible factors HIF-1alpha and HIF-2alpha in prostate cancer cells. *Clin Cancer Res*. 2003;9:3150-3157.
183. Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. *Cancer Res*. 2003;63:1764-1768.
184. Kaur G, Belotti D, Burger AM, et al. Antiangiogenic properties of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. *Clin Cancer Res*. 2004;10:4813-4821.
185. Zhang H, Burrows F. Targeting multiple signal transduction pathways through inhibition of Hsp90. *J Mol Med*. 2004;82:488-499.
186. Kamal A, Thao L, Sensintaffar J, et al. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature*. 2003;425:407-410.
187. Subbarayan V, Sabichi AL, Llansa N, Lippman SM, Menter DG. Differential expression of cyclooxygenase-2 and its regulation by tumor necrosis factor-alpha in normal and malignant prostate cells. *Cancer Res*. 2001;61:2720-2726.
188. Zha S, Gage WR, Sauvageot J, et al. Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. *Cancer Res*. 2001;61:8617-8623.
189. Gupta S, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. *Prostate*. 2000;42:73-78.
190. Yoshimura R, Sano H, Masuda C, et al. Expression of cyclooxygenase-2 in prostate carcinoma. *Cancer*. 2000;89:589-596.
191. Uotila P, Valve E, Martikainen P, Nevalainen M, Nurmi M, Harkonen P. Increased expression of cyclooxygenase-2 and nitric oxide synthase-2 in human prostate cancer. *Urol Res*. 2001;29:23-28.
192. Lee LM, Pan CC, Cheng CJ, Chi CW, Liu TY. Expression of cyclooxygenase-2 in prostate adenocarcinoma and benign prostatic hyperplasia. *Anticancer Res*. 2001;21:1291-1294.
193. Newton R, Kuitert LM, Bergmann M, Adcock IM, Barnes PJ. Evidence for involvement of NF-kappaB in the transcriptional

- control of COX-2 gene expression by IL-1beta. *Biochem Biophys Res Commun.* 1997;237:28-32.
194. von Knethen A, Callsen D, Brune B. NF-kappaB and AP-1 activation by nitric oxide attenuated apoptotic cell death in RAW 264.7 macrophages. *Mol Biol Cell.* 1999;10:361-372.
 195. Allport VC, Slater DM, Newton R, Bennett PR. NF-kappaB and AP-1 are required for cyclo-oxygenase 2 gene expression in amnion epithelial cell line (WISH). *Mol Hum Reprod.* 2000; 6:561-565.
 196. Liu XH, Yao S, Kirschenbaum A, Levine AC. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. *Cancer Res.* 1998; 58:4245-4249.
 197. Kamijo T, Sato T, Nagatomi Y, Kitamura T. Induction of apoptosis by cyclooxygenase-2 inhibitors in prostate cancer cell lines. *Int J Urol.* 2001;8:S35-S39.
 198. Kirschenbaum A, Liu X, Yao S, Levine AC. The role of cyclooxygenase-2 in prostate cancer. *Urology.* 2001;58:127-131.
 199. Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E(2) stimulates prostatic intraepithelial neoplasia cell growth through activation of the interleukin-6/GPI30/STAT-3 signaling pathway. *Biochem Biophys Res Commun.* 2002;290:249-255.
 200. Wen B, Deutsch E, Eschwege P, et al. Cyclooxygenase-2 inhibitor NS398 enhances antitumor effect of irradiation on hormone refractory human prostate carcinoma cells. *J Urol.* 2003; 170:2036-2039.
 201. Liu XH, Kirschenbaum A, Yao S, Lee R, Holland JF, Levine AC. Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer in vivo. *J Urol.* 2000;164:820-825.
 202. Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. *J Biol Chem.* 2000;275:11397-11403.
 203. Song X, Lin HP, Johnson AJ, et al. Cyclooxygenase-2, player or spectator in cyclooxygenase-2 inhibitor-induced apoptosis in prostate cancer cells. *J Natl Cancer Inst.* 2002;94:585-591.
 204. Andrews J, Djakiew D, Krygier S, Andrews P. Superior effectiveness of ibuprofen compared with other NSAIDs for reducing the survival of human prostate cancer cells. *Cancer Chemother Pharmacol.* 2002;50:277-284.
 205. Srinath P, Rao PN, Knaus EE, Suresh MR. Effect of cyclooxygenase-2 (COX-2) inhibitors on prostate cancer cell proliferation. *Anticancer Res.* 2003;23:3923-3928.
 206. Chaudry AA, Wahle KW, McClinton S, Moffat LE. Arachidonic acid metabolism in benign and malignant prostatic tissue in vitro: effects of fatty acids and cyclooxygenase inhibitors. *Int J Cancer.* 1994;57:176-180.
 207. Chen Y, Hughes-Fulford M. Prostaglandin E2 and the protein kinase A pathway mediate arachidonic acid induction of c-fos in human prostate cancer cells. *Br J Cancer.* 2000;82:2000-2006.
 208. Negishi M, Sugimoto Y, Ichikawa A. Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim Biophys Acta.* 1995;1259:109-119.
 209. Garrido C, Saule S, Gospodarowicz D. Transcriptional regulation of vascular endothelial growth factor gene expression in ovarian bovine granulosa cells. *Growth Factors.* 1993;8:109-117.
 210. Damert A, Ikeda E, Risau W. Activator-protein-1 binding potentiates the hypoxia-inducible factor-1-mediated hypoxia-induced transcriptional activation of vascular-endothelial growth factor expression in C6 glioma cells. *Biochem J.* 1997; 327(pt 2):419-423.
 211. Josko J, Mazurek M. Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. *Med Sci Monit.* 2004;10:RA89-RA98.
 212. Lin DW, Nelson PS. The role of cyclooxygenase-2 inhibition for the prevention and treatment of prostate carcinoma. *Clin Prostate Cancer.* 2003;2:119-126.
 213. Liu XH, Kirschenbaum A, Yao S, et al. Upregulation of vascular endothelial growth factor by cobalt chloride-simulated hypoxia is mediated by persistent induction of cyclooxygenase-2 in a metastatic human prostate cancer cell line. *Clin Exp Metastasis.* 1999;17:687-694.
 214. Attiga FA, Fernandez PM, Weeraratna AT, Manyak MJ, Patierno SR. Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases. *Cancer Res.* 2000;60:4629-4637.
 215. Nithipatikom K, Isbell MA, Lindholm PF, Kajdacsy-Balla A, Kaul S, Campell WB. Requirement of cyclooxygenase-2 expression and prostaglandins for human prostate cancer cell invasion. *Clin Exp Metastasis.* 2002;19:593-601.
 216. Pruthi RS, Derksen JE, Moore D. A pilot study of use of the cyclooxygenase-2 inhibitor celecoxib in recurrent prostate cancer after definitive radiation therapy or radical prostatectomy. *BJU Int.* 2004;93:275-278.
 217. Goldmann WH, Sharma AL, Currier SJ, Johnston PD, Rana A, Sharma CP. Saw palmetto berry extract inhibits cell growth and Cox-2 expression in prostatic cancer cells. *Cell Biol Int.* 2001; 25:1117-1124.
 218. Juarranz MG, Bolanos O, Gutierrez-Canas I, et al. Neuroendocrine differentiation of the LNCaP prostate cancer cell line maintains the expression and function of VIP and PACAP receptors. *Cell Signal.* 2001;13:887-894.
 219. Gutierrez-Canas I, Rodriguez-Henche N, Bolanos O, Carmena MJ, Prieto JC, Juarranz MG. VIP and PACAP are autocrine factors that protect the androgen-independent prostate cancer cell line PC-3 from apoptosis induced by serum withdrawal. *Br J Pharmacol.* 2003;139:1050-1058.
 220. Dougherty KM, Blomme EA, Koh AJ, et al. Parathyroid hormone-related protein as a growth regulator of prostate carcinoma. *Cancer Res.* 1999;59:6015-6022.
 221. Bryden AA, Hoyland JA, Freemont AJ, Clarke NW, George NJ. Parathyroid hormone related peptide and receptor expression in paired primary prostate cancer and bone metastases. *Br J Cancer.* 2002;86:322-325.
 222. Tovar Sepulveda VA, Falzon M. Parathyroid hormone-related protein enhances PC-3 prostate cancer cell growth via both autocrine/paracrine and intracrine pathways. *Regul Pept.* 2002; 105:109-120.
 223. Shulkes A, Fletcher DR, Rubinstein C, Ebeling PR, Martin TJ. Production of calcitonin gene related peptide, calcitonin and PTH-related protein by a prostatic adenocarcinoma. *Clin Endocrinol (Oxf).* 1991;34:387-393.
 224. Gkonos PJ, Lokeshwar BL, Balkan W, Roos BA. Neuroendocrine peptides stimulate adenylyl cyclase in normal and malignant prostate cells. *Regul Pept.* 1995;59:43-51.
 225. Tortora G, Ciardiello F. Protein kinase A as target for novel integrated strategies of cancer therapy. *Ann N Y Acad Sci.* 2002; 968:139-147.
 226. Ciardiello F, Tortora G. Interactions between the epidermal growth factor receptor and type I protein kinase A: biological significance and therapeutic implications. *Clin Cancer Res.* 1998;4:821-828.
 227. Cho-Chung YS, Nesterova M, Becker KG, et al. Dissecting the circuitry of protein kinase A and cAMP signaling in cancer genesis: antisense, microarray, gene overexpression, and transcription factor decoy. *Ann N Y Acad Sci.* 2002;968:22-36.
 228. Scala S, Budillon A, Zhan Z, et al. Downregulation of mdr-1 expression by 8-Cl-cAMP in multidrug resistant MCF-7 human breast cancer cells. *J Clin Invest.* 1995;96:1026-1034.
 229. Wang H, Cai Q, Zeng X, Yu D, Agrawal S, Zhang R. Antitumor activity and pharmacokinetics of a mixed-backbone antisense oligonucleotide targeted to the R1alpha subunit of protein kinase A after oral administration. *Proc Natl Acad Sci U S A.* 1999;96:13989-13994.

230. Chen HX, Marshall JL, Ness E, et al. A safety and pharmacokinetic study of a mixed-backbone oligonucleotide (GEM231) targeting the type I protein kinase A by two-hour infusions in patients with refractory solid tumors. *Clin Cancer Res.* 2000; 6:1259-1266.
231. Mami S, Goel S, Nesterova M, et al. Clinical studies in patients with solid tumors using a second-generation antisense oligonucleotide (GEM 231) targeted against protein kinase A type I. *Ann N Y Acad Sci.* 2003;1002:252-262.
232. Ciardiello F, Damiano V, Bianco R, et al. Antitumor activity of combined blockade of epidermal growth factor receptor and protein kinase A. *J Natl Cancer Inst.* 1996;88:1770-1776.
233. Ciardiello F, Damiano V, Bianco C, et al. Cooperative antiproliferative effects of 8-chloro-cyclic AMP and 528 anti-epidermal growth factor receptor monoclonal antibody on human cancer cells. *Clin Cancer Res.* 1995;1:161-167.
234. Ciardiello F, Caputo R, Bianco R, et al. Cooperative inhibition of renal cancer growth by anti-epidermal growth factor receptor antibody and protein kinase A antisense oligonucleotide. *J Natl Cancer Inst.* 1998;90:1087-1094.
235. Sugamoto T, Tanji N, Sato K, et al. The expression of basic fibroblast growth factor and vascular endothelial growth factor in prostatic adenocarcinoma: correlation with neovascularization. *Anticancer Res.* 2001;21:77-88.
236. Stefanou D, Batistatou A, Kamina S, Arkoumani E, Papachristou DJ, Agnantis NJ. Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in benign prostatic hyperplasia and prostate cancer. *In Vivo.* 2004;18:155-160.
237. Trojan L, Thomas D, Knoll T, Grobholz R, Alken P, Michel MS. Expression of pro-angiogenic growth factors VEGF, EGF and bFGF and their topographical relation to neovascularisation in prostate cancer. *Urol Res.* 2004;32:97-103.
238. Strohmeier D, Strauss F, Rossing C, et al. Expression of bFGF, VEGF and c-met and their correlation with microvessel density and progression in prostate carcinoma. *Anticancer Res.* 2004; 24:1797-1804.
239. Melnyk O, Zimmerman M, Kim KJ, Shuman M. Neutralizing anti-vascular endothelial growth factor antibody inhibits further growth of established prostate cancer and metastases in a pre-clinical model. *J Urol.* 1999;161:960-963.
240. Fox WD, Higgins B, Maiese KM, et al. Antibody to vascular endothelial growth factor slows growth of an androgen-independent xenograft model of prostate cancer. *Clin Cancer Res.* 2002;8:3226-3231.
241. Becker CM, Farnebo FA, Iordanescu I, et al. Gene therapy of prostate cancer with the soluble vascular endothelial growth factor receptor Flk1. *Cancer Biol Ther.* 2002;1:548-553.
242. Finkenzeller G, Sparacio A, Technau A, Marme D, Siemeister G. Sp1 recognition sites in the proximal promoter of the human vascular endothelial growth factor gene are essential for platelet-derived growth factor-induced gene expression. *Oncogene.* 1997;15:669-676.
243. Shi Q, Le X, Abbruzzese JL, et al. Constitutive Sp1 activity is essential for differential constitutive expression of vascular endothelial growth factor in human pancreatic adenocarcinoma. *Cancer Res.* 2001;61:4143-4154.
244. Schafer G, Cramer T, Suske G, Kemmner W, Wiedenmann B, Hocker M. Oxidative stress regulates vascular endothelial growth factor-A gene transcription through Sp1- and Sp3-dependent activation of two proximal GC-rich promoter elements. *J Biol Chem.* 2003;278:8190-8198.
245. Niu G, Wright KL, Huang M, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene.* 2002;21:2000-2008.
246. Wei D, Le X, Zheng L, et al. Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene.* 2003;22:319-329.
247. Wei LH, Kuo ML, Chen CA, et al. Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. *Oncogene.* 2003;22:1517-1527.
248. Ruiz M, Pettaway C, Song R, Stoeltzing O, Ellis L, Bar-Eli M. Activator protein 2alpha inhibits tumorigenicity and represses vascular endothelial growth factor transcription in prostate cancer cells. *Cancer Res.* 2004;64:631-638.
249. Milanini-Mongiat J, Pouyssegur J, Pages G. Identification of two Sp1 phosphorylation sites for p42/p44 mitogen-activated protein kinases: their implication in vascular endothelial growth factor gene transcription. *J Biol Chem.* 2002;277:20631-20639.
250. Reisinger K, Kaufmann R, Gille J. Increased Sp1 phosphorylation as a mechanism of hepatocyte growth factor (HGF/SF)-induced vascular endothelial growth factor (VEGF/VPF) transcription. *J Cell Sci.* 2003;116:225-238.
251. Pal S, Claffey KP, Cohen HT, Mukhopadhyay D. Activation of Sp1-mediated vascular permeability factor/vascular endothelial growth factor transcription requires specific interaction with protein kinase C zeta. *J Biol Chem.* 1998;273:26277-26280.
252. Biesiada E, Razandi M, Levin ER. Egr-1 activates basic fibroblast growth factor transcription: mechanistic implications for astrocyte proliferation. *J Biol Chem.* 1996;271:18576-18581.
253. Wang D, Mayo MW, Baldwin AS Jr. Basic fibroblast growth factor transcriptional autoregulation requires EGR-1. *Oncogene.* 1997;14:2291-2299.
254. Gupta S, Srivastava M, Ahmad N, Sakamoto K, Bostwick DG, Mukhtar H. Lipoxygenase-5 is overexpressed in prostate adenocarcinoma. *Cancer.* 2001;91:737-743.
255. Matsuyama M, Yoshimura R, Mitsunashi M, et al. Expression of lipoxygenase in human prostate cancer and growth reduction by its inhibitors. *Int J Oncol.* 2004;24:821-827.
256. Ghosh J, Myers CE. Arachidonic acid stimulates prostate cancer cell growth: critical role of 5-lipoxygenase. *Biochem Biophys Res Commun.* 1997;235:418-423.
257. Ghosh J, Myers CE. Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. *Proc Natl Acad Sci U S A.* 1998;95:13182-13187.
258. Ghosh J. Inhibition of arachidonate 5-lipoxygenase triggers prostate cancer cell death through rapid activation of c-Jun N-terminal kinase. *Biochem Biophys Res Commun.* 2003;307:342-349.
259. Anderson KM, Seed T, Vos M, et al. 5-lipoxygenase inhibitors reduce PC-3 cell proliferation and initiate nonnecrotic cell death. *Prostate.* 1998;37:161-173.
260. Yang P, Collin P, Madden T, et al. Inhibition of proliferation of PC3 cells by the branched-chain fatty acid, 12-methyltetradecanoic acid, is associated with inhibition of 5-lipoxygenase. *Prostate.* 2003;55:281-291.
261. Pidgeon GP, Tang K, Cai YL, Piasentin E, Honn KV. Overexpression of platelet-type 12-lipoxygenase promotes tumor cell survival by enhancing alpha(v)beta(3) and alpha(v)beta(5) integrin expression. *Cancer Res.* 2003;63:4258-4267.
262. Safayhi H, Mack T, Sabieraj J, Anazodo MI, Subramanian LR, Ammon HP. Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase. *J Pharmacol Exp Ther.* 1992;261:1143-1146.
263. Gupta I, Gupta V, Parihar A, et al. Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study. *Eur J Med Res.* 1998;3:511-514.

264. Wenzel SE, Kamada AK. Zileuton: the first 5-lipoxygenase inhibitor for the treatment of asthma. *Ann Pharmacother.* 1996; 30:858-864.
265. O'Flaherty JT, Rogers LC, Chadwell BA, et al. 5(S)-hydroxy-6,8,11,14-E,Z,Z,Z-eicosatetraenoate stimulates PC3 cell signaling and growth by a receptor-dependent mechanism. *Cancer Res.* 2002;62:6817-6819.
266. Ding XZ, Tong WG, Adrian TE. Multiple signal pathways are involved in the mitogenic effect of 5(S)-HETE in human pancreatic cancer. *Oncology.* 2003;65:285-294.
267. Ghosh J. Rapid induction of apoptosis in prostate cancer cells by selenium: reversal by metabolites of arachidonate 5-lipoxygenase. *Biochem Biophys Res Commun.* 2004;315:624-635.
268. Gao X, Porter AT, Honn KV. Involvement of the multiple tumor suppressor genes and 12-lipoxygenase in human prostate cancer: therapeutic implications. *Adv Exp Med Biol.* 1997; 407:41-53.
269. Matsuyama M, Yoshimura R, Mitsuhashi M, et al. Expression of lipoxygenase in human prostate cancer and growth reduction by its inhibitors. *Int J Oncol.* 2004;24:821-827.
270. Pidgeon GP, Kandouz M, Meram A, Honn KV. Mechanisms controlling cell cycle arrest and induction of apoptosis after 12-lipoxygenase inhibition in prostate cancer cells. *Cancer Res.* 2002;62:2721-2727.
271. Kandouz M, Nie D, Pidgeon GP, Krishnamoorthy S, Maddipati KR, Honn KV. Platelet-type 12-lipoxygenase activates NF-kappaB in prostate cancer cells. *Prostaglandins Other Lipid Mediat.* 2003;71:189-204.
272. Nie D, Nemeth J, Qiao Y, et al. Increased metastatic potential in human prostate carcinoma cells by overexpression of arachidonate 12-lipoxygenase. *Clin Exp Metastasis.* 2003;20:657-663.
273. Timar J, Raso E, Dome B, et al. Expression, subcellular localization and putative function of platelet-type 12-lipoxygenase in human prostate cancer cell lines of different metastatic potential. *Int J Cancer.* 2000;87:37-43.
274. Tang DG, Diglio CA, Honn KV. Activation of microvascular endothelium by eicosanoid 12(S)-hydroxyeicosatetraenoic acid leads to enhanced tumor cell adhesion via up-regulation of surface expression of alpha v beta 3 integrin: a posttranscriptional, protein kinase C- and cytoskeleton-dependent process. *Cancer Res.* 1994;54:1119-1129.
275. Tang DG, Renaud C, Stojakovic S, Diglio CA, Porter A, Honn KV. 12(S)-HETE is a mitogenic factor for microvascular endothelial cells: its potential role in angiogenesis. *Biochem Biophys Res Commun.* 1995;211:462-468.
276. Nie D, Tang K, Diglio C, Honn KV. Eicosanoid regulation of angiogenesis: role of endothelial arachidonate 12-lipoxygenase. *Blood.* 2000;95:2304-2311.
277. Eliceiri BP, Cheresh DA. Role of alpha v integrins during angiogenesis. *Cancer J.* 2000;6(suppl 3):S245-S249.
278. Liu JJ, Huang TS, Cheng WF, Lu FJ. Baicalein and baicalin are potent inhibitors of angiogenesis: inhibition of endothelial cell proliferation, migration and differentiation. *Int J Cancer.* 2003;106:559-565.
279. Tong WG, Ding XZ, Witt RC, Adrian TE. Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. *Mol Cancer Ther.* 2002;1:929-935.
280. Fujita M, Hayashi I, Yamashina S, Itoman M, Majima M. Blockade of angiotensin AT1a receptor signaling reduces tumor growth, angiogenesis, and metastasis. *Biochem Biophys Res Commun.* 2002;294:441-447.
281. Miyajima A, Kosaka T, Asano T, et al. Angiotensin II type I antagonist prevents pulmonary metastasis of murine renal cancer by inhibiting tumor angiogenesis. *Cancer Res.* 2002; 62:4176-4179.
282. Abali H, Gullu IH, Engin H, Haznedaroglu IC, Erman M, Tekuzman G. Old antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type I receptor antagonists. *Med Hypotheses.* 2002;59:344-348.
283. Egami K, Murohara T, Shimada T, et al. Role of host angiotensin II type I receptor in tumor angiogenesis and growth. *J Clin Invest.* 2003;112:67-75.
284. Walther T, Menrad A, Orzechowski HD, Siemeister G, Paul M, Schirner M. Differential regulation of in vivo angiogenesis by angiotensin II receptors. *FASEB J.* 2003;17:2061-2067.
285. Uemura H, Ishiguro H, Nakaigawa N, et al. Angiotensin II receptor blocker shows antiproliferative activity in prostate cancer cells: a possibility of tyrosine kinase inhibitor of growth factor. *Mol Cancer Ther.* 2003;2:1139-1147.
286. Dugourd C, Gervais M, Corvol P, Monnot C. Akt is a major downstream target of PI3-kinase involved in angiotensin II-induced proliferation. *Hypertension.* 2003;41:882-890.
287. Touyz RM, He G, Wu XH, Park JB, Mabrouk ME, Schiffrin EL. Src is an important mediator of extracellular signal-regulated kinase 1/2-dependent growth signaling by angiotensin II in smooth muscle cells from resistance arteries of hypertensive patients. *Hypertension.* 2001;38:56-64.
288. Sayeski PP, Ali MS. The critical role of c-Src and the Shc/Grb2/ERK2 signaling pathway in angiotensin II-dependent VSMC proliferation. *Exp Cell Res.* 2003;287:339-349.
289. Lin J, Freeman MR. Transactivation of ErbB1 and ErbB2 receptors by angiotensin II in normal human prostate stromal cells. *Prostate.* 2003;54:1-7.
290. Medeiros R, Vasconcelos A, Costa S, et al. Linkage of angiotensin I-converting enzyme gene insertion/deletion polymorphism to the progression of human prostate cancer. *J Pathol.* 2004;202:330-335.
291. Ronquist G, Rodriguez LA, Ruigomez A, et al. Association between captopril, other antihypertensive drugs and risk of prostate cancer. *Prostate.* 2004;58:50-56.
292. Taub JS, Guo R, Leeb-Lundberg LM, Madden JF, Daaka Y. Bradykinin receptor subtype 1 expression and function in prostate cancer. *Cancer Res.* 2003;63:2037-2041.
293. Clements J, Mukhtar A. Tissue kallikrein and the bradykinin B2 receptor are expressed in endometrial and prostate cancers. *Immunopharmacology.* 1997;36:217-220.
294. Barki-Harrington L, Daaka Y. Bradykinin induced mitogenesis of androgen independent prostate cancer cells. *J Urol.* 2001; 165:2121-2125.
295. Stewart JM, Chan DC, Simkeviciene V et al. Bradykinin antagonists as new drugs for prostate cancer. *Int Immunopharmacol.* 2002;2:1781-1786.
296. Stewart JM. Bradykinin antagonists as anti-cancer agents. *Curr Pharm Des.* 2003;9:2036-2042.
297. Benndorf R, Boger RH, Ergun S, Steenpass A, Wieland T. Angiotensin II type 2 receptor inhibits vascular endothelial growth factor-induced migration and in vitro tube formation of human endothelial cells. *Circ Res.* 2003;93:438-447.
298. Raj GV, Barki-Harrington L, Kue PF, Daaka Y. Guanosine phosphate binding protein coupled receptors in prostate cancer: a review. *J Urol.* 2002;167:1458-1463.
299. Daaka Y. G proteins in cancer: the prostate cancer paradigm. *Sci STKE.* 2004;2004:re2.
300. Xiao D, Qu X, Weber HC. Activation of extracellular signal-regulated kinase mediates bombesin-induced mitogenic responses in prostate cancer cells. *Cell Signal.* 2003;15:945-953.
301. Levine L, Lucci JA III, Pazdrak B, et al. Bombesin stimulates nuclear factor kappa B activation and expression of

- proangiogenic factors in prostate cancer cells. *Cancer Res.* 2003;63:3495-3502.
302. Milovanovic SR, Radulovic S, Groot K, Schally AV. Inhibition of growth of PC-82 human prostate cancer line xenografts in nude mice by bombesin antagonist RC-3095 or combination of agonist [D-Trp6]-luteinizing hormone-releasing hormone and somatostatin analog RC-160. *Prostate.* 1992;20:269-280.
 303. Pinski J, Halmos G, Schally AV. Somatostatin analog RC-160 and bombesin/gastrin-releasing peptide antagonist RC-3095 inhibit the growth of androgen-independent DU-145 human prostate cancer line in nude mice. *Cancer Lett.* 1993;71:189-196.
 304. Kue PF, Daaka Y. Essential role for G proteins in prostate cancer cell growth and signaling. *J Urol.* 2000;164:2162-2167.
 305. Maa MC, Leu TH, McCarley DJ, Schatzman RC, Parsons SJ. Potentiation of epidermal growth factor receptor-mediated oncogenesis by c-Src: implications for the etiology of multiple human cancers. *Proc Natl Acad Sci U S A.* 1995;92:6981-6985.
 306. Tice DA, Biscardi JS, Nickles AL, Parsons SJ. Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc Natl Acad Sci U S A.* 1999;96:1415-1420.
 307. Biscardi JS, Ishizawa RC, Silva CM, Parsons SJ. Tyrosine kinase signalling in breast cancer: epidermal growth factor receptor and c-Src interactions in breast cancer. *Breast Cancer Res.* 2000; 2:203-210.
 308. Biscardi JS, Maa MC, Tice DA, Cox ME, Leu TH, Parsons SJ. c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function. *J Biol Chem.* 1999;274:8335-8343.
 309. Sato K, Nagao T, Iwasaki T, Nishihira Y, Fukami Y. Src-dependent phosphorylation of the EGF receptor Tyr-845 mediates Stat-p21waf1 pathway in A431 cells. *Genes Cells.* 2003; 8:995-1003.
 310. Olayioye MA, Beuving I, Horsch K, Daly JM, Hynes NE. ErbB receptor-induced activation of stat transcription factors is mediated by Src tyrosine kinases. *J Biol Chem.* 1999;274:17209-17218.
 311. Karni R, Jove R, Levitzki A. Inhibition of pp60c-Src reduces Bcl-XL expression and reverses the transformed phenotype of cells overexpressing EGF and HER-2 receptors. *Oncogene.* 1999;18:4654-4662.
 312. Xi S, Zhang Q, Dyer KF, et al. Src kinases mediate STAT growth pathways in squamous cell carcinoma of the head and neck. *J Biol Chem.* 2003;278:31574-31583.
 313. Shi CS, Kehrl JH. Pyk2 amplifies epidermal growth factor and c-Src-induced Stat3 activation. *J Biol Chem.* 2004;279:17224-17231.
 314. Calo V, Migliavacca M, Bazan V, et al. STAT proteins: from normal control of cellular events to tumorigenesis. *J Cell Physiol.* 2003;197:157-168.
 315. Epling-Burnette PK, Liu JH, Catlett-Falcone R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest.* 2001;107:351-362.
 316. Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res.* 2000;60:2132-2135.
 317. Matsuda T, Junicho A, Yamamoto T, et al. Cross-talk between signal transducer and activator of transcription 3 and androgen receptor signaling in prostate carcinoma cells. *Biochem Biophys Res Commun.* 2001;283:179-187.
 318. Lee SO, Lou W, Hou M, de Miguel F, Gerber L, Gao AC. Interleukin-6 promotes androgen-independent growth in LNCaP human prostate cancer cells. *Clin Cancer Res.* 2003; 9:370-376.
 319. de Miguel F, Lee SO, Onate SA, Gao AC. Stat3 enhances transactivation of steroid hormone receptors. *Nucl Recept.* 2003;1:3.
 320. Ni Z, Lou W, Leman ES, Gao AC. Inhibition of constitutively activated Stat3 signaling pathway suppresses growth of prostate cancer cells. *Cancer Res.* 2000;60:1225-1228.
 321. Mora LB, Buettnner R, Seigne J, et al. Constitutive activation of Stat3 in human prostate tumors and cell lines: direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. *Cancer Res.* 2002;62:6659-6666.
 322. Dhir R, Ni Z, Lou W, DeMiguel F, Grandis JR, Gao AC. Stat3 activation in prostatic carcinomas. *Prostate.* 2002;51:241-246.
 323. Ok LS, Lou W, Qureshi KM, Mehraein-Ghomi F, Trump DL, Gao AC. RNA interference targeting Stat3 inhibits growth and induces apoptosis of human prostate cancer cells. *Prostate.* 2004;60:303-309.
 324. Barton BE, Karras JG, Murphy TF, Barton A, Huang HF. Signal transducer and activator of transcription 3 (STAT3) activation in prostate cancer: direct STAT3 inhibition induces apoptosis in prostate cancer lines. *Mol Cancer Ther.* 2004;3:11-20.
 325. Bokemeyer D, Schmitz U, Kramer HJ. Angiotensin II-induced growth of vascular smooth muscle cells requires an Src-dependent activation of the epidermal growth factor receptor. *Kidney Int.* 2000;58:549-558.
 326. Gao Y, Tang S, Zhou S, Ware JA. The thromboxane A2 receptor activates mitogen-activated protein kinase via protein kinase C-dependent Gi coupling and Src-dependent phosphorylation of the epidermal growth factor receptor. *J Pharmacol Exp Ther.* 2001;296:426-433.
 327. Wu W, Graves LM, Gill GN, Parsons SJ, Samet JM. Src-dependent phosphorylation of the epidermal growth factor receptor on tyrosine 845 is required for zinc-induced Ras activation. *J Biol Chem.* 2002;277:24252-24257.
 328. Xiao D, Qu X, Weber HC. Activation of extracellular signal-regulated kinase mediates bombesin-induced mitogenic responses in prostate cancer cells. *Cell Signal.* 2003;15:945-953.
 329. Olayioye MA, Badache A, Daly JM, Hynes NE. An essential role for Src kinase in ErbB receptor signaling through the MAPK pathway. *Exp Cell Res.* 2001;267:81-87.
 330. Dikic I, Tokiwa G, Lev S, Courtneidge SA, Schlessinger J. A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. *Nature.* 1996;383:547-550.
 331. Ma YC, Huang J, Ali S, Lowry W, Huang XY. Src tyrosine kinase is a novel direct effector of G proteins. *Cell.* 2000;102:635-646.
 332. Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ, Lefkowitz RJ. Role of c-Src tyrosine kinase in G protein-coupled receptor- and Gbetagamma subunit-mediated activation of mitogen-activated protein kinases. *J Biol Chem.* 1996; 271:19443-19450.
 333. Luttrell LM, Della Rocca GJ, van Biesen T, Luttrell DK, Lefkowitz RJ. Gbetagamma subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor: a scaffold for G protein-coupled receptor-mediated Ras activation. *J Biol Chem.* 1997;272:4637-4644.
 334. Schmitt JM, Stork PJ. PKA phosphorylation of Src mediates cAMP's inhibition of cell growth via Rap1. *Mol Cell.* 2002;9:85-94.
 335. Stork PJ, Schmitt JM. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol.* 2002;12:258-266.
 336. Frame MC, Fincham VJ, Carragher NO, Wyke JA. v-Src's hold over actin and cell adhesions. *Nat Rev Mol Cell Biol.* 2002;3:233-245.
 337. Slack JK, Adams RB, Rovin JD, Bissonette EA, Stoker CE, Parsons JT. Alterations in the focal adhesion kinase/Src signal transduction pathway correlate with increased migratory capacity of prostate carcinoma cells. *Oncogene.* 2001;20:1152-1163.

338. Sakamoto M, Takamura M, Ino Y, Miura A, Genda T, Hirohashi S. Involvement of c-Src in carcinoma cell motility and metastasis. *Jpn J Cancer Res.* 2001;92:941-946.
339. Kilarski WW, Jura N, Gerwins P. Inactivation of Src family kinases inhibits angiogenesis in vivo: implications for a mechanism involving organization of the actin cytoskeleton. *Exp Cell Res.* 2003;291:70-82.
340. Susva M, Missbach M, Green J. Src inhibitors: drugs for the treatment of osteoporosis, cancer or both? *Trends Pharmacol Sci.* 2000;21:489-495.
341. Missbach M, Altmann E, Widler L, et al. Substituted 5,7-diphenyl-pyrrolo[2,3d]pyrimidines: potent inhibitors of the tyrosine kinase c-Src. *Bioorg Med Chem Lett.* 2000;10:945-949.
342. Recchia I, Rucci N, Festuccia C, et al. Pyrrolopyrimidine c-Src inhibitors reduce growth, adhesion, motility and invasion of prostate cancer cells in vitro. *Eur J Cancer.* 2003;39:1927-1935.
343. Missbach M, Jeschke M, Feyen J, et al. A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption in vitro and in rodent models in vivo. *Bone.* 1999;24:437-449.
344. Nam JS, Ino Y, Sakamoto M, Hirohashi S. Src family kinase inhibitor PP2 restores the E-cadherin/catenin cell adhesion system in human cancer cells and reduces cancer metastasis. *Clin Cancer Res.* 2002;8:2430-2436.
345. Irby RB, Yeatman TJ. Role of Src expression and activation in human cancer. *Oncogene.* 2000;19:5636-5642.
346. Okamoto M, Lee C, Oyasu R. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells in vitro. *Cancer Res.* 1997;57:141-146.
347. Lou W, Ni Z, Dyer K, Tweardy DJ, Gao AC. Interleukin-6 induces prostate cancer cell growth accompanied by activation of stat3 signaling pathway. *Prostate.* 2000;42:239-242.
348. Giri D, Ozen M, Ittmann M. Interleukin-6 is an autocrine growth factor in human prostate cancer. *Am J Pathol.* 2001;159:2159-2165.
349. Culig Z, Bartsch G, Hobisch A. Interleukin-6 regulates androgen receptor activity and prostate cancer cell growth. *Mol Cell Endocrinol.* 2002;197:231-238.
350. Siegall CB, Schwab G, Nordan RP, FitzGerald DJ, Pastan I. Expression of the interleukin 6 receptor and interleukin 6 in prostate carcinoma cells. *Cancer Res.* 1990;50:7786-7788.
351. Spiotto MT, Chung TD. STAT3 mediates IL-6-induced growth inhibition in the human prostate cancer cell line LNCaP. *Prostate.* 2000;42:88-98.
352. Lee SO, Lou W, Hou M, de Miguel F, Gerber L, Gao AC. Interleukin-6 promotes androgen-independent growth in LNCaP human prostate cancer cells. *Clin Cancer Res.* 2003;9:370-376.
353. Yang L, Wang L, Lin HK, et al. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. *Biochem Biophys Res Commun.* 2003;305:462-469.
354. Pu YS, Hour TC, Chuang SE, Cheng AL, Lai MK, Kuo ML. Interleukin-6 is responsible for drug resistance and anti-apoptotic effects in prostatic cancer cells. *Prostate.* 2004;60:120-129.
355. Lee SO, Lou W, Johnson CS, Trump DL, Gao AC. Interleukin-6 protects LNCaP cells from apoptosis induced by androgen deprivation through the Stat3 pathway. *Prostate.* 2004;60:178-186.
356. Hobisch A, Eder IE, Putz T, et al. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. *Cancer Res.* 1998;58:4640-4645.
357. Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res.* 2000;60:2132-2135.
358. Lin DL, Whitney MC, Yao Z, Keller ET. Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression. *Clin Cancer Res.* 2001;7:1773-1781.
359. Smith PC, Keller ET. Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. *Prostate.* 2001;48:47-53.
360. Smith PC, Hobisch A, Lin DL, Culig Z, Keller ET. Interleukin-6 and prostate cancer progression. *Cytokine Growth Factor Rev.* 2001;12:33-40.
361. Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. *Urology.* 2001;58:1008-1015.
362. Shariat SF, Kattan MW, Traxel E, et al. Association of pre- and postoperative plasma levels of transforming growth factor beta(1) and interleukin 6 and its soluble receptor with prostate cancer progression. *Clin Cancer Res.* 2004;10:1992-1999.
363. Zerbini LF, Wang Y, Cho JY, Libermann TA. Constitutive activation of nuclear factor kappaB p50/p65 and Fra-1 and JunD is essential for deregulated interleukin 6 expression in prostate cancer. *Cancer Res.* 2003;63:2206-2215.
364. Bitko V, Velazquez A, Yang L, Yang YC, Barik S. Transcriptional induction of multiple cytokines by human respiratory syncytial virus requires activation of NF-kappa B and is inhibited by sodium salicylate and aspirin. *Virology.* 1997;232:369-378.
365. Campbell CL, Jiang Z, Savarese DM, Savarese TM. Increased expression of the interleukin-11 receptor and evidence of STAT3 activation in prostate carcinoma. *Am J Pathol.* 2001;158:25-32.
366. Zurita AJ, Troncoso P, Cardo-Vila M, Logothetis CJ, Pasqualini R, Arap W. Combinatorial screenings in patients: the interleukin-11 receptor alpha as a candidate target in the progression of human prostate cancer. *Cancer Res.* 2004;64:435-439.
367. Carter BS, Epstein JI, Isaacs WB. ras gene mutations in human prostate cancer. *Cancer Res.* 1990;50:6830-6832.
368. Gumerlock PH, Poonamallee UR, Meyers FJ, deVere White RW. Activated ras alleles in human carcinoma of the prostate are rare. *Cancer Res.* 1991;51:1632-1637.
369. Shiraishi T, Muneyuki T, Fukutome K, et al. Mutations of ras genes are relatively frequent in Japanese prostate cancers: pointing to genetic differences between populations. *Anticancer Res.* 1998;18:2789-2792.
370. Coogan PF, Rosenberg L, Palmer JR, Strom BL, Zaubler AG, Shapiro S. Statin use and the risk of breast and prostate cancer. *Epidemiology.* 2002;13:262-267.
371. Kaye JA, Jick H. Statin use and cancer risk in the General Practice Research Database. *Br J Cancer.* 2004;90:635-637.
372. Sepp-Lorenzino L, Tjaden G, Moasser MM, et al. Farnesyl: protein transferase inhibitors as potential agents for the management of human prostate cancer. *Prostate Cancer Prostatic Dis.* 2001;4:33-43.
373. Head JE, Johnston SR. Protein farnesyltransferase inhibitors. *Expert Opin Emerg Drugs.* 2003;8:163-178.
374. Melisi D, Troiani T, Damiano V, Tortora G, Ciardiello F. Therapeutic integration of signal transduction targeting agents and conventional anti-cancer treatments. *Endocr Relat Cancer.* 2004;11:51-68.
375. Zhang Z, Wang H, Li M, Agrawal S, Chen X, Zhang R. MDM2 is a negative regulator of p21WAF1/CIP1, independent of p53. *J Biol Chem.* 2004;279:16000-16006.
376. Leite KR, Franco MF, Srougi M, et al. Abnormal expression of MDM2 in prostate carcinoma. *Mod Pathol.* 2001;14:428-436.

377. Wang H, Yu D, Agrawal S, Zhang R. Experimental therapy of human prostate cancer by inhibiting MDM2 expression with novel mixed-backbone antisense oligonucleotides: in vitro and in vivo activities and mechanisms. *Prostate*. 2003;54:194-205.
378. Zhang Z, Li M, Wang H, Agrawal S, Zhang R. Antisense therapy targeting MDM2 oncogene in prostate cancer: effects on proliferation, apoptosis, multiple gene expression, and chemotherapy. *Proc Natl Acad Sci U S A*. 2003;100:11636-11641.
379. Mu Z, Hachem P, Agrawal S, Pollack A. Antisense MDM2 sensitizes prostate cancer cells to androgen deprivation, radiation, and the combination. *Int J Radiat Oncol Biol Phys*. 2004;58:336-343.
380. Wang H, Oliver P, Zhang Z, Agrawal S, Zhang R. Chemosensitization and radiosensitization of human cancer by antisense anti-MDM2 oligonucleotides: in vitro and in vivo activities and mechanisms. *Ann N Y Acad Sci*. 2003;1002:217-235.
381. Mu Z, Hachem P, Agrawal S, Pollack A. Antisense MDM2 oligonucleotides restore the apoptotic response of prostate cancer cells to androgen deprivation. *Prostate*. 2004;60:187-196.
382. Bianco R, Caputo R, Caputo R, et al. Combined targeting of epidermal growth factor receptor and MDM2 by gefitinib and antisense MDM2 cooperatively inhibit hormone-independent prostate cancer. *Clin Cancer Res*. 2004;10:4858-4864.
383. Tortora G, Caputo R, Damiano V, et al. Combined blockade of protein kinase A and bcl-2 by antisense strategy induces apoptosis and inhibits tumor growth and angiogenesis. *Clin Cancer Res*. 2001;7:2537-2544.
384. Olie RA, Hall J, Natt F, Stahel RA, Zangemeister-Wittke U. Analysis of ribosyl-modified, mixed backbone analogs of a bcl-2/bcl-xL antisense oligonucleotide. *Biochim Biophys Acta*. 2002;1576:101-109.
385. Bauer JJ, Sesterhenn IA, Mostofi FK, McLeod DG, Srivastava S, Moul JW. Elevated levels of apoptosis regulator proteins p53 and bcl-2 are independent prognostic biomarkers in surgically treated clinically localized prostate cancer. *J Urol*. 1996;156:1511-1516.
386. Lipponen P, Vesalainen S. Expression of the apoptosis suppressing protein bcl-2 in prostatic adenocarcinoma is related to tumor malignancy. *Prostate*. 1997;32:9-15.
387. Keshgegian AA, Johnston E, Cnaan A. Bcl-2 oncoprotein positivity and high MIB-1 (Ki-67) proliferative rate are independent predictive markers for recurrence in prostate carcinoma. *Am J Clin Pathol*. 1998;110:443-449.
388. Kaur P, Kallakury BS, Sheehan CE, Fisher HA, Kaufman RP Jr, Ross JS. Survivin and Bcl-2 expression in prostatic adenocarcinomas. *Arch Pathol Lab Med*. 2004;128:39-43.
389. Chi KN, Gleave ME. Antisense approaches in prostate cancer. *Expert Opin Biol Ther*. 2004;4:927-936.
390. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. *Cancer Res*. 1994;54:805-810.
391. Barreto AM, Schwartz GG, Woodruff R, Cramer SD. 25-hydroxyvitamin D3, the prohormone of 1,25-dihydroxyvitamin D3, inhibits the proliferation of primary prostatic epithelial cells. *Cancer Epidemiol Biomarkers Prev*. 2000;9:265-270.
392. Krill D, DeFlavia P, Dhir R, et al. Expression patterns of vitamin D receptor in human prostate. *J Cell Biochem*. 2001;82:566-572.
393. Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL, Holick MF. Human prostate cells synthesize 1,25-dihydroxyvitamin D3 from 25-hydroxyvitamin D3. *Cancer Epidemiol Biomarkers Prev*. 1998;7:391-395.
394. Young MV, Schwartz GG, Wang L, et al. The prostate 25-hydroxyvitamin D-1 alpha-hydroxylase is not influenced by parathyroid hormone and calcium: implications for prostate cancer chemoprevention by vitamin D. *Carcinogenesis*. 2004;25:967-971.
395. Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality: evidence for a protective effect of ultraviolet radiation. *Cancer*. 1992;70:2861-2869.
396. Luscombe CJ, Fryer AA, French ME, et al. Exposure to ultraviolet radiation: association with susceptibility and age at presentation with prostate cancer. *Lancet*. 2001;358:641-642.
397. Luscombe CJ, French ME, Liu S, et al. Outcome in prostate cancer associations with skin type and polymorphism in pigmentation-related genes. *Carcinogenesis*. 2001;22:1343-1347.
398. Bodiwala D, Luscombe CJ, Liu S, et al. Prostate cancer risk and exposure to ultraviolet radiation: further support for the protective effect of sunlight. *Cancer Lett*. 2003;192:145-149.
399. Bodiwala D, Luscombe CJ, French ME, et al. Associations between prostate cancer susceptibility and parameters of exposure to ultraviolet radiation. *Cancer Lett*. 2003;200:141-148.
400. Grant WB. A multicountry ecologic study of risk and risk reduction factors for prostate cancer mortality. *Eur Urol*. 2004;45:271-279.
401. John EM, Dreon DM, Koo J, Schwartz GG. Residential sunlight exposure is associated with a decreased risk of prostate cancer. *J Steroid Biochem Mol Biol*. 2004;89-90:549-552.
402. Corder EH, Guess HA, Hulka BS, et al. Vitamin D and prostate cancer: a prediagnostic study with stored sera. *Cancer Epidemiol Biomarkers Prev*. 1993;2:467-472.
403. Nomura AM, Stemmermann GN, Lee J, et al. Serum vitamin D metabolite levels and the subsequent development of prostate cancer (Hawaii, United States). *Cancer Causes Control*. 1998;9:425-432.
404. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control*. 2000;11:847-852.
405. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. *Cancer Causes Control*. 2004;15:255-265.
406. Tuohimaa P, Tenkanen L, Ahonen M, et al. Metabolism of 25-hydroxyvitamin D(3) may explain the u-shaped risk curve for prostate cancer. *Int J Cancer*. 2004;111:469.
407. Jacobs ET, Giuliano AR, Martinez ME, Hollis BW, Reid ME, Marshall JR. Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer. *J Steroid Biochem Mol Biol*. 2004;89-90:533-537.
408. Zhao XY, Peehl DM, Navone NM, Feldman D. 1alpha,25-dihydroxyvitamin D3 inhibits prostate cancer cell growth by androgen-dependent and androgen-independent mechanisms. *Endocrinology*. 2000;141:2548-2556.
409. Sung V, Feldman D. 1,25-dihydroxyvitamin D3 decreases human prostate cancer cell adhesion and migration. *Mol Cell Endocrinol*. 2000;164:133-143.
410. Oades GM, Dredge K, Kirby RS, Colston KW. Vitamin D receptor-dependent antitumor effects of 1,25-dihydroxyvitamin D3 and two synthetic analogues in three in vivo models of prostate cancer. *BJU Int*. 2002;90:607-616.
411. Guzey M, Kitada S, Reed JC. Apoptosis induction by 1alpha,25-dihydroxyvitamin D3 in prostate cancer. *Mol Cancer Ther*. 2002;1:667-677.
412. Krishnan AV, Peehl DM, Feldman D. Inhibition of prostate cancer growth by vitamin D: regulation of target gene expression. *J Cell Biochem*. 2003;88:363-371.
413. Vegesna V, O'Kelly J, Said J, Uskokovic M, Binderup L, Koeffle HP. Ability of potent vitamin D3 analogs to inhibit growth of prostate cancer cells in vivo. *Anticancer Res*. 2003;23:283-289.

414. Bauer JA, Thompson TA, Church DR, Ariazi EA, Wilding G. Growth inhibition and differentiation in human prostate carcinoma cells induced by the vitamin D analog 1 α ,25-dihydroxyvitamin D₂. *Prostate*. 2003;55:159-167.
415. Swami S, Zhao XY, Sarabia S, et al. A low-calcemic vitamin D analog (Ro 25-4020) inhibits the growth of LNCaP human prostate cancer cells with increased potency by producing an active 24-oxo metabolite (Ro 29-9970). *Recent Results Cancer Res*. 2003;164:349-352.
416. Stewart LV, Weigel NL. Vitamin D and prostate cancer. *Exp Biol Med (Maywood)*. 2004;229:277-284.
417. Bao BY, Hu YC, Ting HJ, Lee YF. Androgen signaling is required for the vitamin D-mediated growth inhibition in human prostate cancer cells. *Oncogene*. 2004;23:3350-3360.
418. Hsu JY, Feldman D, McNeal JE, Peehl DM. Reduced 1 α -hydroxylase activity in human prostate cancer cells correlates with decreased susceptibility to 25-hydroxyvitamin D₃-induced growth inhibition. *Cancer Res*. 2001;61:2852-2856.
419. Huynh H, Pollak M, Zhang JC. Regulation of insulin-like growth factor (IGF) II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1,25(OH)₂D₃ and its analog EB1089. *Int J Oncol*. 1998;13:137-143.
420. Nickerson T, Huynh H. Vitamin D analogue EB1089-induced prostate regression is associated with increased gene expression of insulin-like growth factor binding proteins. *J Endocrinol*. 1999;160:223-229.
421. Boyle BJ, Zhao XY, Cohen P, Feldman D. Insulin-like growth factor binding protein-3 mediates 1 α ,25-dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1. *J Urol*. 2001;165:1319-1324.
422. Peng L, Malloy PJ, Feldman D. Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. *Mol Endocrinol*. 2004;18:1109-1119.
423. Hong J, Zhang G, Dong F, Rechler MM. Insulin-like growth factor (IGF)-binding protein-3 mutants that do not bind IGF-I or IGF-II stimulate apoptosis in human prostate cancer cells. *J Biol Chem*. 2002;277:10489-10497.
424. Robson CN, Gnanapragasam V, Byrne RL, Collins AT, Neal DE. Transforming growth factor-beta1 up-regulates p15, p21 and p27 and blocks cell cycling in G1 in human prostate epithelium. *J Endocrinol*. 1999;160:257-266.
425. Moustakas A, Pardali K, Gaal A, Heldin CH. Mechanisms of TGF-beta signaling in regulation of cell growth and differentiation. *Immunol Lett*. 2002;82:85-91.
426. Murthy S, Weigel NL. 1 α ,25-dihydroxyvitamin D₃ induced growth inhibition of PC-3 prostate cancer cells requires an active transforming growth factor beta signaling pathway. *Prostate*. 2004;59:282-291.
427. Krishnan AV, Peehl DM, Feldman D. The role of vitamin D in prostate cancer. *Recent Results Cancer Res*. 2003;164:205-221.
428. Tovar SV, Falzon M. Regulation of PTH-related protein gene expression by vitamin D in PC-3 prostate cancer cells. *Mol Cell Endocrinol*. 2002;190:115-124.
429. Tovar SV, Falzon M. Prostate cancer cell type-specific regulation of the human PTHrP gene via a negative VDRE. *Mol Cell Endocrinol*. 2003;204:51-64.
430. Tennant MK, Thrasher JB, Twomey PA, Birnbaum RS, Plymate SR. Insulin-like growth factor-binding protein-2 and -3 expression in benign human prostate epithelium, prostate intraepithelial neoplasia, and adenocarcinoma of the prostate. *J Clin Endocrinol Metab*. 1996;81:411-420.
431. Hampel OZ, Kattan MW, Yang G, et al. Quantitative immunohistochemical analysis of insulin-like growth factor binding protein-3 in human prostatic adenocarcinoma: a prognostic study. *J Urol*. 1998;159:2220-2225.
432. Devi GR, Sprenger CC, Plymate SR, Rosenfeld RG. Insulin-like growth factor binding protein-3 induces early apoptosis in malignant prostate cancer cells and inhibits tumor formation in vivo. *Prostate*. 2002;51:141-152.
433. Grzmil M, Hemmerlein B, Thelen P, Schweyer S, Burfeind P. Blockade of the type I IGF receptor expression in human prostate cancer cells inhibits proliferation and invasion, up-regulates IGF binding protein-3, and suppresses MMP-2 expression. *J Pathol*. 2004;202:50-59.
434. Liu G, Oettel K, Ripple G, et al. Phase I trial of 1 α -hydroxyvitamin d(2) in patients with hormone refractory prostate cancer. *Clin Cancer Res*. 2002;8:2820-2827.
435. Beer TM, Lemmon D, Lowe BA, Henner WD. High-dose weekly oral calcitriol in patients with a rising PSA after prostatectomy or radiation for prostate carcinoma. *Cancer*. 2003;97:1217-1224.
436. Liu G, Wilding G, Staab MJ, et al. Phase II study of 1 α -hydroxyvitamin D(2) in the treatment of advanced androgen-independent prostate cancer. *Clin Cancer Res*. 2003;9:4077-4083.
437. Beer TM. Development of weekly high-dose calcitriol based therapy for prostate cancer. *Urol Oncol*. 2003;21:399-405.
438. Trump DL, Hershberger PA, Bernardi RJ, et al. Anti-tumor activity of calcitriol: pre-clinical and clinical studies. *J Steroid Biochem Mol Biol*. 2004;89-90:519-526.
439. Beer TM, Eilers KM, Garzotto M, Egorin MJ, Lowe BA, Henner WD. Weekly high-dose calcitriol and docetaxel in metastatic androgen-independent prostate cancer. *J Clin Oncol*. 2003;21:123-128.
440. Dunlap N, Schwartz GG, Eads D, et al. 1 α ,25-dihydroxyvitamin D(3) (calcitriol) and its analogue, 19-nor-1 α ,25(OH)₂D(2), potentiate the effects of ionising radiation on human prostate cancer cells. *Br J Cancer*. 2003;89:746-753.
441. Signoretti S, Loda M. Estrogen receptor beta in prostate cancer: brake pedal or accelerator? *Am J Pathol*. 2001;159:13-16.
442. Ho SM. Estrogens and anti-estrogens: key mediators of prostate carcinogenesis and new therapeutic candidates. *J Cell Biochem*. 2004;91:491-503.
443. Zhu X, Leav I, Leung YK, et al. Dynamic regulation of estrogen receptor-beta expression by DNA methylation during prostate cancer development and metastasis. *Am J Pathol*. 2004;164:2003-2012.
444. Linja MJ, Savinainen KJ, Tammela TL, Isola JJ, Visakorpi T. Expression of ER α and ER β in prostate cancer. *Prostate*. 2003;55:180-186.
445. Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ER β) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. *Prostate*. 2003;54:79-87.
446. Leav I, Lau KM, Adams JY, et al. Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *Am J Pathol*. 2001;159:79-92.
447. Cheng J, Lee EJ, Madison LD, Lazennec G. Expression of estrogen receptor beta in prostate carcinoma cells inhibits invasion and proliferation and triggers apoptosis. *FEBS Lett*. 2004;566:169-172.
448. Krege JH, Hodgin JB, Couse JF, et al. Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *Proc Natl Acad Sci U S A*. 1998;95:15677-15682.
449. Weihua Z, Makela S, Andersson LC, et al. A role for estrogen receptor beta in the regulation of growth of the ventral prostate. *Proc Natl Acad Sci U S A*. 2001;98:6330-6335.

450. Paruthiyil S, Parmar H, Kerekatte V, Cunha GR, Firestone GL, Leitman DC. Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Res.* 2004;64:423-428.
451. Speir E, Yu ZX, Takeda K, Ferrans VJ, Cannon RO III. Competition for p300 regulates transcription by estrogen receptors and nuclear factor-kappaB in human coronary smooth muscle cells. *Circ Res.* 2000;87:1006-1011.
452. Kanda N, Watanabe S. 17beta-estradiol inhibits the production of RANTES in human keratinocytes. *J Invest Dermatol.* 2003;120:420-427.
453. Lau KM, LaSpina M, Long J, Ho SM. Expression of estrogen receptor (ER)-alpha and ER-beta in normal and malignant prostatic epithelial cells: regulation by methylation and involvement in growth regulation. *Cancer Res.* 2000;60:3175-3182.
454. Kim IY, Seong do H, Kim BC, et al. Raloxifene, a selective estrogen receptor modulator, induces apoptosis in androgen-responsive human prostate cancer cell line LNCaP through an androgen-independent pathway. *Cancer Res.* 2002;62:3649-3653.
455. Kim IY, Kim BC, Seong do H, et al. Raloxifene, a mixed estrogen agonist/antagonist, induces apoptosis in androgen-independent human prostate cancer cell lines. *Cancer Res.* 2002;62:5365-5369.
456. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology.* 1998;139:4252-4263.
457. Bektic J, Berger AP, Pfeil K, Dobler G, Bartsch G, Klocker H. Androgen receptor regulation by physiological concentrations of the isoflavonoid genistein in androgen-dependent LNCaP cells is mediated by estrogen receptor beta. *Eur Urol.* 2004;45:245-251.
458. Takimoto CH, Glover K, Huang X, et al. Phase I pharmacokinetic and pharmacodynamic analysis of unconjugated soy isoflavones administered to individuals with cancer. *Cancer Epidemiol Biomarkers Prev.* 2003;12:1213-1221.
459. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. *J Nutr.* 1999;129:1628-1635.
460. Aronson WJ, Tymchuk CN, Elashoff RM, et al. Decreased growth of human prostate LNCaP tumors in SCID mice fed a low-fat, soy protein diet with isoflavones. *Nutr Cancer.* 1999;35:130-136.
461. Mentor-Marcel R, Lamartiniere CA, Eltoum IE, Greenberg NM, Elgavish A. Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP). *Cancer Res.* 2001;61:6777-6782.
462. deVere White RW, Hackman RM, Soares SE, Beckett LA, Li Y, Sun B. Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. *Urology.* 2004;63:259-263.
463. Butler R, Mitchell SH, Tindall DJ, Young CY. Nonapoptotic cell death associated with S-phase arrest of prostate cancer cells via the peroxisome proliferator-activated receptor gamma ligand, 15-deoxy-delta12,14-prostaglandin J2. *Cell Growth Differ.* 2000;11:49-61.
464. Shappell SB, Gupta RA, Manning S, et al. 15S-hydroxy-eicosatetraenoic acid activates peroxisome proliferator-activated receptor gamma and inhibits proliferation in PC3 prostate carcinoma cells. *Cancer Res.* 2001;61:497-503.
465. Nwankwo JO, Robbins ME. Peroxisome proliferator-activated receptor-gamma expression in human malignant and normal brain, breast and prostate-derived cells. *Prostaglandins Leukot Essent Fatty Acids.* 2001;64:241-245.
466. Segawa Y, Yoshimura R, Hase T, et al. Expression of peroxisome proliferator-activated receptor (PPAR) in human prostate cancer. *Prostate.* 2002;51:108-116.
467. Gann PH, Ma J, Giovannucci E, et al. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res.* 1999;59:1225-1230.
468. Grant WB. An ecologic study of dietary links to prostate cancer. *Altern Med Rev.* 1999;4:162-169.
469. Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A prospective study of tomato products, lycopene, and prostate cancer risk. *J Natl Cancer Inst.* 2002;94:391-398.
470. Giovannucci E. A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp Biol Med (Maywood).* 2002;227:852-859.
471. Kucuk O, Sarkar FH, Djuric Z, et al. Effects of lycopene supplementation in patients with localized prostate cancer. *Exp Biol Med (Maywood).* 2002;227:881-885.
472. Bowen P, Chen L, Stacewicz-Sapuntzakis M, et al. Tomato sauce supplementation and prostate cancer: lycopene accumulation and modulation of biomarkers of carcinogenesis. *Exp Biol Med (Maywood).* 2002;227:886-893.
473. Kim HS, Bowen P, Chen L, et al. Effects of tomato sauce consumption on apoptotic cell death in prostate benign hyperplasia and carcinoma. *Nutr Cancer.* 2003;47:40-47.
474. Wu K, Erdman JW Jr, Schwartz SJ, et al. Plasma and dietary carotenoids, and the risk of prostate cancer: a nested case-control study. *Cancer Epidemiol Biomarkers Prev.* 2004;13:260-269.
475. Etmnan M, Takkouche B, Caamano-Isorna F. The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev.* 2004;13:340-345.
476. Siler U, Barella L, Spitzer V, et al. Lycopene and vitamin E interfere with autocrine/paracrine loops in the Dunning prostate cancer model. *FASEB J.* 2004;18:1019-1021.
477. Willett WC, Polk BF, Morris JS, et al. Prediagnostic serum selenium and risk of cancer. *Lancet.* 1983;2:130-134.
478. Clark LC, Dalkin B, Krongrad A, et al. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. *Br J Urol.* 1998;81:730-734.
479. Nomura AM, Lee J, Stemmermann GN, Combs GF Jr. Serum selenium and subsequent risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 2000;9:883-887.
480. Brooks JD, Metter EJ, Chan DW, et al. Plasma selenium level before diagnosis and the risk of prostate cancer development. *J Urol.* 2001;166:2034-2038.
481. Vogt TM, Ziegler RG, Graubard BI, et al. Serum selenium and risk of prostate cancer in U.S. blacks and whites. *Int J Cancer.* 2003;103:664-670.
482. Waters DJ, Shen S, Cooley DM, et al. Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. *J Natl Cancer Inst.* 2003;95:237-241.
483. Duffield-Lillico AJ, Dalkin BL, Reid ME, et al. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. *BJU Int.* 2003;91:608-612.
484. van den Brandt PA, Zeegers MP, Bode P, Goldbohm RA. Toenail selenium levels and the subsequent risk of prostate cancer: a prospective cohort study. *Cancer Epidemiol Biomarkers Prev.* 2003;12:866-871.
485. Corcoran NM, Najdovska M, Costello AJ. Inorganic selenium retards progression of experimental hormone refractory prostate cancer. *J Urol.* 2004;171:907-910.

486. Li H, Stampfer MJ, Giovannucci EL, et al. A prospective study of plasma selenium levels and prostate cancer risk. *J Natl Cancer Inst.* 2004;96:696-703.
487. Combs GF. Status of selenium in prostate cancer prevention. *Br J Cancer.* 2004;91:195-199.
488. 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;96:239-243.
489. Gupta S, Hastak K, Ahmad N, Lewin JS, Mukhtar H. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. *Proc Natl Acad Sci U S A.* 2001;98:10350-10355.
490. Zhou JR, Yu L, Zhong Y, Blackburn GL. Soy phytochemicals and tea bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice. *J Nutr.* 2003;133:516-521.
491. Jatoi A, Ellison N, Burch PA, et al. A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. *Cancer.* 2003;97:1442-1446.
492. Adhami VM, Ahmad N, Mukhtar H. Molecular targets for green tea in prostate cancer prevention. *J Nutr.* 2003;133:2417S-2424S.
493. Jian L, Xie LP, Lee AH, Binns CW. Protective effect of green tea against prostate cancer: a case-control study in southeast China. *Int J Cancer.* 2004;108:130-135.
494. Saleem M, Adhami VM, Siddiqui IA, Mukhtar H. Tea beverage in chemoprevention of prostate cancer: a mini-review. *Nutr Cancer.* 2003;47:13-23.
495. Singh RP, Tyagi AK, Dhanalakshmi S, Agarwal R, Agarwal C. Grape seed extract inhibits advanced human prostate tumor growth and angiogenesis and upregulates insulin-like growth factor binding protein-3. *Int J Cancer.* 2004;108:733-740.
496. Fritz WA, Wang J, Eltoum IE, Lamartiniere CA. Dietary genistein down-regulates androgen and estrogen receptor expression in the rat prostate. *Mol Cell Endocrinol.* 2002;186:89-99.
497. Wang J, Eltoum IE, Lamartiniere CA. Dietary genistein suppresses chemically induced prostate cancer in Lobund-Wistar rats. *Cancer Lett.* 2002;186:11-18.
498. Jarred RA, Keikha M, Dowling C, et al. Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones. *Cancer Epidemiol Biomarkers Prev.* 2002;11:1689-1696.
499. Messina MJ. Emerging evidence on the role of soy in reducing prostate cancer risk. *Nutr Rev.* 2003;61:117-131.
500. Lee MM, Gomez SL, Chang JS, Wey M, Wang RT, Hsing AW. Soy and isoflavone consumption in relation to prostate cancer risk in China. *Cancer Epidemiol Biomarkers Prev.* 2003;12:665-668.
501. Ozasa K, Nakao M, Watanabe Y, et al. Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men. *Cancer Sci.* 2004;95:65-71.
502. Hussain M, Banerjee M, Sarkar FH, et al. Soy isoflavones in the treatment of prostate cancer. *Nutr Cancer.* 2003;47:111-117.
503. Wang J, Eltoum IE, Lamartiniere CA. Genistein alters growth factor signaling in transgenic prostate model (TRAMP). *Mol Cell Endocrinol.* 2004;219:171-180.
504. Singh RP, Agarwal R. Prostate cancer prevention by silibinin. *Curr Cancer Drug Targets.* 2004;4:1-11.
505. Singh RP, Sharma G, Mallikarjuna GU, Dhanalakshmi S, Agarwal C, Agarwal R. In vivo suppression of hormone-refractory prostate cancer growth by inositol hexaphosphate: induction of insulin-like growth factor binding protein-3 and inhibition of vascular endothelial growth factor. *Clin Cancer Res.* 2004;10:244-250.
506. Godley PA, Campbell MK, Gallagher P, Martinson FE, Mohler JL, Sandler RS. Biomarkers of essential fatty acid consumption and risk of prostatic carcinoma. *Cancer Epidemiol Biomarkers Prev.* 1996;5:889-895.
507. Harvei S, Bjerve KS, Tretli S, Jellum E, Robsahm TE, Vatten L. Prediagnostic level of fatty acids in serum phospholipids: omega-3 and omega-6 fatty acids and the risk of prostate cancer. *Int J Cancer.* 1997;71:545-551.
508. Norrish AE, Skeaff CM, Arribas GL, Sharpe SJ, Jackson RT. Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study. *Br J Cancer.* 1999;81:1238-1242.
509. Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wolk A. Fatty fish consumption and risk of prostate cancer. *Lancet.* 2001;357:1764-1766.
510. Augustsson K, Michaud DS, Rimm EB, et al. A prospective study of intake of fish and marine fatty acids and prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 2003;12:64-67.
511. Dewailly E, Mulvad G, Sloth PH, Hansen JC, Behrendt N, Hart Hansen JP. Inuit are protected against prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 2003;12:926-927.
512. Karmali RA, Reichel P, Cohen LA, et al. The effects of dietary omega-3 fatty acids on the DU-145 transplantable human prostatic tumor. *Anticancer Res.* 1987;7:1173-1179.
513. Aronson WJ, Glaspy JA, Reddy ST, Reese D, Heber D, Bagga D. Modulation of omega-3/omega-6 polyunsaturated ratios with dietary fish oils in men with prostate cancer. *Urology.* 2001;58:283-288.
514. McCarty MF. Fish oil may impede tumour angiogenesis and invasiveness by down-regulating protein kinase C and modulating eicosanoid production. *Med Hypotheses.* 1996;46:107-115.
515. Rose DP, Connolly JM. Antiangiogenicity of docosahexaenoic acid and its role in the suppression of breast cancer cell growth in nude mice. *Int J Oncol.* 1999;15:1011-1015.
516. Murota SI, Onodera M, Morita I. Regulation of angiogenesis by controlling VEGF receptor. *Ann NY Acad Sci.* 2000;902:208-212.
517. Rose DP, Connolly JM. Regulation of tumor angiogenesis by dietary fatty acids and eicosanoids. *Nutr Cancer.* 2000;37:119-127.
518. Tsuji M, Murota SI, Morita I. Docosapentaenoic acid (22:5, n-3) suppressed tube-forming activity in endothelial cells induced by vascular endothelial growth factor. *Prostaglandins Leukot Essent Fatty Acids.* 2003;68:337-342.
519. Hardman WE. Omega-3 fatty acids to augment cancer therapy. *J Nutr.* 2002;132:3508S-3512S.
520. Ireson CR, Jones DJ, Orr S, et al. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol Biomarkers Prev.* 2002;11:105-111.
521. Ciardiello F, Caputo R, Bianco R, et al. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin Cancer Res.* 2000;6:2053-2063.
522. Dixit M, Yang JL, Poirier MC, Price JO, Andrews PA, Arteaga CL. Abrogation of cisplatin-induced programmed cell death in human breast cancer cells by epidermal growth factor antisense RNA. *J Natl Cancer Inst.* 1997;89:365-373.
523. Mendelsohn J, Fan Z. Epidermal growth factor receptor family and chemosensitization. *J Natl Cancer Inst.* 1997;89:341-343.
524. Nakao-Hayashi J, Ito H, Kanayasu T, Morita I, Murota S. Stimulatory effects of insulin and insulin-like growth factor I on migration and tube formation by vascular endothelial cells. *Atherosclerosis.* 1992;92:141-149.
525. Grant MB, Mames RN, Fitzgerald C, Ellis EA, Aboufrikha M, Guy J. Insulin-like growth factor I acts as an angiogenic agent in rabbit cornea and retina: comparative studies with basic fibroblast growth factor. *Diabetologia.* 1993;36:282-291.

526. Smith LE, Shen W, Perruzzi C, et al. Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. *Nat Med.* 1999; 5:1390-1395.
527. Chantelau E. Evidence that upregulation of serum IGF-1 concentration can trigger acceleration of diabetic retinopathy. *Br J Ophthalmol.* 1998;82:725-730.
528. Ziche M, Jones J, Gullino PM. Role of prostaglandin E1 and copper in angiogenesis. *J Natl Cancer Inst.* 1982;69:475-482.
529. Pan Q, Kleer CG, van Golen KL, et al. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. *Cancer Res.* 2002;62:4854-4859.
530. Fujita M, Hayashi I, Yamashina S, Itoman M, Majima M. Blockade of angiotensin AT1a receptor signaling reduces tumor growth, angiogenesis, and metastasis. *Biochem Biophys Res Commun.* 2002;294:441-447.
531. Tamarat R, Silvestre JS, Durie M, Levy BI. Angiotensin II angiogenic effect in vivo involves vascular endothelial growth factor- and inflammation-related pathways. *Lab Invest.* 2002; 82:747-756.
532. Egami K, Murohara T, Shimada T, et al. Role of host angiotensin II type 1 receptor in tumor angiogenesis and growth. *J Clin Invest.* 2003;112:67-75.
533. Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresch DA. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. *Mol Cell.* 1999;4:915-924.
534. Cao Y, Cao R. Angiogenesis inhibited by drinking tea. *Nature.* 1999;398:381.
535. Cao Y, Cao R, Brakenhielm E. Antiangiogenic mechanisms of diet-derived polyphenols. *J Nutr Biochem.* 2002;13:380-390.
536. Kojima-Yuasa A, Hua JJ, Kennedy DO, Matsui-Yuasa I. Green tea extract inhibits angiogenesis of human umbilical vein endothelial cells through reduction of expression of VEGF receptors. *Life Sci.* 2003;73:1299-1313.
537. Maiti TK, Chatterjee J, Dasgupta S. Effect of green tea polyphenols on angiogenesis induced by an angiogenin-like protein. *Biochem Biophys Res Commun.* 2003;308:64-67.
538. Sartippour MR, Heber D, Henning S, et al. cDNA microarray analysis of endothelial cells in response to green tea reveals a suppressive phenotype. *Int J Oncol.* 2004;25:193-202.
539. Fassina G, Vene R, Morini M, et al. Mechanisms of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. *Clin Cancer Res.* 2004;10:4865-4873.
540. Kanayasu T, Morita I, Nakao-Hayashi J, et al. Eicosapentaenoic acid inhibits tube formation of vascular endothelial cells in vitro. *Lipids.* 1991;26:271-276.