Using Curcumin (Yu Jin/Jiang Huang) to Treat Cancer and Benign Tumors

A Five Hour CEU/PDA Course

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Introduction -

So many rich, powerful, intelligent, and/or famous people have died just of pancreatic cancer, including the following:

- *Steve Jobs, founder of Apple*
- *Aretha Franklin, singer*
- *Alan Rickman, actor*
- *Patrick Swayze, actor*
- *Joan Crawford, actor*
- *Jack Benny, comedian*
- *Luciano Pavarotti, tenor (who swore by his Miami acupuncturist Richard Browne for treatment of his sciatica)*
- *Sally Ride, astronaut*
- *Donna Reed, actress*
- *John Hurt, actor*
- *Count Basie, musician*
- *Syd Barret, musician*
- *Michael Landon, actor*
- *Rex Harrison, actor*
- *Alan Bates, actor*
- *Henry Mancini, musician*
- *Benoit Mandelbrot, mathematician*
- *Werner Von Braun, rocket scientist*
- *Ralph Ellison, author*
- *Dizzy Gillespie, musician*
- *Margaret Mead, anthropologist*
- *Don Hewitt, director of 60 Minutes*
- *Krishnamurti, philosopher*
- *René Magritte, painter*
- *Griffin Bell, US. Attorney General*
- *Frank Church, U.S. Senator*
- *Lee Remick, actress*

This had left me to wonder, “Is there no way to overcome this dreaded disease? Surely many of those who died had resources to explore alternatives, but none lived.” My own uncle died of pancreatic cancer in 1980, and I have been researching methods for treating it and other cancers ever since. Prior to discovering the power of curcumin, my efforts had been mostly fruitless, although I did find treatments with Electro-Cancer Therapy and Hyperthermia to be quite promising. However, those treatments are not available in the United States, with perhaps the

sole exception being at the Cancer Treatment Centers of America.

Then I read a study by Indian medical doctors who had some very limited success treating pancreatic cancer with curcumin. That sparked my interest, and thereafter, by some cosmic chance, a woman suffering from advanced pancreatic cancer came into my practice. The year was 2016, she had a golf-ball-sized tumor, and her doctors had given her only three months to live. Desperate, she was turning to me as a last resort. Having read the study by Indian doctors on the efficacy of treating pancreatic cancer with curcumin, I suggested that approach to her, with the minor modifications which you will read about later.

The result? After three months, instead of dying, she reported that her pancreatic tumor had dissolved, and now, three years later, she is still alive. The total cost to her was less than $200. Our experience together led me to do further research on curcumin, the research which comprises the bulk of this course.

My purpose in writing it is to help you help your patients prevent and treat cancer on their own, at home, for relatively little expense with the near miraculous power of curcumin that is bonded with B-lactoglobulin. In case you have doubts about this claim, this course contains summaries of numerous peer-reviewed medical research studies that were published in respected medical journals. They attest to the efficacy of treating cancers with curcumin, as do some anecdotal reports which I've also included.

This information is so important that as a writer I am humbled trying to present it properly. If nothing else, please take this one understanding away from your reading: **In many cases conventional chemotherapy and radiation treatments can ultimately spawn new, harder to treat metastatic tumors, tumors which curcumin can prevent.** I base this assertion on a study included in Part Three of this course entitled "Curcumin and Cancer Stem Cells: Curcumin Has Asymmetrical Effects on Cancer and Normal Stem Cells."

Of course, prevention is the best cure for cancer, and to that end I recommend you read my CEU course **X-Rayed to Death, Ionizing Radiation and Human Health**.

Julius Caesar once wrote that "*Omnes Gallia in tres partes divisa est."* Translation? "All Gaul is divided into three parts." This course is also divided into three parts.

Part One - Traditional Chinese Medical (TCM) Qualities of Curcumin

Taste, Property, Channels, Contraindications, Functions, and Chemical Components of Curcumin and How They Are Anti-Carcinogenic

Part Two - Preparation Methods and Bio-Availability

How the bioavailability of curcumin, which is markedly affected by preparation methods, influences its efficacy on treating cancers.

Part Three - Peer-Reviewed Clinical Studies on the Efficacy of Curcumin

A. This section presents research, cancer treatment protocols, and case studies from peer-reviewed scientific and medical journals which document the effectiveness of curcumin in preventing and treating cancers and benign tumors. For the most part, just read the abstract, results, conclusions, and discussion sections of the studies. Some studies are presented in full, including their footnotes, which point to many, many other studies that deal with the effectiveness of curcumin on cancers. I have just presented excerpts from other studies. I have often emphasized the most relevant portions of text by putting them in bold. In most cases you can access the entire studies online.

B. Seventeen Supplemental Research Studies on Curcumin's Health Benefits.
Optional Reading, Not Required. Read them for your pleasure…This information will not be tested on the exam.

Exam Tip: Print out the exam before starting your reading. Have it near you as you read so you can mark answers as they emerge from the text. Then mark your answers on the digital exam form for submission back to me.

Part One - Traditional Chinese Medical (TCM) Qualities of Curcumin

In 1993 I was asked by U.S. Senator Tom Harkin (D-Iowa), who served as Chair of the Senate Subcommittee for Healthcare Appropriations, to testify before the Senate about the health care savings which could be achieved using acupuncture and Traditional Chinese Medicine (TCM). That testimony can be read in full in my CEU course Acupuncture Works at the following web link: https://hkacup.com/courses/acupuncture-works/

Or at this web link:
https://archive.org/details/alternativemedic00unit

Essentially, I testified that in 1990 Americans were spending $3200 per person on healthcare, whereas the Chinese were spending $71 per person. A big factor in those huge cost savings was the Chinese ongoing use of traditional Chinese herbal remedies from among the 25,000 herbal formulae which they had developed over the past 5,000 years. Curcumin has been a principal herb used in these Chinese remedies for the treatment of tumors since at least 600 A.D. We know this since it was written about that year in the *Yao Xing Ben Cao* (Materia Medica of Medicinal Properties) by the physician Zhen Quan.

Curcumin comes from both the rhizome (Jiang Huang) and the root (Yu Jin) of the Curcuma Longa plant, commonly known as turmeric, which is from the Zingiberacea or ginger family. It is curcumin derived from the root of the plant (Yu Jin) which has proven, through millennia of documented use, to be most effective for treating tumors. The Chinese understanding is that stagnation of qi or energy contributes to the creation of cancers. Qi in TCM is considered to be more than just energy. It is also oxygen. Thus the TCM theory of cancer creation deems lack of fresh qi or lack of oxygen in the cells to be a critical factor. Herbs which move stagnant qi and get fresh, oxygen rich qi flowing through an area are known through clinical experience to be anti-carcinogenic.

The Nobel Laureate Otto Warburg expressed similar ideas. He hypothesized that damage to the mitochondria of some cells resulted in a hypoxic state or low level of oxygen in the core of the cells and that "the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar."

In an article published in the February, 2007 issue of the *Journal of Bioenergetics and Biomembranes. **39** (1): 1–12* entitled *"The cancer cell's "power plants" as promising therapeutic targets: an overview,"* Peter Pedersen echoes Warburg's hypothesis. Pedersen concludes that often the body kills cancer cells by programmed cell death or apoptosis, a way of cancer cell destruction that involves the mitochondria. However, this way does not work on cancer cells when their mitochondria [which are central to production of energy in cells via respiration of oxygen] are damaged and not functioning. Reviving mitochondria in cancer cells gets their process of apoptosis and hence cancer cell death going again.

One of the main causes of damage to the mitochondria is exposure to radiation, such as from the Sun, which leads to skin cancer. A more widespread but greatly under-reported source is ionizing radiation from medical imaging such as X-Rays or and especially CT scans, but that is the subject of another one of my courses: **X-Rayed to Death: Ionizing Radiation and Human Health**.

That course is available at https://hkacup.com/courses/medical-imaging/

These are the properties of Yu Jin. Note that it promotes the circulation of qi and removal of stagnation to eliminate tumors:

Yu Jin:
Taste: Pungent and bitter
Property: Cold
Therapeutic channel: Heart, lung, liver and gall bladder
Function:
To relieve pain by removing blood and qi (energy) stasis
To regulate circulation of qi and disperse stagnation
To clear pathogenic heat from the pericardium, cool the blood, calm shen
To increase bile production and excretion, reduce jaundice

Medical Uses:
1. Pain in the abdomen, chest, or intercostals; amenorrhea or dysmenorrheal;
2. Tumors
3. Angina, heart disease
4. Jaundice from hepatitis
5. Convulsions, epilepsy, mania with delirium from phlegm in the heart

Pharmacological Effects:
1. protects the liver, reduces SGOT and SGPT, stimulates the immune system
2. Reduces cholesterol and triglycerides
3. Cholagogic – It increases bile production and excretion
4. Lowers pH in the duodenum and stomach, increases stomach acid,

Contraindications: For patients with no qi and blood stagnation or when pregnant. Not for patients with bleeding and/or qi deficiency. Not for patients taking anticoagulant medications such as Coumadin, enoxaparin, or heparin or antiplatelets such as aspirin, Persantine, or Plavix

Chemical composition:
Essential oils: d-camphene, d-camphor, l-d-curcumene, l-B-curcume; bisdemethoxycurcumin, carvone, curcumin, demethoxycurcumin, fatty oil,
P-tolylmethylcarbinolddifereryloymeethane, Ar-turmerone, starch, and turmerone.

Dosage: 3 to 9 grams, depending on patient's body weight. 20 mg/kg for tumors

Jiang Huang:
Taste: Pungent and bitter
Property: Warm
Therapeutic channel: Spleen and liver
Function: To regulate circulation of blood and qi with qi and blood stasis
To remove stagnation
To relieve pain by clearing the channels and collaterals
To reduce swelling from accumulation of toxic heat with qi and blood stasis
Medical Uses:
1. Pain in the abdomen and chest, dysmenorrheal
2. Traumatic injuries
3. Arthritis - Bi zheng syndrome, especially of the upper limbs
4. Hypochondriac pain from hepatitis
5. Sores and lesions caused by toxic heat buildup

Pharmacological Effects:
1. Reduces both cholesterol and triglyceride levels
2. Inhibits aggregation of platelets
3. Cholagogic – It increases bile production and excretion
4. Abortifacient – at levels of 10 gm/kg by stimulating and contracting the uterus

Contraindications:
For patients with no qi and blood stagnation or when pregnant
For patients taking anticoagulant or antiplatelet medications

Chemical composition:
Essential oil containing borneol, cineole phellandrene, sabinene, turmerone, and zingerene; arabinose, arturmerone, bisdemethoxycurcumin, caryophyllene, curcumin, curcumoids, demethoxyucurcumin, fatty oil, fructose, glucose, and starch

Dosage: 3 to 9 grams, depending on patient's body weight. 20 mg/kg for tumors

Part Two - Preparation Methods and Bio-Availability
(Or How My Patient Recovered from Pancreatic Cancer)

April 1, 2019 I presented the following observations about curcumin at the 2nd Annual International Conference of TCM and Acupuncture in Washington, D.C. It was attended by Doctors of Acupuncture and TCM from 15 countries and all 50 United States.

Much of the research presented later in this course confirms how important preparation methods are to achieving bio-availability and maximum clinical efficacy. The key to whether curcumin is remarkably effective is its bio-availability, which is directly a function of the methods used to prepare it, long known to be so important in traditional Chinese herbal medicine. Unfortunately, until very recently limited bio-availability has been a major obstacle to the clinical efficacy of curcumin.

Walls and Barriers

In *Mending Wall*, Robert Frost once wrote: *"Good fences make good neighbors."*
In the same poem, he also observed: *"Something there is that doesn't love a wall."*

This dichotomy exists in the human body as well. Walls or barriers are important elements of human physiology, needed to keep out unwanted substances, but at times they also prevent the entry of healthy and crucially needed nutrients such as curcumin, which can prevent or cure even the most serious of ailments if adequately absorbed.

When it comes to Curcumin, the walls we are most concerned with are the wall of the intestinal tract and the blood /brain barrier. It is very difficult for the body to absorb curcumin. Indian doctors found that the most they could see absorbed by a patient was **40 nanograms per milliliter of blood.** However, even at that low level, they saw dramatic improvement in 10% of patients with pancreatic cancer. The Indian doctors wished that more curcumin could be absorbed, because they saw it as key to curing pancreatic cancer, which has a very low survival rate.

In 2015, the Japanese Theracumin company developed a more easily absorbable form of curcumin labeled "Theracumin". It delivers up to 40 nanograms per milliliter of blood.

https://doi.org/10.1016/j.phanu.2015.08.002

But Theracumin ONLY delivered 40 nanograms per milliliter of blood, the same level Indian doctors thought was promising but still insufficient to cure pancreatic cancer. Several of the clinical studies cited in this course were funded in part by the Theracumin Company of Japan, and they used Theracumin in their studies. Even at the increased though still low bio-availability afforded by Theracumin, curcumin nevertheless showed promising clinical results. Most studies, however, still lamented the limited bio-availability of curcumin. In fact, one study by Verbeck and Kiselak of the University of North Texas observed that very little commercially available curcumin passed the blood/brain barrier.

Some nutritional companies have mixed curcumin with piperine from black pepper to increase absorption of curcumin. Blending curcumin with the alkaloid piperene from black pepper does increase absorption by 154%, but at a price: piperine inhibits the detoxification functions of the liver and makes the curcuminoids, although absorbed, much less effective. Such inhibition is discussed in the following study:

Piperine, a Major Constituent of Black Pepper, Inhibits Human P-glycoprotein and CYP3A4

Rajinder K. Bhardwaj, Hartmut Glaeser, Laurent Becquemont, Ulrich Klotz, Suresh K. Gupta and Martin F. Fromm

Journal of Pharmacology and Experimental Therapeutics August 2002, 302 (2) 645-650; DOI: https://doi.org/10.1124/jpet.102.034728

A National Institutes of Health report concluded as follows: "This study showed that there might have been a considerable damage to the liver with piperine extract. Further research may be required to prove this damage to liver function."

https://www.ncbi.nlm.nih.gov/pubmed/26205799

Moreover, the study by Verbeck and Kiselak found that because piperine acts as a metabolism inhibitor, it actually weakens or prevents the effects of curcumin in the body.

Importantly, the same study, **"Determining Apparent Permeability of Curcumin and Curcumin bound to B-Lactoglobulin using a Parallel Artificial Membrane Permeability Assay (PAMPA),"** observed a marked difference between how well the body absorbs unbound curcumin . The types commonly available in the marketplace of supplements barely permeated the intestinal or blood brain barriers, whereas curcumin bound to B-lactoglobulin - a form available in only very limited commercial supply - did so remarkably:

"After consulting with the University of North Texas Molecular Chemistry Department, we determined a more scientific, and accurate, way to measure whether curcumin, or more specifically, our curcumin/protein conjugate, was transporting / assimilating in the body. We ran a Parallel Artificial Membrane Permeability Assay (PAMPA) study on the raw curcumin we use to make our products, and the CPRO conjugate we produce. This method provides an in-vitro model for passive diffusion. This is an important factor in determining transport through the gastrointestinal tract (GI), penetration of the blood-brain barrier (BBB), as well as transport across cell membranes. This test is commonly used by the DEA in determining drug transport."

Using the PAMPA method, Verbeck and Kiselak determined that the amount of curcumoids from curcumin found in the blood after taking curcumin bound to B-lactoglobulin was 1000 nanograms per milliliter of blood – twenty-five times that of the curcumoids bio-available compared to even Theracumin's 40 nanograms per milliliter. That's a whopping increase in bio-availability of 2500%**!**

With The Verbeck and Kiselak studies in mind and guided by the conclusions of the Dhillon study - "**Phase II trial of curcumin in patients with advanced pancreatic cancer"** – in April, 2016 I treated a patient with a golf ball sized pancreatic cancer tumor who was not responding to chemo. Her oncologists said she had only three months left to live.

My treatment protocol for her? It was simply this. I recommended that each day she take 1800 mg. of curcumin bound to B-lactoglobulin, the form of curcumin tested in Kiselek and Verbeck's study. It is commercially available from Amazon as CurcuminPro® Complete®. I also recommended the Health Concerns product, Power Mushrooms (Ganoderma - Reishi), three capsules a day for three months, which had been suggested to me by Dr. Carolyn Schoner, a licensed acupuncturist from Englewood, Florida. By the end of three months, my patient's pancreatic tumor had totally dissolved. She is alive and well now, more than three years later.

These results affirmed the hypothesis of the Dhillon study, that with enough absorbed curcuminoids, pancreatic cancers would reduce or disappear. I have since treated skin cancers successfully the same way. Also, the PAMPA studies by Kiselak and Verbeck show that while unbound curcumin itself did not significantly cross the gastrointestinal or blood-brain barriers, curcumin bound with B-lactoglobulin passed both barriers at levels significant for clinical efficacy. The implications are profound for treating both Alzheimer's and Chronic Traumatic Encephalopathy (CTE) with curcumin bound with B-lactoglobulin. These ailments are much more widespread than pancreatic cancer.

In addition to binding curcumin with B-lactoglobulin, researchers have now found remarkable efficacy by delivering curcumin as **nanoparticles** or as **encapsulated in exosomes**. The implications for stroke prevention and treatment (discussed later in this course in a study entitled **Epigenetic Impact of Curcumin on Stroke Prevention)** are especially profound, since such delivery system cross the blood/brain barrier even more effectively than when curcumin is bound with B-lactoglobulin. However, curcumin as **nanoparticles** or as **encapsulated in exosomes** have not yet come onto the market. For now, curcumin bound with B-lactoglubulin is the best form available.

Part Three A., Peer-Reviewed Clinical Studies on the Efficacy of Curcumin

(Where you see bold-faced type in the text, I have added it for emphasis) HK

1. *The following abstract summarizes one of my favorite, and what I consider to be one of the most important studies on curcumin and cancer. I have emphasized significant parts in bold. Simply put, chemo and radiation therapies, while killing cancer cells, create cytokines which stimulate surviving cancer stem cells (CSC) possibly to go on to create metastatic tumors which are particularly resistant to conventional treatment methods. However, curcumin kills cancer cells, with little cytotoxic effects to normal cells, and does not spur the growth of CSC's. Moreover, curcumin kills Cancer Stem Cells but has no harmful, cytotoxic effect on normal stem cells. HK*

ANTICANCER RESEARCH 35: 599-614 (2015) by PETER P. SORDILLO and LAWRENCE HELSON

Curcumin and Cancer Stem Cells: Curcumin Has Asymmetrical Effects on Cancer and Normal Stem Cells

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Abstract. **Curcumin has been shown to have numerous cytotoxic effects on cancer stem cells (CSCs**). This is due to its suppression of the release of cytokines, particularly interleukin (IL)-6, IL-8 and IL-1, which stimulate CSCs, and also to its effects at multiple sites along CSC pathways, such as Wnt, Notch, Hedgehog and FAK. In spite of its multiple actions targeting CSCs, **curcumin has little toxicity against normal stem cells (NSCs**). This may be due to curcumin's different effects on CSCs and NSCs.

The use of cytotoxic therapies remains the standard treatment for patients with metastatic cancer. The efficacy of these treatments is limited, with recurrence common. According to the cancer stem cell paradigm, cancers contain distinct subpopulations of cancer stem/progenitor cells (CSCs) characterized by self-renewal mechanisms and resistance to conventional treatments (1-3). When CSCs are transferred to an immune-deficient mouse, these cells can reconstitute the original cancer in the animal (4-6). Even a small number of stem cells (as few as 100) can be effective in bringing about the transplantation (7). However, tumors depleted of stem cells do not grow as xenografts (8). **These CSCs have been shown to be resistant to chemotherapy (9), radiation (10) and hormone therapy (11).** For this reason, metastases from solid tumors, in particular, will re-appear even after initially successful treatments and prolonged periods of complete remission. Further, an unintended consequence of induced cancer cell death is the release of inflammatory cytokines, which can stimulate replication of CSCs (12-14). The percentage of CSCs in the cancer has been shown to increase in patients receiving neoadjuvant chemotherapy (9, 15, 16). Thus, an "equilibrium" may be formed where **chemotherapy-induced tumor cell death results in increased stimulation of tumor growth** (12). In addition, the **cytokines secreted during induced cancer cell death can result in resistance to cytotoxic agents, so that metastases, when they occur, may be refractory to therapy** (14, 17, 18). This suggests, for therapy to be effective on a consistent basis, it must eliminate both CSCs and non-stem cell cancer cells.

2. *This abstract is from **Cancer Science**, the official journal of the Japanese Cancer Association. It documents how curcumin suppresses glioblastoma brain cancers. HK*

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Open Access

Curcumin promotes differentiation of glioma-initiating cells by inducing autophagy

Wenzhuo Zhuang, Linmei Long , Bingxin Zheng, Wenjun Ji , Neng Yang , Qingqing Zhang, Zhongqin Liang

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Abstract

"Glioblastoma (GBM) is a highly aggressive brain tumor characterized by increased proliferation and resistance to chemotherapy and radiotherapy. Recently, a growing body of evidence suggests that glioma-initiating cells (GICs) are responsible for the initiation and recurrence of GBM. However, the factors determining the differential development of GICs remain poorly defined. In the present study, we show that **curcumin, a natural compound with low toxicity in normal cells, significantly induced differentiation of GICs *in vivo* and *in vitro* by inducing autophagy. Moreover, curcumin also suppressed tumor formation on intracranial GICs implantation into mice.** Our results suggest that autophagy plays an essential role in the regulation of GIC self-renewal, differentiation, and tumorigenic potential, suggesting autophagy could be a promising therapeutic target in a subset of glioblastomas. **This is the first evidence that curcumin has differentiating and tumor-suppressing actions on GICs.** (*Cancer Sci* 2012; 103: 684–690)"

3. *The following study on treating glioblastomas suggests using curcumin, especially in nano-size, along with conventional chemotherapy. Using nano-sized curcumin makes sense to me, since it is more easily transported across the brain/blood barrier, but the conventional radiation and chemotherapy approaches seem silly. Without treatment, survival is only a few months. With radiation, this article observes, survival is 12.1 month on average. With radiation and chemo, survival time increases to 14.6 months. I say this is silly, because the side effects so seriously compromise quality of life with no regard fr other non-toxic alternatives. At the Klink St. Georg in Bad Aibling, Germany I interviewed a man in his early 20's who had already lived three years after receiving only hypothermia for his glioblastoma. CT scans showed that his tumor had completely shrunk. Since there was no surgery involved, his normal brain tissue had regrown in place of the tumor, and the patient had zero neurological deficits. You can read about this case in my article "**Too Hot For Cancer"** which originally appeared in Alternative Medicine magazine and now can be found at this web link*: http://www.chidvd.com/layout/images/HotHouse/Too_Hot_for_Cancer.pdf

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Curcumin for the Treatment of Glioblastoma

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Abstract

Glioblastoma multiforme is a highly aggressive primary cancer of the brain associated with a poor prognosis. Modest increases in survival can sometimes be achieved with the use of temozolomide and radiation therapy after surgery, but second-line therapy after recurrence has a limited efficacy. Curcumin has demonstrated promising results against this form of cancer in experimental models. The reported activity of curcumin against cancer stem cells, a major cause of glioblastoma resistance to therapy, and its ability to augment the apoptotic effects of ceramides, suggest it would have a synergistic effect with cytotoxic chemotherapy agents currently used in second-line therapy, such as lomustine.

Standard Chemotherapy

Temozolomide is an oral alkylating agent which is an imidazotetrazine derivative of dacarbazine. It crosses the blood–brain barrier and is, in combination with radiation, the most frequently used first-line treatment given following surgery for malignant glioma (12-15). The addition of temozolomide to radiation therapy increases patient median survival by 2 to 3 months. In one randomized trial, median survival with this combination was 14.6 months compared to 12.1 months with radiation therapy alone (16-18). Two other agents, bevacizumab, which suppresses angiogenesis, and lomustine, have frequently been used as second-line therapy (19-23). Lomustine is a lipid-soluble, alkylating nitrosourea which also crosses the blood–brain barrier (21-25). However, treatment with these agents results in only minor increases in survival, and overall survival rates remain low, with fewer than 10% of patients alive at 5 years after diagnosis (18, 26). Due to the highly resistant and aggressive nature of GBM, new treatments are required.

Section Next

Curcumin

Curcumin (diferuloylmethane) is the principal curcuminoid of turmeric, the Indian spice derived from the plant *Curcuma longa* Linn. Curcumin absorbs light with a wavelength maximum at approximately 420 nm, thus giving turmeric its yellow color. Curcumin has been shown to have antioxidant, anti-infective and anticancer effects, and its use is being investigated in diseases as diverse as diabetes (27), Alzheimer's disease (28), hepatitis (29) and rheumatoid arthritis (30). When orally administered, it is non-toxic and safe (31-35). Curcumin has numerous mechanisms of action, including suppression of pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin (IL)1, IL6, IL8, and affects multiple signaling pathways including wingless-related integration site (WNT), NOTCH, mitogen-activated protein kinase, hedgehog and Janus kinase/signal transducer and activator of transcription (JAK/STAT) (36-41). Curcumin is highly lipophilic, and crosses the blood–brain barrier (42, 43).

Curcumin and GBM

The potential benefits of curcumin as a treatment for GBM have been studied by numerous groups (44-49). Aoki *et al.* showed that curcumin induced autophagy by suppression of the protein kinase B (AKT)/mammalian target of rapamycin (mTOR)/p70S6K and activation of the extracellular-signal-regulated kinase (ERK1/2) pathways in U87-MG and U373-MG human malignant glioma cells harboring a phosphatase and tensin homolog (*PTEN*) mutation. Similar results were seen in KBM-5 human leukemia cells (50). Choi *et al.* reported that curcumin activates p21 in U87-MG human GBM cells *via* ERK and c-JUN N-terminal protein kinase signaling (51). Senft *et al.* studied cell lines from human primary and recurrent GBM, and showed that curcumin reduced cell growth, inhibited migration and decreased invasiveness due to its inhibition of the JAK/STAT3 pathway (52). Similarly, Dhandapani *et al.* showed that curcumin enhanced cell death by reducing the activity of activator protein 1 and nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 binding in human and rat glioma cell lines (53). Zanotto-Filho *et al.* showed that in the C6 implant rat glioma model, curcumin caused reduction in brain tumor volume (54). Perry *et al.* showed that curcumin can suppress growth of human glioma U87 cells xenografted into athymic mice (55).

The effects of curcumin on GBM stem cells may also be important. Beier *et al.*showed that detoxifying enzymes such as O6-methylguanine-DNA-methyltransferase may confer intrinsic resistance of cancer stem cells to alkylating agents (56). Other researchers have also suggested a key role for stem cells in GBM formation and resistance to alkylating agent therapy (57, 58). Fong *et al.* studied rat C6 glioma cells, and showed that curcumin may have the potential to target cancer stem cells (59). Zhuang *et al.* found that curcumin induced differentiation of glioma-initiating cells and inhibited their growth *via* autophagy (60).

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Curcumin: Alternate Delivery Mechanisms

Recently, new mechanisms have emerged, and engendered methods of improving the efficacy of curcumin (61-65). These methods may prove superior because of their ability to deliver greater doses of curcumin to the tumor. Nano-sized capsules of curcumin have been used as a treatment of GBM cells. Lim *et al.* have shown that curcumin nanoparticles can slow-down GBM growth through the inhibition of cell proliferation and a reduction in stem-like tumor cells (66). Langone *et al.* have shown that curcumin coupled to a monoclonal antibody caused a 120-fold increase in the death of human GBM cells in culture compared to curcumin alone. In addition, mice implanted with GBM cells had an extended survival time and a reduction in the size of the brain tumor mass with this treatment (67).

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Rationale for Combination therapy

It is proposed that the optimal method of using curcumin is not as a single agent, but rather in combination with cytotoxic chemotherapy. Ramachandran *et al.* have shown that curcumin could be used to increase the therapeutic potential of temozolomide or of etoposide in brain tumor cell lines (68). Yin *et al.* investigated the use of a combination of curcumin and temozolomide in U87-MG GBM cell lines and in xenograft mouse models, and found that **curcumin enhanced the effects of temozolomide by generating reactive oxygen species production, and by suppressing phosphorylated AKT and mTOR, thus causing cell death** (69). Zanotto-Filho *et al.* showed that **curcumin increased the cytotoxic effects of doxorubicin and cisplatin on GBM cells** (70). Wu *et al.* showed that **curcumin enhanced temozolomide cytotoxicity against human GBM cells** (71). It has been reported that curcumin and paclitaxel act synergistically with much greater activity than seen with each individual agent in increasing the B-cell lymphoma like protein 4/B-cell lymphoma 2 ratio, increasing cytochrome *c*, reducing angiogenesis and causing apoptosis of HBTSC, LN18 and U138-MG cells (72).

These results suggest that the use of curcumin should be investigated in clinical trials of patients with GBM, ideally as a second-line therapy after failure of radiation therapy and temozolomide, and that the optimal method for using curcumin in this setting may be in combination with an established cytotoxic chemotherapy agent with activity against GBM such as carmustine or lomustine. As noted, it appears that a major reason for the very limited efficacy of alkylating agents in established tumors is the resistance of GBM stem cells to therapy. Our previous work and those of others has suggested that curcumin may be effective in reducing or eliminating the population of cancer stem cells, either by causing apoptosis or differentiation (73-76), while conventional chemotherapy alone is ineffective against stem cells, resulting in tumor recurrence even following initial response (77). Furthermore, curcumin may also increase the activity of cytotoxic chemotherapy against mature tumor cells. Curcumin has been shown to enhance ceramide production by increasing the

activity of enzyme ceramide synthase (78). It has been suggested that the progression of GBM is caused by a decrease in ceramide levels (79). Increased activity of glucosylceramide synthase, an enzyme that causes a decrease in ceramides, has been associated with GBM progression and resistance to temozolomide (80). In contrast, acid sphingomyelinase, which hydrolyzes sphingomyelin to ceramide and phosphorylcholine, has been shown to sensitize glioma cell lines to chemotherapy or radiation therapy (81, 82). The combination of curcumin and chemotherapy has also been shown to have a synergistic effect on the generation of reactive oxygen species in GBM cell lines and in mouse xenografts (69). This may be an additional mechanism by which GBM cell destruction might be enhanced, since reactive oxygen species are known to increase acid sphingomyelinase activity and, in consequence, ceramide levels (83-85).

Previous SectionNext Section

- This article is freely accessible online.
- **Conflicts of Interest**

  Dr. Peter Sordillo is a member of the Scientific Advisory Board of SignPath Pharma, a developmental stage biotechnology company that is studying liposomal curcumin, liposomes and other agents. Dr. Helson is CEO of SignPath Pharma. Laura Sordillo reports no conflicts.

4. Abstract Title:

Paraptosis in human glioblastoma cell line induced by curcumin.

Abstract Source:

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Abstract Author(s):

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Abstract:

Curcumin is a polyphenol compound extracted from Curcuma longa plant, is a molecule with pleiotropic effects that suppresses transformation, proliferation and metastasis of malignant tumors. Curcumin can cause different kinds of cell death depending of its concentration on the exposed cell type. Here we show that exposure of the glioblastoma cell line A172 to curcumin at 50 μM, the IC50, causes morphological change characteristic of paraptosis cell-death. Vesicles derived from the endoplasmic reticulum (ER) and low membrane potential of the mitochondria were constantly found in the exposed cells. Furthermore, changes in expression of the ER Stress Response (ERSR) genes IRE1 and ATF6, and the microRNAs (miRNAs) miR-27a, miR-222, miR-449 was observed after exposure to curcumin. AKT-Insulin and p53-BCL2 networks were predicted being modulated by the affected miRNAs. Furthermore, AKT protein levels reduction was confirmed. Our data, strongly suggest that curcuminexerts its cell-death properties by affecting the integrity of the reticulum, leading to paraptosis in the glioblastoma cells. **These data unveils the versatility of curcumin to control cancer progression.**

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5.

European Journal of Pharmaceutics and Biopharmaceutics

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Research paper

Nanomedicine based curcumin and doxorubicin combination treatment of glioblastoma with scFv-targeted micelles: *In vitro* evaluation on 2D and 3D tumor models

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Abstract

NF-κB is strongly associated with poor prognosis of different cancer types and an important factor responsible for the malignant phenotype of glioblastoma. **Overcoming chemotherapy-induced resistance caused by activation of PI3K/Akt and NF-κB pathways is crucial for successful glioblastoma therapy**. We developed an all-in-one nanomedicine formulation for co-delivery of a chemotherapeutic agent (topoisomerase II inhibitor, doxorubicin) and **a multidrug resistance modulator (NF-κB inhibitor, curcumin)** for treatment of glioblastoma due to their synergism. Both agents were incorporated into PEG-PE-based polymeric micelles. The glucose transporter-1 (GLUT1) is overexpressed in many tumors including glioblastoma. The micellar system was decorated with GLUT1 antibody single chain fragment variable (scFv) as the ligand **to promote blood brain barrier transport** and glioblastoma targeting. The combination treatment was synergistic (combination index, CI of 0.73) against U87MG glioblastoma cells. This synergism was improved by micellar encapsulation (CI: 0.63) and further so with GLUT1 targeting (CI: 0.46). Compared to non-targeted micelles, GLUT1 scFv surface modification increased the association of micelles (>20%, $P < 0.01$) and the nuclear localization of doxorubicin(~3-fold) in U87MG cells, which also translated into enhanced cytotoxicity. **The increased caspase 3/7 activation by targeted micelles indicates successful apoptosis enhancement by combinatory treatment.** Moreover, GLUT1 targeted micelles resulted in deeper penetration into the 3D spheroid model. The increased efficacy of combination nanoformulations on the spheroids compared to a single agent loaded, or to non-targeted formulations, reinforces the rationale for selection of this combination and successful utilization of GLUT1 scFv as a targeting agent for glioblastoma treatment.

6.

Cancer Prevention Research

1. Cancer Prev Res April 2014 7; 466

Curcumin Suppresses Proliferation of Colon Cancer Cells by Targeting CDK2

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1. T.-G. Lim and S.-Y. Lee contributed equally to this work.

Abstract

Curcumin, the yellow pigment of turmeric found in Southeast Indian food, is one of the most popular phytochemicals for cancer prevention. Numerous reports have demonstrated modulation of multiple cellular signaling pathways by curcumin and its molecular targets in various cancer cell lines. To identify a new molecular target of curcumin, we used shape screening and reverse docking to screen the Protein Data Bank against curcumin. Cyclin-dependent kinase 2 (CDK2), a major cell-cycle protein, was identified as a potential molecular target of curcumin. **Indeed, *in vitro* and *ex vivo* kinase assay data revealed a dramatic suppressive effect of curcumin on CDK2 kinase activity. Furthermore, curcumin induced G_1 cell-cycle arrest,** which is regulated by CDK2 in HCT116 cells. Although the expression levels of CDK2 and its regulatory subunit, cyclin E, were not changed, the phosphorylation of retinoblastoma (Rb), a well-known CDK2 substrate, was reduced by curcumin. Because curcumin induced cell-cycle arrest, we investigated the antiproliferative effect of curcumin

on HCT116 colon cancer cells. In this experiment, **curcumin suppressed HCT116 cell proliferation effectively.** To determine whether CDK2 is a direct target of curcumin, CDK2 expression was knocked down in HCT116 cells. As expected, HCT116 sh-CDK2 cells exhibited G_1 arrest and reduced proliferation. Because of the low levels of CDK2 in HCT116 sh-CDK2 cells, the effects of curcumin on G_1 arrest and cell proliferation were not substantially relative to HCT116 sh-control cells. From these results, we identified CDK2 as a direct target of curcumin in colon cancer cells. *Cancer Prev Res; 7(4); 466–74. ©2014 AACR*.

Footnotes

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Normal human colon epithelial cells (HCEC) comprise an immortalized epithelial cells derived from human colon biopsies with properties similar to normal colon epithelial cells.

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7. Clinical Cancer Research *[You only need to read the abstract, its results, & conclusions. First is the abstract of the study which provoked my interest in curcumin as an anti-cancer agent. The full study follows with all the accompanying footnotes. Charts and figures have been left out. - HK]*

Clin Cancer Res. 2008 Jul 15;14(14):4491-9. doi: 10.1158/1078-0432.CCR-08-0024.

Phase II trial of curcumin in patients with advanced pancreatic cancer.

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Author information

Abstract

PURPOSE:

Pancreatic cancer is almost always lethal, and the only U.S. Food and Drug Administration-approved therapies for it, gemcitabine and erlotinib, produce objective responses in <10% of patients. We evaluated the clinical biological effects of curcumin (diferuloylmethane), a plant-derived dietary ingredient with potent nuclear factor-kappaB (NF-kappaB) and tumor inhibitory properties, against advanced pancreatic cancer.

EXPERIMENTAL DESIGN:

Patients received 8 g curcumin by mouth daily until disease progression, with restaging every 2 months. Serum cytokine levels for interleukin (IL)-6, IL-8, IL-10, and IL-1 receptor antagonists and peripheral blood mononuclear cell expression of NF-kappaB and cyclooxygenase-2 were monitored.

RESULTS:

Twenty-five patients were enrolled, with 21 evaluable for response. Circulating curcumin was detectable as drug in glucuronide and sulfate conjugate forms, albeit at low steady-state levels, suggesting poor oral bioavailability. Two patients showed clinical biological activity. One had ongoing stable disease for >18 months; interestingly, one additional patient had a brief, but marked, tumor regression (73%) accompanied by significant increases (4- to 35-fold) in serum cytokine levels (IL-6, IL-8, IL-10, and IL-1 receptor antagonists). No toxicities were observed. Curcumin down-regulated expression of NF-kappaB, cyclooxygenase-2, and phosphorylated signal transducer and activator of transcription 3 in peripheral blood mononuclear cells from patients (most of whom had baseline levels considerably higher than those found in healthy volunteers). Whereas there was considerable interpatient variation in plasma curcumin levels, drug levels peaked at 22 to 41 ng/mL and remained relatively constant over the first 4 weeks.

CONCLUSIONS:

Oral curcumin is well tolerated and, despite its limited absorption, has biological activity in some patients with pancreatic cancer.

Free full text

Phase II Trial of Curcumin in Patients with Advanced Pancreatic Cancer

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Abstract

Purpose: Pancreatic cancer is almost always lethal, and the only U.S. Food and Drug Administration–approved therapies for it, gemcitabine and erlotinib, produce objective responses in <10% of patients. We evaluated the clinical biological effects of curcumin (diferuloylmethane), a plant-derived dietary ingredient with potent nuclear factor-κB (NF-κB) and tumor inhibitory properties, against advanced pancreatic cancer.

Experimental Design:Patients received 8 g curcumin by mouth daily until disease progression, with restaging every 2 months. Serum cytokine levels for interleukin (IL)-6, IL-8, IL-10, and IL-1 receptor antagonists and peripheral blood mononuclear cell expression of NF-κB and cyclooxygenase-2 were monitored.

Results: Twenty-five patients were enrolled, with 21 evaluable for response. Circulating curcumin was detectable as drug in glucuronide and sulfate conjugate forms, albeit at low steady-state levels, suggesting **poor oral bioavailability.** Two patients showed clinical biological activity. **One had ongoing stable disease for >18 months; interestingly, one additional patient had a brief, but marked, tumor regression (73%)** accompanied by significant increases (4- to 35-fold) in serum cytokine levels (IL-6, IL-8, IL-10, and IL-1 receptor antagonists). No toxicities were observed. Curcumin down-regulated expression of NF-κB, cyclooxygenase-2, and phosphorylated signal transducer and activator of transcription 3 in peripheral blood mononuclear cells from patients (most of whom had baseline levels considerably higher than those found in healthy volunteers). **Whereas there was considerable interpatient**

variation in plasma curcumin levels, drug levels peaked at 22 to 41 ng/mL and remained relatively constant over the first 4 weeks.

Conclusions: **Oral curcumin is well tolerated and, despite its limited absorption, has biological activity in some patients with pancreatic cancer.**

Pancreatic adenocarcinoma is one of the most lethal cancers, with most patients dying of their disease within 1 year (1). The only currently available U.S. Food and Drug Administration–approved treatments for this disease are gemcitabine and erlotinib, both of which produce responses only in a minority of patients, and their effect on survival is measured in weeks only (2, 3). Therefore, better therapies are urgently needed.

Numerous studies have indicated that the inflammatory transcription factor nuclear factor-κB (NF-κB) is constitutively active in patients with pancreatic cancer (4). The role of NF-κB in suppression of apoptosis, tumor growth, invasion, angiogenesis, and metastasis, via a variety of downstream effectors, is well documented (5–8). Therefore, an agent that can target NF-κB is of interest for the treatment of pancreatic cancer.

Curcumin (diferuloylmethane) is derived from turmeric (Curcuma longa). We and others have shown that it suppresses NF-κB activation (9) as well as a multitude of other biological signals pertinent to cancer (5, 10, 11). Recent work in our laboratory showed that treatment of human pancreatic cancer cells with curcumin leads to down-regulation of constitutive NF-κB activation, suppression of NF-κB-regulated gene products, and inhibition of cell growth associated with apoptosis (12). In addition, administration of liposome-encapsulated curcumin systemically suppresses the growth of human pancreatic cancer xenografts in a mouse model, and this antitumor activity is accompanied by an antiangiogenic effect (13).

Phase I studies of curcumin have shown that this agent can be administered safely at oral doses of up to 8 g/d (14, 15). There was no dose-limiting toxicity; dosing was limited by the number of pills that patients could or would swallow daily. However, the usefulness of curcumin may be attenuated because of its poor oral bioavailability (16). Therefore, we did the present phase II study to determine whether oral curcumin has biological activity in patients with pancreatic cancer.

Patients and Methods

Eligibility.Patients were eligible if they were at least ≥18 years old and had histologically confirmed adenocarcinoma of the pancreas. Patients had to have a Karnofsky performance status of >60 at study entry (17) and preserved hepatic function (bilirubin ≤ 2.0 mg/dL) and renal function (creatinine ≤ 2.0 mg/dL). Patients with an unstable medical condition or intercurrent illness, including uncontrolled diabetes mellitus or hypertension, active infections requiring treatment with systemic antibiotics, unstable congestive heart failure, uncontrolled arrhythmias, unstable coagulation disorders, brain metastases, and uncontrolled seizure disorder, were excluded. In addition, pregnant women and those who were breastfeeding were excluded as were individuals who underwent radiotherapy or chemotherapy <4 weeks beforehand. All subjects gave their written informed consent in keeping with the policies of the Surveillance Committee of The University of Texas M. D. Anderson Cancer Center.

Curcumin.Curcumin was obtained as a generous donation from Sabinsa in 1 g caplet form. Each capsule contained 1 g curcuminoids (900 mg curcumin, 80 mg desmethoxycurcumin, and 20 mg bisdesmethoxycurcumin) confirmed by high-performance liquid chromatography tandem mass spectrometry.

Study design.This was a nonrandomized, open-label, phase II trial of curcumin, and we are reporting on the first 25 patients who satisfied all inclusion and exclusion criteria. Patients took oral curcumin daily for 8 weeks. The starting dose was 8 g/d. The patients could not receive any concomitant chemotherapy or radiotherapy, although they could receive supportive care. Patients who had stable disease or better after 8 weeks received continued therapy with curcumin at the same dose and schedule.

Evaluation during study.A complete history [pathologic confirmation of malignancy, disease staging, prior therapy/surgery, and prior response(s)] and a physical examination, as well as blood tests (including a complete blood count with differential, platelet counts, and electrolytes), renal and hepatic function tests, a serum pregnancy test for female patients of childbearing potential, tumor markers (CA 19-9, CA 27.29, and CA125 as well as carcinoembryonic antigen), an electrocardiogram, a chest radiograph, and diagnostic imaging, were done at baseline. All of these procedures were repeated every ∼4 weeks and at the end of therapy for all patients enrolled on the study, except for diagnostic imaging, which was repeated every ∼8 weeks during the course of therapy.

Safety evaluation.Systemic and local adverse events were assessed using the National Cancer Institute expanded Common Toxicity Criteria version 3.0 (18). Patients could continue treatment until disease progression unless grade 3 toxicity supervened.

Tumor response.Tumor response was defined as a complete response, partial response, stable disease, or progressive disease as per the classic Response Evaluation Criteria in Solid Tumors criteria (19). In addition, tumor markers were evaluated at the beginning of and every ∼8 weeks during treatment.

Correlative studies.Correlative studies were done on blood samples of patients if they gave informed consent for optional blood draws for research purposes. Correlative studies done include serum cytokine assessment; the effect of orally administered curcumin on constitutive and tumor necrosis factor-α–induced binding expression of NF-κB, cyclooxygenase-2 (COX-2), and phosphorylated signal transducer and activator of transcription 3 (pSTAT3) in peripheral blood mononuclear cells (PBMC) pretherapy and on day 8 by using electrophoretic mobility shift assay (EMSA); and pharmacokinetics of curcumin.

Cytokines.Cytokines [interleukin (IL)-6, IL-8, and IL-10 and IL-1 receptor antagonists (IL-1RA)] have been implicated previously in the pathophysiology of pancreatic cancer (20). Serum samples were drawn by a phlebotomist prestudy at 24 h, 8 days, 4 weeks, and 8 weeks to assess these cytokine levels, measured using an ELISA with commercially available kits (Quantikine; R&D Systems). The lower limits of the assay sensitivity are as follows: IL-6 (0.7 pg/mL), IL-8 (3.5 pg/mL), IL-10 (3.9 pg/mL), and IL-1RA (22.0 pg/mL), respectively.

As controls, cytokine levels were measured in 48 to 62 healthy volunteers depending on the cytokine assessed. Volunteers provided informed consent in accordance with institutional policy. The control samples were frozen and stored in a manner identical to the handling of patient samples.

All serum samples were aliquoted and stored at −80°C. Baseline samples were obtained within 48 h before starting therapy. Samples were thawed and assayed in duplicate with all values expressed as the mean of the two determinations. A standard curve was generated using known concentrations of recombinant cytokines according to the manufacturer's instructions and the samples were read using a plate reader (Molecular Devices). Results were calculated by generating a four-variable, logistic curve fit using the SOFTmax Pro software program (version 2.6; Molecular Devices). The concentration of a particular cytokine was then determined using the standard curve.

Materials.The mouse monoclonal antibody (sc-8059) against pSTAT3, which detects STAT3 phosphorylated at tyrosine residue 705, and antibody against the epitope corresponding to amino acids mapping within the amino-

terminal domain of human NF-κB p65 (anti-p65) were obtained from Santa Cruz Biotechnology. Anti-COX-2 antibody was purchased from Transduction Labs (now Invitrogen). The liquid 3,3′-diaminobenzidine substrate chromogen system-horseradish peroxidase used for immunocytochemistry was obtained from DakoCytomation. Bacteria-derived human tumor necrosis factor, purified to homogeneity with a specific activity of 5 × 107units/mg, was provided by Genentech. Penicillin, streptomycin, RPMI 1640 and fetal bovine serum were obtained from Invitrogen. Tris, glycine, sodium chloride, SDS, and bovine serum albumin were obtained from Sigma.

NF-κB activation.To determine NF-κB activation status, we isolated the nuclei from PBMC derived from patients with pancreatic cancer and healthy volunteers, homogenates were prepared, and EMSA was carried out essentially as described previously (21). Briefly, nuclear extracts prepared from PBMC (1 × 106/mL) were incubated with 32P-end-labeled 45-mer double-stranded NF-κB oligonucleotides (4 g protein with 16 fmol DNA) from the HIV long terminal repeat (5′-TTGTTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGG-3′; italics indicates NF-κB binding sites) for 15 min at 37°C. The DNA-protein complex formed was separated from free oligonucleotides on 6.6% native polyacrylamide gels. A double-stranded mutated oligonucleotide (5′-TTGTTACAACTCACTTTCCGCTGCTCACTTTCCAGGGAGGCGTGG-3′) was used to examine the specificity of binding of NF-κB to the DNA. The specificity of binding was also examined by competition with an unlabeled oligonucleotide. For supershift assays, nuclear extracts were incubated with antibody against the p65 subunit of NF-κB for 30 min at room temperature before the complex was analyzed using EMSA. Antibodies against cyclin D1 and preimmune serum were included as negative controls. The gels were dried and visualized, and radioactive bands were quantitated using a PhosphorImager (Molecular Dynamics) with the ImageQuant software program.

Immunolocalization of NF-κB p65, pSTAT3, and COX-2.The nuclear localization of p65, pSTAT3, and COX-2 was examined using an immunocytochemical method as described previously (22). Briefly, PBMC derived from patients with pancreatic cancer were plated on glass slides, allowed to adhere overnight, and fixed with paraformaldehyde. After a brief washing with PBS, slides were blocked with a protein block solution (DakoCytomation) for 20 min and then incubated with rabbit polyclonal anti-human p65 antibody, mouse monoclonal anti-human pSTAT3, and anti-COX-2 antibodies (at dilutions of 1:100, 1:50, and 1:75, respectively). After incubation overnight, the slides were washed and then incubated with biotinylated link universal antiserum and then a horseradish peroxidase-streptavidin conjugate using a labeled streptavidin-biotin system kit. Slides were rinsed and developed using 3,3′-diaminobenzidine as a chromogen. Finally, slides were rinsed in distilled water, counterstained with Mayer's hematoxylin, and mounted for evaluation using digital picture exchange. Photographs of the stained PBMC were captured using the Photometrics Coolsnap CF color camera (Nikon) and the MetaMorph software program (version 4.6.5 software; Universal Imaging).

Curcumin pharmacology

Analysis of clinical curcumin product.Curcumin used for cancer prevention is seldom administered in a pure chemical form. Rather, it typically consists of three separate curcuminoids consisting of curcumin itself as well as demethoxycurcumin and bisdesmethoxycurcumin (23, 24). To determine the qualitative and quantitative presence of these curcuminoids in the curcumin product used for this clinical trial, the drug material was separated on a Gemini 5 μm C18 (2 × 100 mm) analytic column (Phenomenex) using a linear acetonitrile/methanol/0.2% formic acid gradient. The amount of curcuminoid was detected using a Waters Quattro Ultima tandem mass spectrometer equipped with electrospray-positive ionization capability. All three compounds were quantified by using standard calibration curves prepared from reference standard materials obtained from Sigma-Aldrich. Calibration curves were prepared by making a 1 mg/mL stock solution of the authentic materials in methanol and then serially diluting the stock solutions to 1,000, 500, 100, 50, 10, 5, and 1 ng/mL in 50:50 methanol/0.2% formic acid. Calibration curves

were then prepared using the mass spectrometry quantification software program. The percentages of the three curcuminoids in the curcumin product used in this study were as follows: curcumin, 87.2% (detected at m/z367); demethoxycurcumin, 10.5% (detected at m/z337); and bisdesmethoxycurcumin, 2.3% (m/z307).

Analysis of curcumin pharmacology by mass spectrometry.Despite the use of doses of curcumin as high as 8 g/d, very little free curcumin is typically found in patient plasma samples (14, 16). Rather, curcumin is present in plasma in conjugated (glucuronide and sulfate) forms, thereby necessitating appropriate enzymatic hydrolysis of the plasma before detection of free curcumin (24). In the present study, plasma samples were obtained from patients before they received their initial dose of curcumin as well as at 1, 2, 6, 24, 48, and 72 h, day 8, and 4 weeks after day 1 while still receiving the same daily dose of curcumin. Aliquots (200 L) of plasma were mixed with 600 L Dulbecco's PBS (Sigma-Aldrich) and 200 L of 100 units/L type II β-glucuronidase/sulfatase (total 20,000 units) in Dulbecco's PBS and incubated at 37°C. After 1 h, the incubated plasma was mixed with 1 mL of 0.2% formic acid to acidify the solution and samples were then extracted three times with 3 mL ethyl acetate. Ethyl acetate extracts were combined and evaporated to dryness with nitrogen gas; dried samples were then reconstituted with 200 L of 50:50 methanol/0.2% formic acid and analyzed for curcumin content using high-performance liquid chromatography-tandem mass spectrometry. The instrument used was an Agilent 1100 binary high-performance liquid chromatography system with a temperature-controlled autosampler connected to a Waters Quattro Ultima tandem mass spectrometer. Extracted curcumin was chromatographed using a linear gradient consisting of solution A (0.2% formic acid) and solution B (80:20 acetonitrile/methanol). The initial mobile phase conditions consisting of 30% solution A and 70% solution B were switched to 5% solution A and 95% solution B at 3 min. These conditions were maintained for 2 min before switching back to the initial conditions, which were maintained for an additional 3 min before additional sample analyses. The analytical column, Gemini 5 μm C18 (150 × 2 mm), was obtained from Phenomenex. The mass spectrometer was run in the electrospray-positive mode with curcumin being detected and quantified using the following mass transitions m/z= 369.2 > 285.0 mass transition. Quantification was done using a standard curve constructed from extracted human plasma spiked with known amounts of Sigma curcuminoid standards. The extracted curves were prepared using spiked plasma standards ranging in concentration from 1 to 1,000 ng/mL. These standard curves typically had correlation coefficients of >0.98.

Patient characteristics.Of the 25 patients enrolled, 24 patients were evaluable for toxicity and 21 were evaluable for response (Table 1). Their median age was 65 years (range, 43-77 years). Thirteen patients were men. The median number of prior therapies was 2 (range, 0-4) and the median time from diagnosis to enrollment into the trial was 8 months (range, 1-67 months).

Toxicity and response.We observed no treatment-related toxic effects. **To date, one patient remains stable for >18 months and another patient had a dramatic but brief tumor response**. The former patient had previously undergone a failed Whipple's surgery followed by gemcitabine and radiation for locally advanced disease. He had an elevated CA125 level but not an elevated CA19.9 level. With treatment (curcumin 8 g by mouth daily), the CA125 level in this patient has slowly decreased over 1 year (Fig. 1). His weight remains unchanged (and he does not have ascites or edema). His lesions are stable in size by serial positron emission tomography/computed tomography scans and there has been a decrease in the standardized uptake value in those lesions from a baseline level of 10.6 to a level of 5.7 after 12 months of therapy. **One patient had a brief but marked response (73% reduction in tumor size** by Response Evaluation Criteria in Solid Tumors) that lasted 1 month (Fig. 2). Interestingly, at the time of progression, the lesions that had regressed remained small, but other lesions grew larger (data not shown). Finally, one patient remained on study for ∼8 months with stable weight and a feeling of well-being, albeit with progression in nontarget lesions.

Levels of CA125 in patient 14. Serum levels of CA125 increased transiently before dropping again when the dose of curcumin was held briefly (for 3 wk) for surgery for correction of an abdominal wall defect associated with his previous surgery and radiation therapy. CA125 then dropped when curcumin was restarted. CA 19-9 was not elevated.

Computed tomography scan of the abdomen showing hepatic lesions in patient 8. The computed tomography scans on the left were done pretherapy; the one on the right were done at 2 mo after starting curcumin. There was an overall 73% decrease in the size of liver lesions by Response Evaluation Criteria in Solid Tumors.

Cytokine levels in healthy volunteers.The majority of healthy volunteers (n= 48-62 participants depending on the cytokine being measured) had undetectable serum levels of IL-6, IL-8, and IL-10. In contrast, they all had detectable serum levels of IL-1RA (20).

Cytokine, NF-κB, and COX-2 levels in patients who received curcumin.As per our previous studies, baseline serum cytokines were measurable and elevated in most of the patients with pancreatic cancer (Table 2; ref. 20), including the two patients who appeared to have biological activity of curcumin after treatment. Notably, the levels were below the median for both patients for IL-6 and above the median for both patients for IL-1RA. The patient who benefited most (≥18 months with slowly improving disease) had the highest IL-1RA level among all patients.

Baseline cytokine levels and responses

After treatment, we detected variable changes in cytokine levels (Fig. 3A-D). Of interest, the patient who had a marked, albeit short-lived, tumor response (patient 8) had significant increases in all cytokine levels. These increases were greater than those seen in any of the other patients. Specifically, the IL-6 level reached 35-fold of the baseline level for this patient. The patient who had stable disease for ≥18 months (patient 14) experienced slow improvement over 1.5 years and had decreases in all cytokine levels. NF-κB is constitutively active in patients with pancreatic cancer.

Ato D,ratio of cytokines IL-6, IL-8, IL-10, and IL-1RA respectively. Numbers are calculated by the following formula: (higher number / lower number × 100) - 100. If the pretherapy number was the higher number (cytokine levels decreased after treatment), a negative sign was given to the number calculated by the above formula. Cytokine levels were measured by ELISA as per methods. Of interest, patient 8 with the marked, albeit brief, response (depicted in Fig. 2) had a marked increase in cytokine levels after treatment. Patient 14 had stable disease for ≥12 mo (CA125 levels for patient 14 are shown in Fig. 1).

Because NF-κB has been shown to play a critical role in the growth and angiogenesis of pancreatic cancer (25, 26), we examined the expression of this transcription factor in PBMC by immunocytochemistry (22) and by EMSA. Immunocytochemistry showed constitutively active NF-κB as indicated by nuclear localization of p65 (Fig. 4A). In contrast, neither EMSA nor immunocytochemistry showed NF-κB activation in healthy volunteers (Fig. 4; representative data shown). NF-κB was constitutively active in PBMC derived from patients with pancreatic cancer as examined using a DNA-binding assay (Fig. 4B). NF-κB binding was comparable with that after tumor necrosis factor stimulation. Of the 25 patients, 19 consented to optional blood draws to look for correlative markers and cytokines. All 19 patients examined had constitutively active NF-κB (see Table 3). Ten of the 19 patients also donated their blood samples for research blood draw for immunocytochemistry at day 8 of therapy (Table 3). On treatment of patients with curcumin, immunocytochemistry showed a decline (without reaching statistical

significance; P= 0.1, Student's ttest) in nuclear NF-κB compared with that in normal volunteers (Table 3; Fig. 4C), but EMSA did not (data not shown).

A,PBMC from pancreatic cancer patients express constitutively activated NF-κB. Cells from patients at baseline (top) and from normal volunteers (bottom) were analyzed for nuclear p65 as described in Materials and Methods. There were high baseline levels of p65. B,PBMC from patients with pancreatic cancer show constitutive binding of NF-κB by EMSA, whereas PBMC from normal volunteers do not. KBM5 (myeloid leukemia line) with and without treatment with tumor necrosis factor (0.1 nmol/L for 30 min) were used as positive and negative controls (last two lanes). Nuclear extracts were prepared and analyzed for DNA binding by EMSA as described in Materials and Methods. Patients with pancreatic cancer showed high levels of constitutive NF-κB binding, whereas normal volunteers did not (results expressed as fold). C,representative example of a patient with pancreatic cancer who had baseline (day 0) increased expression of NF-κB (p65), COX-2, and pSTAT3 compared with the decrease in immunohistochemical staining after only 8 d of oral curcumin treatment.

Expression of NF-κB (p65), COX-2, and pSTAT3 in PBMC derived from patients with pancreatic cancer by immunohistochemical staining

We also examined COX-2 expression in PBMC because COX-2 is activated by NF-κB, is overexpressed in many tumors, including pancreatic cancer, and plays a role in tumorigenesis (6–8). PBMC in all 19 patients examined expressed COX-2 by immunocytochemistry (Table 3; Fig. 4). The COX-2 expression levels declined post-treatment with curcumin (P< 0.03, Student's ttest).

Additionally, we examined patient blood samples for expression of activated pSTAT3 in PBMC, because pSTAT3 is regulated by growth factors such as epidermal growth factor and because IL-6 is overexpressed in many tumors, including pancreatic cancer, plays a role in tumorigenesis, and can be regulated by curcumin (27). All 19 patients examined had activated STAT3 expression at baseline. There was a statistically significant decline (P= 0.009, Student's ttest) in the percentage of pSTAT3-positive cells by immunocytochemistry after treatment with curcumin (Table 3; Fig. 4C).

Curcumin pharmacology.Plasma curcumin levels were determined in 19 patients, all of whom received a daily dose of 8 g curcumin. Although we found little, if any, free or unconjugated curcumin in these patients' plasma, we easily detected levels of curcumin following digestion of plasma with combined glucuronidase and sulfatase enzymes. This is consistent with data suggesting that curcumin is present in plasma in conjugated (glucuronide and sulfate) forms, thereby necessitating appropriate enzymatic hydrolysis before detection of free curcumin (24, 28). Plasma levels of drug released from conjugated derivatives of curcumin on day 1 of dosing decreased on average to 22 to 41 ng/mL from 2 to 6 h after the first dose of curcumin, although the Cmaxrange across patients was considerable. For example, at 2 h, curcumin released from conjugate forms ranged from 0 (not detectable) to 125 ng/mL; at 24 h (before treatment on day 2), drug levels ranged from 1.8 to 117.0 ng/mL. The peak level in the patient with prolonged stable disease was only 2.6 ng/mL at 6 h, whereas the peak level in the patient with tumor regression was only 14.9 ng/mL. We found no evidence of a cumulative increase in drug levels throughout the 4-week sampling period. We detected an apparent steady-state level of conjugated curcumin in plasma that was achieved by day 3; this level was 22 to 41 ng/mL. Interestingly, three patients had small but detectable levels of curcumin in their pretreatment plasma, suggesting that a dietary source of curcumin was already present.

The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of malignant cell types, as well as tumor initiation, promotion, and metastasis, presumably due to its myriad biological properties (29). These properties include down-regulation of transcription factors such as NF-κB as well as other growth-

regulatory molecules including, but not limited to, STAT3 and COX-2, cyclin D1, and growth factor receptors (such as epidermal growth factor receptor; ref. 30).

Based on our in vitroand in vivo(animal) work showing activity of curcumin and liposome-encapsulated curcumin in cell lines and models of pancreatic cancer (12, 13), and the fact that this activity was associated with down-regulation of NF-κB binding, we initiated the present study of oral curcumin. To date, this agent has been well tolerated, with no systemic toxic effects. We have seen antitumor effects in two patients, one of whom had 73% tumor reduction (Fig. 2), which was, however, short-lived. Surprisingly, this patient had a rapid and dramatic increase in cytokine levels (IL-6, IL-8, IL-1RA, and IL-10; Fig. 3). Conceivably, this occurred because of release of cytokines from the tumor associated with shrinkage. Also, of potential importance in this patient is the observation that the tumors that originally regressed continued to show regression during the follow-up period on curcumin, whereas the tumors that grew were the ones that had been small originally. This observation suggests that there was a malignant clone responsive to curcumin, whereas another resistant clone emerged. In contrast, the patient who has appeared to have benefited most from treatment with curcumin (patient 14) has had slow improvement over 1 year and a gradual decrease in cytokine levels (Fig. 3). Of interest, patient 14 had the highest baseline levels of IL-1RA of any of the study patients. This may be biologically relevant because IL-1RA is a naturally occurring IL-1 antagonist, and we have seen in vitrogrowth promotion of pancreatic cancer cell lines by IL-1β.6

Cheng et al. (15) gave tablets containing curcumin to patients with premalignant conditions for as long as 3 months and did not record any treatment-related toxic effects up to doses of 8 g/d. Beyond 8 g/d, the bulky volume of curcumin was unacceptable to the patients. Other studies have shown a similar lack of toxicity at daily doses of curcumin of up to 12 g (14–16, 23).

A key question related to treatment with curcumin is its poor bioavailability after being taken orally (23). Our results also indicated that only low levels of curcumin are detectable in plasma (steady-state level at day 3 is ~22-41 ng/mL). Nevertheless, some of the patients had biological activity of curcumin as evidenced by the antitumor effects noted above in two patients and by effects on cytokine levels and on NF-κB, COX-2, and pSTAT3, as described above. Conceivably, the limited bioavailability of curcumin attenuated the response rate, because **exposure to microgram amounts of curcumin is required to show antiproliferative effects in vitro(12).** It is also possible that circulating curcumin levels do not reflect tumor tissue curcumin levels. Our results also showed that PBMC derived from almost all patients expressed constitutively active NF-κB (n= 18), whereas none of the PBMC from normal subjects did (n= 5; representative data in Fig. 4). NF-κB may have been activated in these patients because of high levels of cytokine expression, as multiple cytokines can induce NF-κB (20). Indeed, most of our patients had high baseline levels of IL-6, IL-8, IL-10, and IL-1RA expression (Table 2). On the other hand, the high levels of NF-κB expression may have been responsible for the elevated baseline cytokine levels, because NF-κB can be found in the promoter regions of these cytokines and thus drives their expression. The expression of NF-κB correlated well with the expression of COX-2. This is not surprising as NF-κB is also a transcription factor for COX-2. The majority of the patients showed down-regulation of NF-κB and COX-2 after treatment with curcumin, but this down-regulation did not reach statistical significance for NF-κB (Table 3; representative data in Fig. 4C). This is the first study to show that curcumin can down-regulate the expression of these molecules in humans. However, the ability of curcumin to do this is consistent with earlier preclinical data from our group (9, 11, 13). Down-regulation of these factors was not associated with clinical response in many patients. It may be that down-regulation in PBMC does not reflect what occurs at the level of the tumor itself, and this may be one explanation for why many patients did not respond.

We also found that pSTAT3 was constitutively active in PBMC from almost all of the patients with pancreatic cancer

(Table 3) but not in normal subjects. Curcumin treatment led to a decrease in constitutive pSTAT3 activation in most patients (Table 3; representative data in Fig. 4C). These results agree with reports from our group showing that curcumin can modulate pSTAT3 activation (22), a molecule implicated in tumorigenesis and chemoresistance (22, 31).

One important puzzle that arises from this study relates to **why we see biological activity despite limited absorption** and low nanogram levels of circulating curcumin. Low systemic bioavailability of curcumin after oral dosing is consistent with findings in preclinical models and in humans. It is now well established that curcumin exists in rodent and human plasma largely in conjugated forms, with the glucuronide conjugate present in much greater abundance than the sulfate conjugate (32). **Little "free" or unconjugated drug is therefore typically found in plasma after oral dosing.** Even plasma concentrations of curcumin released from conjugated forms, however, were surprisingly low. In the present study, these levels also varied widely among patients. It has been suggested previously that systemic levels of drug may not reflect drug levels actually present in tissues of interest (14). Although at least one study has examined curcumin levels in colon tissue of mice after oral administration (33), few, if any, studies have analyzed curcumin or curcumin metabolites in malignant human tissues. Furthermore, although all three forms of curcuminoids (curcumin, demethoxycurcumin, and bisdesmethoxycurcumin) have been shown to have important pharmacologic activity against malignant cell growth in vitro(34–37), few studies have reported the relative activity of curcumin glucuronide against malignant cell growth (38). Such information is, of course, of great importance as this form of curcumin represents the major circulating form of this drug. Therefore, **further investigations are needed to elucidate the relationship between the form of curcumin, its relative pharmacologic activity, and circulating versus tumor tissue levels.**

In conclusion, our current study shows that oral curcumin is tolerated without toxicity at doses of 8 g/d for up to 18 months. Although this molecule is **poorly absorbed**, with low nanogram levels of circulating curcumin detected at steady-state, biological activity is evident. Preclinical data suggest that curcumin has potent activity against pancreatic cancer (12, 13), but higher levels of exposure need to be achieved. Curcumin is hydrophobic and therefore cannot be given i.v. However, because it is lypophilic, it can be encapsulated in a liposome, and such a preparation would allow i.v. administration, leading presumably to higher circulating levels of curcumin. We have reported previously that systemically administered liposomal curcumin has antitumor activity both in vitroand in vivo(13) and has no overt toxicity in animal models**. Our current results suggest, therefore, that our plan to develop liposomal curcumin for clinical trials in cancer patients is a worthwhile strategy. This or other better formulations of curcumin may provide more consistent blood levels with better pharmacologic effect.**

Disclosure of Potential Conflicts of Interest

V. Ba**dmaev is employed by Sabinsa.**

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An Anticancer Effect of Curcumin Mediated by Down-Regulating Phosphatase of Regenerating Liver-3 Expression on Highly Metastatic Melanoma Cells

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Abstract

Phosphatase of regenerating liver-3 (PRL-3) has been suggested as a potential target for anticancer drugs based on its involvement in tumor metastasis. However, little is known about a small-molecule inhibitor against PRL-3. In this study, we report that **curcumin, the component of the spice turmeric, shows its antitumor effect by selectively down-regulating the expression of PRL-**3 but not its family members PRL-1 and -2 in a p53-independent way. Curcumin inhibited the phosphorylation of Src and stat3 partly through PRL-3 down-regulation. Cells with PRL-3 stably knocked down show less sensitivity to curcumin treatment, which reveals **that PRL-3 is the much further upstream target of curcumin**. **Curcumin treatment also remarkably prevented B16BL6 from invading the draining lymph nodes in the spontaneous metastatic tumor model,** which is probably of relevance to PRL-3 down-regulation. **Our results reveal a novel capacity of curcumin to down-regulate oncogene PRL-3, raising its possibility in therapeutic regimen against malignant tumor.**

The phosphatase of regenerating liver (PRL) as a tyrosine phosphatase family includes 3 members: PRL-1, PRL-2, and PRL-3. In 2001, PRL-3 was first reported to be overexpressed in metastatic lesions derived from colorectal cancers, but it was expressed at lower levels in primary tumors and normal colorectal epithelium (Saha et al., 2001). The elevated PRL-3 expression was then found in other highly metastatic cancers such as gastric carcinomas (Miskad et al., 2004), Hodgkin's lymphoma (Schwering et al., 2003), melanomas (Wu et al., 2004), and breast (Polato et al., 2005) and ovarian tumors (Zeng et al., 2003), suggesting that PRL-3 may be a molecular marker for metastatic tumor cells. **Indeed, several in vitro and in vivo studies support a causal link between PRL-3 and tumor metastasis.** Overexpressing PRL-3 promotes motility and invasion of both tumor cell

lines and normal cell lines (Zeng et al., 2003; Wu et al., 2004), whereas knocking down endogenous PRL-3 with small interfering RNA attenuates cancerous cell motility and metastatic tumor formation (Qian et al., 2007). Treatment with monoclonal antibody of PRL-3 massively inhibited the tumor growth in vivo (Li et al., 2005; Guo et al., 2008). **Therefore, PRL-3 is considered a tractable target for anticancer drugs,** and regulating its expression and function may become a new strategy to prevent or treat tumor metastasis. However, there is no report on the natural small-molecule compounds that can regulate PRL-3 expression.

Curcumin is a polyphenol derived from dietary spice turmeric. It possesses wide-ranging anti-inflammatory and anticancer properties (Sharma et al., 2005). **The abilities of curcumin to induce apoptosis of cancer cells and to inhibit angiogenesis and cell adhesion contribute to its chemotherapeutic potential in the treatment of cancer.** Several phase I and phase II clinical trials indicate that **curcumin is quite safe and may exhibit therapeutic efficacy in patients with progressive advanced cancers** (Dhillon et al., 2008). Although inhibition of several cell signaling pathways involving Akt (Woo et al., 2003), nuclear factor-κB (Aggarwal et al., 2006), activator protein-1 (Balasubramanian and Eckert, 2007), or c-Jun N-terminal kinase (Chen and Tan, 1998) have been implicated in the biological effects of curcumin, its direct molecular target and mechanism of inhibition in tumor metastasis remain to be well clarified.

In the present study, **we showed a novel activity of curcumin in this first report of specific down-regulation of PRL-3 expression**, which contributes to **the in vivo antimetastatic effect of curcumin**. Such activity of curcumin was further demonstrated to be at the transcriptional level without affecting the stability of either PRL-3 mRNA or protein and to result in the inhibition of the phosphorylation of Src and stat3.

.....other sections deleted

Discussion

It is well accepted that PRL-3 is a metastasis-associated gene. In the initial study, we observed that curcumin inhibited cell proliferation and adhesion of mouse melanoma B16BL6, in which high invasive and metastatic activity is closely correlated with its high level of PRL-3 expression. These findings implicate that curcumin might show anticancer effects at least partially by regulating PRL-3. **Indeed, curcumin decreased PRL-3 mRNA of B16B16 in a dose- and time-dependent manner,** [NOTE - curcumin decreases PRL in a dose dependent manner! HK] and the inhibitory effect occurred at the transcriptional level. The cells with PRL-3 stably knocked down were less susceptible to curcumin inhibition. PRL-3 expression in other cell lines was also inhibited by curcumin, suggesting that this mechanism is not unique to B16B16 highly expressing PRL-3. It is noteworthy that curcumin had no effect on the expression of PRL-1 and PRL-2, which share a high degree (>75%) of amino acid sequence identity. It is likely that curcumin down-regulates PRL-3 transcription through a pathway different from PRL-1 or PRL-2. The 5′-noncoding regions of mouse PRLs are much more divergent, and the expression pattern of the PRLs differs among tissues, which supports the possibility of differential transcription regulation (Zeng et al., 1998).

The exact mode of PRL-3 transcription regulation is unclear. Although recent study has demonstrated that PRL-3 is a p53 target gene and induces G_1 cell-cycle arrest in a p53-dependent manner in primary cells (Basak et al., 2008), we found that curcumin had no effect on p53 despite the PRL-3 down-regulation. Upon exposure to curcumin, no changes were detected in either p53 protein level or p53 binding to the corresponding site in the

promoter of PRL-3 (Supplemental Fig. 1A). **Moreover, curcumin still decreased PRL-3 even in p53-deficient murine embryonic fibroblasts (Supplemental Fig. 1B). Curcumin has ever been reported to accelerate the p53 accumulation in some tumor cell lines,** such as MCF-7 (Choudhuri et al., 2002). In our test, PRL-3 down-regulation in MCF-7 cells seemed to be less sensitive to curcumin treatment than in B16BL6 cells. That is, the PRL-3 level of MCF-7 cells showed no significant change after exposure to 20 μM curcumin for 12 h (Fig. 1A), which may be caused by the accumulation of wild type of p53. However, such accumulation of p53 did not up-regulate PRL-3, because the target transcription factor of curcumin could be more active than p53 in the transcription regulation of PRL-3. Taken together, these results suggest that there are other transcription factors, rather than p53, involving in regulating PRL-3 transcription in tumor cells, and they are targeted by curcumin. Some tumor cells were consistently not arrested by up-regulated PRL-3 in a p53-dependent manner like primary cells (Ryan et al., 2001). On the other hand, the p53 tumor suppressor plays a critical role in protecting organisms from developing cancer (Liang et al., 2007). Degrading wild-type p53 might lead to the accumulation of DNA-damaged cells by inhibiting their p53-induced apoptosis (Ryan et al., 2001; Vousden and Lu, 2002). Therefore, it seems unreasonable to down-regulate PRL-3 expression by targeting the degradation of p53 in tumor cells. Further study is in progress to elucidate the mechanism of PRL-3 transcription regulation by using curcumin as a tool.

Considerable studies suggest that curcumin shows wide-ranging anti-inflammatory and anticancer properties and is able to affect multiple targets (Anand et al., 2008). In this study, we demonstrated that PRL-3 is not only a normal target of curcumin, but a trigger one. Elevated PRL-3 will lead to Src activation through down-regulating the synthesis of C-terminal Src kinase protein, which in turn leads to tyrosine phosphorylation of a number of proteins in human embryonic kidney 293 cells (Liang et al., 2007). In highly metastatic melanoma cells, we were surprised to find decreases in tyrosine phosphorylation in PRL-3 stably knocked down cell lines (P1 and P9), compared with L1 and L13 cells, which stably express luciferase siRNA. However, unlike that in human embryonic kidney 293 cells, we have not noticed significant change of the Tyr527 but the Tyr416 phosphorylation of Src (Supplemental Fig. S2). This result indicates that there might be other relationships between PRL-3 and Src activation that are independent of C-terminal Src kinase. As an important substrate of Src, stat3 can be also elevated by PRL-3, and the Src/stat3 pathway has been demonstrated to be implicated in tumor metastasis, including proliferation, invasion, and motility (Darnell, 2002) As shown in Fig. 3C, the inhibition of the activity of Src and stat3 made by curcumin was approximately 45 and 39% through down-regulating PRL-3 expression, respectively. Cells with PRL-3 stably knocked down by siRNA proved to be less susceptible to the anticancer effect of curcumin. **These findings suggest that PRL-3 is the much further upstream target of curcumin.** This study is the first to reveal the relationship between the inhibition effect of curcumin on stat3 phosphorylation and PRL-3 and **provide a possible mechanism by which curcumin inhibits the metastasis of different cancers.**

In vivo study showed that curcumin dose-dependently inhibited the tumor growth and prevented B16BL6 cells in primary tumor from invading the draining lymph nodes. As expected, the PRL-3 expression in the tumor tissues was remarkably decreased by curcumin. These results are similar to those in our previous study using PRL-3 siRNA (Qian et al., 2007). Because of the high level of PRL-3 mRNA in heart, therapeutic targeting of PRL-3 might exhibit cardiotoxicity (Stephens et al., 2005). However, we detected no changes either of PRL-3 mRNA level in the cardiac muscle tissues or of myocardial function in the mice treated with curcumin. Moreover, we did not detect visible protein of PRL-3 in the heart and muscle of adult mice like the tumor cell lines, which indicates that the role of PRL-3 protein synthesis system is unique in normal tissues, and **it also supports the idea that targeting the expression of endogenous PRL-3 by curcumin is safe and feasible as a novel therapy for cancer.**

Footnotes

- ⌟S The online version of this article (available at http://molpharm.aspetjournals.org) contains supplemental material.

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- ABBREVIATIONS:

  PRL = phosphatase of regenerating liver

  DMEM = Dulbecco's modified Eagle's medium

  DMSO = dimethyl sulfoxide

  FBS = fetal bovine serum

  GAPDH = glyceraldehyde-3-phosphate dehydrogenase

  PBS = phosphate-buffered saline

  PCR = polymerase chain reaction

  siRNA = small interfering RNA

  RT-PCR = reverse transcription-polymerase chain reaction

  Bp = base pair.

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9. Carcinogenesis *[This study - NOT about curcumin - points to the marked efficacy of other Chinese herbs taken by mouth in treating breast cancer. Apparently it is as good as Taxol. HK]*

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Oral administration of penta-O-galloyl-β-D-glucose suppresses triple-negative breast cancer xenograft growth and metastasis in strong association with JAK1-STAT3 inhibition

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Abstract

There is an urgent clinical need for chemotherapeutic and chemopreventive drugs for triple-negative breast cancer (TNBCa). Extending on our recent work, **we hypothesize that the herbal compound 1,2,3,4,6-penta-O-galloyl-beta-D-glucose (PGG) can inhibit the growth and metastasis of TNBCa xenograft** and target Janus-activated kinase (JAK)-

signal transducer and activator of transcription (STAT) 3-signaling axis. Daily oral gavage of 10 mg PGG/kg body wt decreased MDA-MB-231 xenograft weight by 49.3% ($P < 0.01$) at 40 days postinoculation, whereas weekly intraperitoneal injections of Taxol at the same dosage resulted in a 21.4% reduction ($P > 0.1$). **PGG treatment also decreased the incidence of lung metastasis.** Immunohistochemical staining detected decreased Ki-67 (proliferation) index and increased terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (apoptosis) index in PGG-treated and Taxol-treated xenografts. However, **the CD34 (angiogenesis) index was decreased only in PGG-treated xenografts** along with decreased phospho-STAT3. In cell culture of MDA-MB-231 cells, PGG decreased pSTAT3 and its downstream target proteins, decreased its upstream kinase pJAK1 and induced the expression of SHP1, a JAK1 upstream tyrosine phosphatase, within as early as 1 h of exposure. The phosphatase inhibitor pervanadate reversed the PGG-induced downregulation of pSTAT3 and caspase activation. Orally administered PGG can inhibit TNBCa growth and metastasis, probably through anti-angiogenesis, antiproliferation and apoptosis induction. Mechanistically, PGG-induced inhibition of JAK1-STAT3 axis may contribute to the observed in vivo efficacy and the effects on the cellular processes.

Introduction

Breast cancer (BCa) remains the major cause of cancer-related deaths in women in the USA (1) and worldwide. Approximately 60–70% of BCa cases express estrogen receptor-α (ERα) and/or progesterone receptor, and another ∼20% of cases have amplified human epidermal growth factor receptor (HER)-2 proto-oncogene and express high levels of the HER-2 protein (2). For the majority of organ-confined BCa, which express ERα and progesterone receptor, lumpectomy/mastectomy is often curative. Molecularly targeted therapies that inhibit the estrogen/ERα pathway or that target amplified HER-2 are quite effective in treating the residual diseases in patients whose cancer expresses these targets in an adjuvant therapy context.

Approximately 15–20% of BCa cases are in the category of triple-negative phenotype (2–4), i.e. they lack of ERα and progesterone receptor and do not have amplification of HER-2. These patients have a very poor prognosis because there is no clinically validated molecularly targeted therapy. When surgical and radiation options are no longer applicable to these triple negative breast cancer (TNBCa) patients, treatment with available cytotoxic and genotoxic chemotherapy drugs produces limited efficacy and significant side effects. There remains a strong and urgent need for safer anticancer compounds for the treatment/management of the TNBCa and their metastasis. Novel agents with multiple-targeting ability distinct from the known drugable targets could be useful for circumventing the limitations of current treatment options.

Mammary development occurs through highly coordinated and precise expression/activation of a variety of transcription factors. Inappropriate or constitutive activation of many of these transcription factors is found in BCa and may contribute directly to its pathogenesis (5). Especially, signal transducer and activator of transcription (STAT) proteins have been shown to play an important role in tumor cell survival and proliferation (6). STAT3 is often constitutively active in many human cancer cells and is highly expressed in the TNBCa MDA-MB231 cells (7–9). STAT3 is a latent transcription factor that resides in the cytoplasm. Upon activation by tyrosine phosphorylation, STAT3 dimerizes, translocates to the nucleus and binds to nuclear DNA to modulate transcription of target genes. STAT3 phosphorylation is principally mediated through the activation of non-receptor protein tyrosine kinase family of Janus-activated kinases (JAKs), which include many members JAK1, JAK2, JAK3 and tyrosine kinase 2 (10,11). Additionally, the STAT3 phosphorylation can also be mediated by crosstalk with c-Src kinase (11,12). The major phosphorylation sites in STAT3 include tyrosine and serine residues at positions Tyr705 and Ser727, respectively, located in the transactivation domain. The activation of STAT3 results in expression of many target genes required

for tumor cell survival (e.g. Bcl-xL, Mcl-1 and survivin), proliferation (e.g. cyclin D1 and c-myc) and angiogenesis [e.g. vascular endothelial growth factor (VEGF)] as well as metastasis (13). **Thus, STAT3-signaling pathway has been a favorite therapeutic target for drug development (14,15).**

Natural herbal products are potential rich source of chemical inhibitors of STAT3 signaling. 1,2,3,4,6-penta-O-galloyl-beta-D-glucose (PGG) (see structure in Figure 1A) is **a naturally occurring gallotannin polyphenolic compound in oriental herbs such as Galla Rhois, the gallnut of Rhus chinensis MILL and the root of peony Paeonia suffruticosa** Andrews (16). Our collaborative team has recently reported that **PGG inhibits both constitutive and cytokine-induced STAT3 phosphorylation in prostate cancer cells in vitro** and decreased in vivo xenograft growth with decreased pSTAT3 in PGG-treated mice (17). The mechanisms of STAT3 inactivation by PGG have not been elucidated.

TNBCa xenograft growth-suppressing activity of PGG in female athymic nude mice. (A) Chemical structure of PGG. (B-E) Effect of PGG administration by oral gavage on MDA-MB-231 tumor growth. Starting 1 day after cell inoculation, PGG (10 mg/kg body wt) was delivered by feeding needle with 2% Tween-80 as vehicle once daily. Taxol injection (10 mg/kg body wt) was given intraperitoneally once per a week. (B) Body weights of mice. (C) Tumor growth in a time course. (D) Final tumor weight at termination of experiment. Values are means ± standard deviations, n = 8. *P < 0.05 compared with control. (E) Photographs of selected tumor bearing mice and their dissected tumors.

Earlier work from our group has reported an anti-angiogenic effect of PGG (18) through downregulation of cyclo-oxygenase-2 and VEGF, the latter being a well-recognized target gene of STAT3 (19) and that PGG potently inhibits mouse Lewis lung carcinoma allograft growth in syngenic mice in a dose-dependent manner (18). Recently, PGG has been shown to inhibit PC-3 xenograft growth established in the mouse tibia and to decrease matrix metalloproteinase-9 expression (20), which is a target of STAT3 and is crucial for cancer cell invasion and metastasis. **Our collaborative team has recently shown, for the first time, an impressive oral efficacy of PGG at 20 mg/kg daily single dosage against xenograft growth established from human MDA-MB-231 TNBCa cells (21).**

Here, we confirmed and extended the efficacy evaluation for suppressing not only MDA-MB-231 TNBCa xenograft growth, but also on lung metastasis and established the involvement of suppression of STAT3 signaling in vivo. Using cell culture model with this cell line, we aimed at elucidating the molecular mechanisms of PGG-induced STAT3 pathway inactivation.

….Sections Deleted

Discussion

Our in vivo data demonstrate a growth inhibitory efficacy of orally administered PGG against MDA-MB231 TNBCa xenograft and lung metastasis (Figure 1). The fact that PGG is orally available and therefore can be self administered by patients will have a major impact on reducing the health care delivery cost, compared with injection-only drugs (such as Taxol) that have to be given by health care professionals. In addition to the suppression efficacy against the growth of inoculated primary cancer, PGG also decreased the incidence of metastasis to the lung (Figure 2A). The STAT3 pathway has been strongly linked to cancer angiogenesis, inflammation and metastasis (13,19,27). The

observed suppression by PGG treatment on pSTAT3 in tumor tissues (detected by IHC and western blotting) and its target proteins such as VEGF and Bcl-2, but not by Taxol treatment, suggest a potential in vivo molecular target pathway of PGG for contributing to/mediating antiproliferation, anti-angiogenesis, apoptosis and anti-metastasis .

Our investigation with the cell culture model (Figures 3–6) confirmed our early report of inhibition of STAT3 signaling by PGG in prostate cancer cells (17). We showed not only reduction in pSTAT3 and its DNA-binding activity in MDA-MB231 cells in vitro (detected by western blotting and electrophoretic mobility shift assay) (Figure 3), but also downregulation of many of its important targets involved in angiogenesis, cell survival and proliferation (Figure 4). Furthermore, our data produced additional insights into how PGG inactivates the STAT3 pathway. The simultaneous downregulation of pJAK1 with pSTAT3 suggested targeting this upstream activator of STAT3 by PGG (Figure 6). The ability of the PTP inhibitor pervanadate to reverse pSTAT3 dephosphorylation strongly suggested the induction of one or more PTPs by PGG. Our results (Figure 5D and E) suggested SHP-1 as one such PTP that could be rapidly induced by PGG to deactivate pJAK and the pSTAT3-signaling axis.

In summary, our in vivo data support oral efficacy of PGG against the growth and metastasis of a triple-negative BCa model, rivaling that of Taxol and with mechanistic differences from this first line chemotherapy drug. The inhibition of the activated STAT3 pathway contributed, at least in part, to the tumor growth efficacy and anti-metastasis through antiproliferative, anti-angiogenic and apoptosis induction.

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Conflict of Interest Statement: None declared.

Abbreviations: BCa = breast cancer

ERK = extracellular signal-related kinase

ERα = estrogen receptor-α

HER = human epidermal growth factor receptor

JAK = Janus-activated kinase

PARP = poly (adenosine diphosphate-ribose) polymerase

PBS = phosphate-buffered saline

PGG = 1,2,3,4,6-penta-O-galloyl-beta-D-glucose

PTP = protein tyrosine phosphatase

STAT = signal transducer and activator of transcription

TNBCa = triple-negative breast cancer

TUNEL = deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling

VEGF = vascular endothelial growth factor

10. Molecular Cancer Therapeutics

1. Mol Cancer Ther September 2008 7; 2681

Penta-1,2,3,4,6-*O*-galloyl-β-D-glucose induces p53 and inhibits STAT3 in prostate cancer cells *in vitro* and suppresses prostate xenograft tumor growth *in vivo*

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Abstract: Penta-1,2,3,4,6-*O*-galloyl-β-D-glucose (PGG) is a naturally occurring gallotannin from some Oriental herbs. Several cell culture studies suggested a potential for PGG as a novel agent for the chemoprevention and treatment of cancer. .....**Our data support PGG as a multitargeting agent for chemoprevention and therapy of prostate cancer by activating the p53 tumor suppressor pathway and by inhibiting STAT3 oncogenic signaling. [Mol Cancer Ther 2008;7(9):2681-91]**

Introduction: Prostate cancer is the most commonly diagnosed cancer that affects one in six American men (1). Notable features of prostate cancer that make chemoprevention a rational and cost-effective approach include its ubiquity and its long latency between premalignant lesions and clinically evident cancers (2). The induction of tumor suppressor signaling such as the p53 axis (3) and/or an inhibition of oncogenic signaling such as signal transducer and activator of transcription 3 (STAT3; refs. 4, 5) in precancerous or cancer cells could be potential molecular mechanisms of prostate cancer primary or secondary chemoprevention.....Therefore, p53 is considered to be a rational molecular target not only for cancer therapy but also for chemoprevention. **Indeed, several phytochemical cancer chemopreventive agents such as epigallocatechin-3-gallate (8), resveratrol (9), and curcumin (10), have been reported to induce apoptosis through the p53-dependent signaling pathway...**

10. ↵*Choudhuri T, Pal S, Das T, Sa G. Curcumin selectively induces apoptosis in deregulated cyclin D1-expressed cells at G_2 phase of cell cycle in a p53-dependent manner. J Biol Chem 2005;280:20059-68.*
Abstract/FREE Full Text

11. Molecular Cancer Therapeutics - June 2015. 14 (6)

Cancer Therapy: Preclinical

Clinical

Dietary Curcumin Attenuates Glioma Growth in a Syngeneic Mouse Model by Inhibition of the JAK1,2/STAT3 Signaling Pathway

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Abstract

Purpose: Glioblastomas are the most common and most deadly primary brain tumors. Here, we evaluated the chemotherapeutic effect of the natural polyphenol curcumin on glioma cells *in vitro* and *in vivo* using an immunocompetent orthotopic mouse model.

Conclusion: This preclinical study shows that curcumin is capable of suppressing malignant glioma growth *in vitro* and *in vivo.* Our data suggest that the pharmacologically safe agent curcumin holds promise for clinical application in glioma therapy.

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12. Interdiscip Toxicol. 2014 Jun; 7(2): 85–88. **EXCERPTED**

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Radiosensitive effect of curcumin on thyroid cancer cell death induced by radioiodine-131

Seyed Jalal Hosseinimehr and Seyed Amir Hossein Hosseini

Introduction: "Radioiodine-131 (^{131}I) has been used as the first line of treatment for hyperthyroidism, Graves' disease and differentiated thyroid cancer. It has a physical half-life of 8.02 days and emits gamma rays and beta particles (Sawin *et al.*, 1997, Zanzonico, 1997, Robbins *et al.*, 2005). It concentrates in thyroid cells and kills tumor cells, yet it has several side effects such as sialadenitis, gastrointestinal symptoms, xerostomia, temporary bone-marrow suppression and neoplasia (Bushnell *et al.*, 1992, Noaparast *et al.*, 2013). ^{131}I may also induce genetic damage and chromosomal instability in normal cells that may result in secondary malignancies (Baugnet-Mahieu *et al.*, 1994, Watanabe *et al.*, 2004, Hosseinimehr *et al.*, 2013). The cytotoxic effect of ^{131}I is mainly related to beta particles. Ionizing radiation causes cellular injury mainly by producing reactive oxygen species (ROS). ROS can induce lipid peroxidation and damage to cellular membranes and critical macromolecules such as DNA (Little, 2000, Noaparas *et al.*, 2013). Curcumin is a major component of turmeric, produced from the rhizome of the plant Curcuma *longa* (Chendil *et al.*, 2004). **Many studies have indicated that curcumin has strong pharmacological activities such as anti-oxidant, anti-cancer (Kuttan *et al.*, 1985), anti-microbial effects** (Negi *et al.*, 1999). Curcumin can scavenge free radicals and protect the cellular macromolecules against oxidative stress (Kalpana *et al.*, 2004, Polasa *et al.*, 2004, Singh *et al.*, 2012). Recently we showed that curcumin protected human lymphocytes against genotoxicity induced by ^{131}I and it significantly reduced the DNA damage induced by ^{131}I *in vitro*(Shafaghati *et al.*, 2014). Although curcumin exhibited protective effects on chromosome damage induced by ^{131}I in normal cells, its effect on thyroid cancer cells during ^{131}I treatment is not clear.

"The aim of this study was to determine the therapeutic effect of curcumin on cell death induced by ^{131}I in thyroid human cancer cells and human non-malignant fibroblast cells *in vitro.*"

RESULTS: Effect of curcumin on cell proliferation in thyroid cancer and HFFF2 cells

The effect of curcumin on cell proliferation in thyroid cancer and HFFF2 cells is shown in Figure 1. **In thyroid cancer cells, a statistically significantly reduced cell proliferation was observed in curcumin treatments** at concentrations of 5, 10 and 25 µg/ml ($p<0.02$). The percentage of survival in thyroid cancer cells was 92.5±2.4, 95±4.9 and 89.4±5.3 at concentrations of 5, 10 and 25 µg/ml, respectively. A statistically significant difference was observed between the doses of 5, 10 and 25 µg/ml of curcumin with control for cellular anti-proliferation (Figure 1A). **No significant toxicity was observed** in HFFF2 cells treated by any of the doses of curcumin (Figure 1B).

13. BMJ Case Reports - Volume 2017, Issue

CASE REPORT

Long-term stabilisation of myeloma with curcumin

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Summary

“Myeloma is a haematological malignancy which typically follows a relapsing-remitting course. While treatment can control the myeloma and improve quality of life for given periods of time, remissions generally become progressively shorter with subsequent relapses, and patients ultimately enter a final refractory phase. To help control symptoms and enhance quality of life, some patients use complementary therapies as an adjunct to their conventional therapy. **Here, we describe a myeloma patient who started a daily dietary supplement of curcumin when approaching her third relapse. In the absence of further antimyeloma treatment, the patient plateaued and has remained stable for the last 5 years with good quality of life.**

View Full Text

http://dx.doi.org/10.1136/bcr-2016-218148

Background

“Myeloma is a B-cell malignancy that is characterised by the monoclonal expansion and accumulation of abnormal plasma cells within the bone marrow. Clinical manifestations include bone pain, renal impairment, recurrent infections and anaemia.[1] Over the past decade, advances in the understanding of the

disease, together with the development of several novel treatments, have led to significant improvements in overall survival.[2]

“Despite this, myeloma remains incurable, with a median overall survival of 5.2 years from diagnosis.[3] The course of the disease is typically one of recurrent remission and relapse. However, patients progressively acquire resistance to treatment and subsequent remissions become shorter and shorter. Eventually, either they run out of treatment options or become refractory to them.

“In an effort to improve long-term outcomes, some myeloma patients seek to use dietary supplements, mostly for palliative purposes. While they may help to improve quality of life, there is little evidence they can increase survival.[4] Among them, curcumin, the active constituent of turmeric, has gained popularity as a complementary therapy in several cancers.

“Here, we present a case of a heavily pretreated relapsing myeloma patient who, in the absence of further treatment options at the time, started daily curcumin and has since remained stable for the past 5 years.

Case presentation

“A woman aged 57 years was initially diagnosed with monoclonal gammopathy of undetermined significance (MGUS) in 2007 following an incidental finding of M-protein (18 g/L) during investigation for hypertension.

“Within 15 months, the patient had rapidly progressed to ISS stage 3 myeloma with M-protein 49 g/L, urinary protein 1.3 g/24-hour, Bence-Jones protein 1.0 g/24-hour, Hb 9.7 g/dL and increasing back pain. She initially declined antimyeloma treatment but 6 months later, following vertebral collapse at T5 and T12, started cyclophosphamide, thalidomide and dexamethasone (CTD) treatment. However, after a week, the patient was admitted with idiosyncratic syndrome including hyponatraemia, a fall in albumin and worsening of blood counts. She received red cell transfusion and her electrolyte abnormalities were carefully corrected.

“Although there was evidence of a response to CTD (M-protein 34 g/L), bortezomib and dexamethasone treatment was initiated as an alternative, but this was discontinued after three cycles due to progressive disease (M-protein 49 g/L). The patient was then treated with lenalidomide and dexamethasone with the aim of reducing disease burden prior to high-dose therapy and autologous stem cell transplantation. Treatment was frequently interrupted and dose adjusted to account for neutropenia and despite a minor response after six cycles (starting M-protein 47 g/L, finishing M-protein 34 g/L), in October 2009, she proceeded with stem cell mobilisation. However, neither cyclophosphamide nor plerixafor/GCSF priming were successful. A bone marrow biopsy revealed 50%

myeloma cells and a course of CTD was restarted with cautious titration of thalidomide.

“The patient achieved a partial response with CTD retreatment over the course of 17 cycles (M-protein 13 g/L) with no further episodes of idiosyncratic syndrome. However, attempts to harvest stem cells in February 2011 and again there months later, both failed. By then, her M-protein had risen to 24 g/L and the patient was too neutropenic to be considered for a clinical trial.

At this point, the patient began a daily regime of oral curcumin complexed with bioperine (to aid absorption), as a single dose of 8 g each evening on an empty stomach. A few months later, she also embarked on a once-weekly course of hyperbaric oxygen therapy (90 min at 2 ATA) which she has maintained ever since. Her paraprotein levels gradually declined to a nadir of 13 g/L, her blood counts steadily improved and **there was no evidence of further progressive lytic bone disease.**

Outcome and follow-up

“The patient continues to take oral curcumin 8 g daily without further antimyeloma treatment. Over the last 60 months, her myeloma has remained stable with minimal fluctuation in paraprotein level, her blood counts lie within the normal range and she has maintained good quality of life throughout this period. Repeat bone imaging in 2014 identified multiple lucencies <1 cm in the right hip and degenerative changes in both hips, but these were attributed to osteoarthritis rather than the myeloma. Recent cytogenetic analysis revealed she had no abnormal cytogenetics by fluorescent in situ hybridisation.

Discussion

“A small but significant number of myeloma patients consume dietary supplements in conjunction with conventional treatment primarily to help cope with the side effects of treatment, manage symptoms and enhance general well-being. Few, if any, use dietary supplementation as an alternative to standard antimyeloma therapy. **Here, we describe a case in which curcumin has maintained long-term disease control in a multiply-relapsed myeloma patient. To the best of our knowledge, this is the first report in which curcumin has demonstrated an objective response in progressive disease in the absence of conventional treatment.**

“Curcumin is a polyphenol derived from the perennial herb *Curcuma longa* (turmeric) and has, for centuries, been used as a traditional Indian medicine. Several reports published over the two decades have claimed various

health benefits of curcumin and this has led to its increasing popularity as a dietary supplement to prevent or treat a number of different diseases.[5] ,[6]

“The biological activity of curcumin is indeed remarkable. It is a highly pleiotropic molecule which possesses **natural antioxidant, anti-inflammatory, antiseptic and analgesic properties.**[7] More recently, it has demonstrated **antiproliferative effects in a wide variety of tumour cells** including myeloma cells and exerts its antiproliferative effects through multiple cellular targets that regulate cell growth and survival.

“In vitro, curcumin prevents myeloma cell proliferation through inhibition of IL-6-induced STAT-3 phosphorylation and through modulation of the expression of NF-kB-associated proteins such as IkB〈,Bcl-2, Bcl-xL, cyclin D1 and IL-6[8] and apoptosis-related molecules including p53 and Bax.[9] In other studies, curcumin was shown to circumvent resistance to dexamethasone, doxorubicin and melphalan as well as potentiate the effects of bortezomib, thalidomide[10] and lenalidomide.[11] **Furthermore, curcumin-induced cell death was not influenced by myeloma molecular heterogeneity.**[12]

“The antimyeloma effects of curcumin in the clinical setting however are less clear. Only one phase I/II study has evaluated curcumin treatment in myeloma patients. These patients were either asymptomatic, relapsed or had plateau phase disease. Treatment with curcumin downregulated the expression of NFkB, COX-2 and STAT3 in peripheral blood mononuclear cells, but no objective responses were observed in any subgroup of patients.[13] **This may be as a result of small sample size in this study,** follow-up was limited to 3 months and clinical responses may have been observed with longer follow-up. However, downregulation of NFkB, COX-2 and STAT3 expression may not correlate with the clinical activity of curcumin and there may be further mechanisms of action that remain unclear, possibly through the modulation of another target. We would not be able to identify any patient-specific mechanisms of activity in this case study, as the patient has been taking curcumin for some time now and baseline bone marrow or peripheral blood samples are not available. However, in the setting of a clinical trial, it may be possible to use next-generation sequencing to help identify a mutation that may be a potential target for curcumin.

“Another study examined its effects in preventing the progression of MGUS and smouldering myeloma to myeloma.[14] ,[15] **The results showed that curcumin exerted a trace of biological activity** with modest decreases in free light chain and paraprotein levels and a reduction in a marker of bone resorption with curcumin treatment, suggesting the therapeutic potential of curcumin in MGUS and smouldering myeloma. However, more studies are needed to address this further.

“Whether such effects are observed in patients with active disease remains to be seen. **The fact that our patient, who had advanced stage disease and was effectively salvaged while exclusively on curcumin, suggests a potential antimyeloma effect of curcumin.** She continues to take daily curcumin and remains in a very satisfactory condition with good quality of life. This case provides further evidence of the potential benefit for curcumin in myeloma. We would recommend further evaluation of curcumin in myeloma patients in the context of a clinical trial.

“Learning points

- Myeloma is a relapsing-remitting cancer for which there is currently no cure.
- Curcumin, a polyphenol derived from turmeric, has been used for many years in some herbal remedies.
- **We report a case of a myeloma patient with advanced myeloma who, in the absence of conventional treatment, plateaued and has remained stable for many years with daily curcumin.**
- **Dietary supplements, such as curcumin, may be beneficial for some myeloma patients.”**

14. https://www.ncbi.nlm.nih.gov/pubmed/9973206/

Cancer Res. 1999 Feb 1;59(3):597-601.

Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer.

Kawamori T[1], Lubet R, Steele VE, Kelloff GJ, Kaskey RB, Rao CV, Reddy BS.
Author information

Abstract

"**Curcumin, derived from the rhizome of Curcuma longa L. and having both antioxidant and anti-inflammatory properties, inhibits chemically induced carcinogenesis in the skin, forestomach, and colon** when it is administered during initiation and/or postinitiation stages. This study was designed to investigate the chemopreventive action of curcumin when it is administered (late in the premalignant stage) during the promotion/progression stage of colon carcinogenesis in male F344 rats. We also studied the modulating effect of this agent on apoptosis in the tumors. At 5 weeks of age, groups of male F344 rats were fed a control diet containing no curcumin and an experimental AIN-76A diet with 0.2% synthetically derived curcumin (purity, 99.9%). At 7 and 8 weeks of age, rats intended for carcinogen treatment were given s.c. injections of azoxymethane (AOM) at a dose rate of 15 mg/kg body weight per week. Animals destined for the promotion/progression study received the AIN-76A control diet for 14 weeks after the second AOM treatment and were then switched to diets containing 0.2 and 0.6% curcumin. Premalignant lesions in the colon would have developed by week 14 following AOM treatment. They continued to receive their respective diets until 52 weeks after carcinogen treatment and were then sacrificed. **The results confirmed our earlier study in that administration of 0.2% curcumin during both the initiation and postinitiation periods significantly inhibited colon tumorigenesis.** In addition, administration of 0.2% and of 0.6% of the synthetic curcumin in the diet during the promotion/progression stage **significantly suppressed the incidence and multiplicity of noninvasive adenocarcinomas** and **also strongly inhibited the multiplicity of invasive adenocarcinomas of the colon. The inhibition of adenocarcinomas of the colon was, in fact, dose dependent. Administration of curcumin to the rats during the initiation and postinitiation stages and throughout the promotion/progression stage increased apoptosis** in the colon tumors as compared to colon tumors in the groups receiving AOM and the control diet. Thus, **chemopreventive activity of curcumin is observed when it is administered prior to, during, and after carcinogen treatment** as well as when it is given only during the promotion/progression phase (starting late in premalignant stage) of colon carcinogenesis."

15. J Breast Cancer. 2013 Jun; 16(2): 133–137.

Published online 2013 Jun 28. doi: 10.4048/jbc.2013.16.2.133

The Effect of Curcumin on Breast Cancer Cells

Dongwu Liu and Zhiwei Chen

"INTRODUCTION

"It is known that **breast cancer is the most common cancer for women worldwide, and accounts for approximately 25% of all female malignancies** with a higher prevalence in developed countries. Breast cancer is the second leading cause of cancer-related death among females in the world [1]. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) (Figure 1), which is extracted from the plant *Curcuma longa*, is an antioxidant that exerts antiproliferative and apoptotic effects. The expressions and activities of various proteins, such as inflammatory cytokines and enzymes, transcription factors, and gene-products linked with cell survivals and proliferation, can be modified by curcumin [2]. **Since curcumin possesses anti-inflammatory, antioxidant, and antitumoral effects, it has been studied vigorously as a chemopreventative agent in some cancer models and used in the therapeutic arsenal in clinical oncology. However, curcumin is insoluble and instable in water. The solubility of curcumin could be enhanced by utilizing the solubilizing properties of rubusoside. In addition, the selective delivery of synthetic analogs or nanotechnology-based formulations of curcumin to tumors may improve the chemopreventive and chemotherapeutic effects.** The focus of this short review is to describe how curcumin participates in the regulation of oncogene protein expression in breast cancer cells." ……Deleted…..

"The effect of curcumin on other oncogenes: **"Curcumin has been studied vigorously as a chemopreventative in several cancer models. ….deleted….** It was found that curcumin inhibited the phosphorylation of Src and stat3 partly through PRL-3 down-regulation, raising its possibilities in therapeutic regimen against malignant tumor **(Figure 3) [24]."**

"THE EFFECT OF CURCUMIN AND MITOMYCIN C COMBINATION TREATMENT ON BREAST CANCER CELLS: **"Mitomycin C (MMC) (Figure 4), a potent DNA cross-linker and antineoplastic agent, is usually used to fight various cancers. However, the use of MMC is limited because the prolonged use of MMC will result in permanent kidney or bone marrow damage and secondary tumors in normal cells.** It has been found that curcumin improves MMC-based chemotherapy **by simultaneously sensitizing cancer cells to MMC and reducing MMC-associated side-effects, increasing cell viability, and further decreasing lipid peroxidation and DNA damage [25,26].** The combination treatment of MMC and curcumin reduces the toxic effect of MMC **by inhibiting glucose regulatory protein (GRP58)-mediated DNA cross-linking**

through the ERK/p38 MAPK pathway (Figure 5) [27]. Another report indicated that curcumin enhanced antiproliferative effect of MMC in human breast cancer MCF-7 cells via the p38 MAPK pathway [25]. ….”

“ENHANCEMENT THE SOLUBILITY AND STABILITY OF CURCUMIN

“Though curcumin has been indicated as **highly cytotoxic towards various cancer cell** lines, its insolubility and instability in water contributes to **low bioavailability**. On the other hand, photodegradation and low bioavailability are major hurdles for the therapeutic use of curcumin. However, the solubility of curcumin could be enhanced by utilizing the solubilizing properties of rubusoside, and the rubusoside-solubilized curcumin successfully inhibited cell viability in human colon, breast, and pancreatic cancer cell lines [28]. In order to increase curcumin photostability and enhance its anticancer activity against MCF-7 breast cancer cells, Mulik et al. [29] formulated the transferrin-mediated solid lipid nanoparticles (Tf-C-SLN), which enhances the anticancer effect of curcumin in breast cancer cells *in vitro*. Moreover, it was found that curcumin conjugated with phosphatidylcholine increased curcumin bioavailability five-fold compared to original curcumin [30]. The prodrugs, which are produced by mono-PEGylation of curcumin, are stable in buffer at physiological pH and released curcumin readily in human plasma [31].

“The polycurcumins have high drug loading efficiency and can be used as backbone-type conjugates. The polycurcumins could fix drug loading contents, stabilize curcumin in their backbones, and tailor water-solubility. Tang et al. [32] made the high molecular weight curcumin polymers (polycurcumins) through condensation polymerization of curcumin. It was found that the polyacetal-based polycurcumin was not only highly cytotoxic to MCF-7 breast cancer cell lines but also showed significant antitumor activities in SKOV-3 intraperitoneal xenograft tumor models [32].

“CONCLUSION: **Recently, the potential effect of curcumin on cancer cells has been recognized by the scientific community in the world, and the molecular biological approaches help to elucidate the underlying mechanisms of actions on curcumin in tumor cells. However, the molecular mechanisms underlying the antitumor activity of curcumin have not been very clear until now. Another thing is that the molecular mechanism of curcumin on tumor cells was usually studied with tumor cell lines *in vitro*, and the molecular mechanisms *in vivo* need to be further investigated. More sophisticated technologies will have to be applied in conjunction so that curcumin derivatives could be used for rational cancer therapy.”**

16. Curcumin blocks autophagy and activates apoptosis of malignant mesothelioma cell lines and increases the survival of mice intraperitoneally transplanted with a malignant mesothelioma cell line

www.impactjournals.com/Oncotarget/ 2017

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“Malignant mesothelioma (MM) is a primary tumor arising from the serous membranes. The resistance of MM patients to conventional therapies, and the poor patients’ survival, encouraged the identification of molecular targets for MM treatment. Curcumin (CUR) is a “multifunctional drug”. We explored the in vitro effects of CUR on cell proliferation, cell cycle regulation, pro-survival signaling pathways, apoptosis, autophagy of human (MM-B1, H-Meso-1, MM-F1), and mouse (#40a) MM cells. In addition, we evaluated the in vivo anti-tumor activities of CUR in C57BL/6 mice intraperitoneally transplanted with #40a cells forming ascites. **CUR in vitro inhibited MM cells survival in a dose- and time-dependent manner and increased reactive oxygen species’intracellular production and induced DNA damage**. CUR triggered autophagic flux, but the process was then blocked and was coincident with caspase 8 activation which activates apoptosis. CUR-mediated apoptosis was supported by the increase of Bax/Bcl-2 ratio, increase of p53 expression, activation of caspase 9, cleavage of PARP-1, increase of the percentage of cells in the sub G1 phase which was reduced (MM-F1 and #40a) or abolished (MM-B1 and H-Meso-1) after MM cells incubation with the apoptosis inhibitor Z-VAD-FMK. CUR treatment stimulated the phosphorylation of ERK1/2 and p38 MAPK, inhibited that of p54 JNK and AKT, increased c-Jun expression and phosphorylation and prevented NF-κB nuclear translocation. Intraperitoneal administration of CUR increased the median survival of C57BL/6 mice intraperitoneally transplanted with #40a cells and reduced the risk of developing tumors. **Our findings may have important implications for the design of MM treatment using CUR.”**

17. Curcumin vs. Benign Uterine Tumors

Here are two case reports from one of my colleagues. HK

Patient 1.

Patient presented herself in office with four very large non-cancerous tumors. Two 2 cm tumors on the left portion of the uterus and two tumors on the right 1cm and 4 cm respectively. Patient had heard that Myofascial Release methods would reduce the problems associated with those types of tumors; such as pain and cramps.

After three treatments of MFR to affected area the edema associated with the tumors had improved for 4+ to 1+ edema but size of tumors relatively unchanged. After research on the subject the patient agreed to start a dosage of Complete Curcumin Pro of three tabs to begin and building to tolerance of six a day. Within one week of usage the cramping and pain disappeared. After one month of usage, the tumors had reduced to less than 1 cm and after six weeks three of the tumors were no longer detectable. The large tumor was about 10mm in size and hard to the touch.

Patient discharged with instructions to continue Curcumin Pro until there were no more associated issues.

Patient 2.

Patient presented to doctor with large tumors on her uterus; with continuous vagina bleeding, cramps and pain.

Patient consulted with this office about her issues.

Patient was placed on 8 tabs of Curcumin Pro per day; and told to monitor her progress. After one week the bleeding stopped and her regular menstruation returned. After four weeks the tumors had greatly reduced and pain stopped. After eight weeks the tumors were no longer determinable.

Patient was asked to monitor self for any changes.

18. *What follows is a curious tale of how cures can be found by serendipitous routes. On an off the beaten path web site called "OddityCentral" I read a story of a man who cured himself of pervasive terminal cancers simply by taking a safe and commonly used anti-worm medicine, fat soluble vitamins, and curcumin. I was about to disregard the story when I decided to do a Google search on the anti-parasitics involved - fenbendazole or mebendazole. Here is the "Oddity Central" article followed by the more serious medical research on the topic. You can decide if the protocol is worth recommending to your patients. I've already suggested it to some of mine. HK*

18. A. Man Claims Cheap Dog Deworming Medicine Cured His Terminal Cancer

By Spooky on May 2nd, 2019 Category: News - from Oddity Central

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"An Oklahoma man who was once diagnosed with small-cell lung cancer and told that he only had three months live claims he is now tumor-free thanks to a $5 deworming drug usually meant for dogs.

"Joe Tippens was diagnosed with small-cell lung cancer in 2016. Despite undergoing treatment for the disease, by January of 2017, the cancer had spread to other organs, including his stomach, neck, pancreas and even his bones. The cancer was everywhere and doctors advised him to go home and say his goodbyes because he only had three months to live. When small-cell cancer spreads as wide as it had in his case, the chances of survival are around one percent. Tippens thought he was going to die, and with nothing left to lose, he was willing to try anything in hopes of a miracle, even a dog dewormer called fenbendazole.

"The desperate cancer sufferer stumbled upon the bizarre treatment while browsing a forum of his alma mater, Oklahoma State University. The post that caught his eye read "If you have cancer or know someone who does, give me a shout". Joe had

already signed up for an experimental treatment that doctors said wouldn't save him but might extend his life expectancy from three months to a year, enough to at least meet his grandson. But he decided that contacting that forum poster couldn't hurt either. To his surprise, that person was a veterinarian who had a very interesting story to tell.

"The vet told Joe that scientists had accidentally discovered that a dog de-worming drug seemed to attack cancer cells in mice. One of the scientists who conducted the research had been diagnosed with stage 4 brain cancer and had been giving the same grim prognosis as Joe, but she started popping dog deworming pills and within six weeks her cancer was gone. Obviously, Joe was intrigued.

"Tippens stayed in the clinical trial his doctors had suggested, but also placed an order for fenbendazole, the canine drug the veterinarian mentioned. He didn't tell his physicians about it though. Three months later, when he had another PET scan to check the spread of his cancer, he was shocked to learn that there was no sign of the tumors anywhere in his body.

""Three months earlier…There was cancer in my body from head to toe. And it was a terrifyingly dangerous metastasis that leaves virtually 100% of its victims dead within 3 months," Joe said. "Here I was 3 months later and the PET scan was completely dark……void of any light…..anywhere."

"In September 2017, Joe went for yet another scan which again showed he was cancer-free. This time he told his doctor about the fenbendazole, but there was no way to prove that the deworming drug had cured him. **He had also been taking vitamin E supplements, CBD and bioavailable curcumin** *[I have added the bold emphasis - HK*], plus there was also the clinical trial the doctors suggested. The funny thing about that experimental drug was that out of the 1,100 patients on that clinical trial, Joe was the only one cleared of cancer.

""My insurance company spent $1.2 million on me with traditional means before I switched to a $5 a week medicine that actually saved me," Joe said, making sure to mention that he is not a doctor. "I am not prescribing medicine and I am not qualified to

give advice on medical treatments. BUT…..I am qualified to tell my story to as many people as possible."

"Joe Tippens story has caught the attention of the president of the Oklahoma Medical Research Foundation, Dr Stephen Prescott, who said he is working on case study report about the cancer-fighting properties of fenbendazole.

""We're going to do it and see if we can confirm, in a very rigorous and clinical sort of way, that these patients had that kind of response," Prescott said. "I'm usually skeptical, and I was and maybe still am about this one, but there's interesting background on this."

"Tippens, who is still cancer free, says he has at least 40 success stories other than his, all of which involve the use of fenbendazole. There are also studies that suggest the compound essentially starves cancer cells and kills them. Still, many doctors are skeptical about the drug and recommend that patients stick to classical treatments. Tippens himself has been accused of giving cancer patients false hope, but he seems unfazed by critics.

""Oh, how do I answer that? I mean, if I've saved one other person other than me, it's worth it to me," he told KOKO News, adding that he plans to take fenbendazole for the rest of his life."

18. B J Am Assoc Lab Anim Sci. 2008 Nov; 47(6): 37–40. Published online 2008 Nov.

Unexpected Antitumorigenic Effect of Fenbendazole when Combined with Supplementary Vitamins

Ping Gao,[1] Chi V Dang,[1] and Julie Watson[2,*]

Author information Article notes Copyright and License information Disclaimer

Abstract

"Diet containing the anthelminthic fenbendazole is used often to treat rodent pinworm infections because it is easy to use and has few reported adverse effects on research. However, during fenbendazole treatment at our institution, an established human lymphoma xenograft model in C.B-17/Icr-*prkdc*^{scid}/Crl (SCID) mice failed to grow. Further investigation revealed that the fenbendazole had been incorporated into a sterilizable diet supplemented with additional vitamins to compensate for loss during autoclaving, but the diet had not been autoclaved. To

assess the role of fenbendazole and supplementary vitamins on tumor suppression, 20 vendor-supplied 4-wk-old SCID mice were assigned to 4 treatment groups: standard diet, diet plus fenbendazole, diet plus vitamins, and diet plus both vitamins and fenbendazole. Diet treatment was initiated 2 wk before subcutaneous flank implantation with 3×10^7 lymphoma cells. Tumor size was measured by caliper at 4-d intervals until the largest tumors reached a calculated volume of 1500 mm^3. Neither diet supplemented with vitamins alone nor fenbendazole alone caused altered tumor growth as compared with that of controls. However, the group supplemented with both vitamins and fenbendazole exhibited significant inhibition of tumor growth. The mechanism for this synergy is unknown and deserves further investigation. Fenbendazole should be used with caution during tumor studies because it may interact with other treatments and confound research results."

18.C. Fenbendazole acts as a moderate microtubule destabilizing agent and causes cancer cell death by modulating multiple cellular pathways

- Nilambra Dogra,
- Ashok Kumar &
- Tapas Mukhopadhyay

Scientific Reports **volume 8**, Article number: 11926 (2018) | Download Citation

Abstract

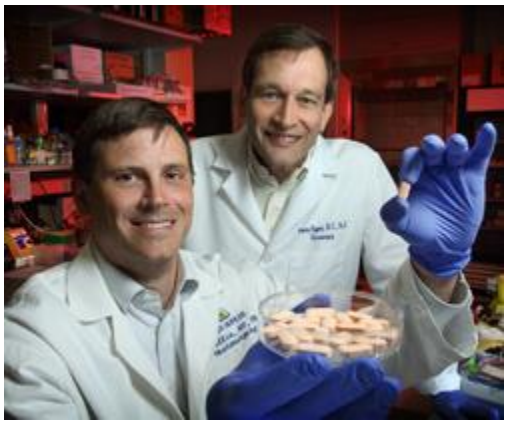
"Drugs that are already clinically approved or experimentally tested for conditions other than cancer, but are found to possess previously unrecognized cytotoxicity towards malignant cells, may serve as fitting anti-cancer candidates. Methyl N-(6-phenylsulfanyl-1H benzimidazol-2-yl) carbamate [Fenbendazole, FZ], **a benzimidazole compound, is a safe and inexpensive anthelmintic drug possessing an efficient anti-proliferative activity.** In our earlier work, we reported a potent growth-inhibitory activity of FZ caused partially by impairment of proteasomal function. Here, we show that FZ demonstrates moderate affinity for mammalian tubulin and exerts cytotoxicity to human cancer cells at micromolar concentrations. Simultaneously, it caused mitochondrial translocation of p53 and effectively inhibited glucose uptake, expression of *GLUT* transporters as well as hexokinase (*HK II*) - a key glycolytic enzyme that most cancer cells thrive on. **It blocked the growth of human xenografts in *nu/nu* mice model when mice were fed with the drug orally.** The results, in conjunction with our earlier data, suggest that FZ is **a new microtubule interfering agent that displays anti-neoplastic activity** and may be evaluated as a potential therapeutic agent because of its effect on multiple cellular pathways leading to effective elimination of cancer cells.

18.D. This is from the Johns Hopkins website:

https://clinicalconnection.hopkinsmedicine.org/news/surprise-finding-yields-a-possible-tumor-fighting-drug

"Surprise Finding Yields a Possible Tumor-Fighting Drug

"**Date:** November 11, 2014



"Gallia, left, and Riggins found what they'd been looking for with mebendazole.

Photo by Keith Weller

"A serendipitous finding in lab mice made by research professor **Gregory Riggins** and neurosurgeon **Gary Gallia** is creating excitement as a possible treatment for glioblastoma, an aggressive brain tumor.

Riggins' lab is known for discovering cancer-causing gene mutations and for assessing new drugs at the preclinical phase. Ordinarily, he and his colleagues have no trouble triggering glioblastoma cells to proliferate in mice. But over the course of several months in 2009, they encountered one group of mice in which the tumors would not grow. After some investigating, the scientists discovered that the mice had been treated with the veterinary antiparasitic drug fenbendazole.

"Searching the literature, they found reports that fenbendazole had been shown to inhibit cancer growth. Then, by trial and error, they determined that the related drug mebendazole—which has been used for the last 60 years to treat parasites in the human gastrointestinal tract—might also hold potential for stalling glioblastoma.

""We screened a lot of drugs in this family of compounds, and it was mebendazole that worked best," says Gallia. "This is what we'd been looking for."

"With that, Riggins, Gallia and their research team launched a program to understand the drug, improve its effectiveness against glioblastoma cells and have it manufactured for testing in patients. "The drug company that previously supplied mebendazole in the U.S. was no longer

manufacturing it," says Riggins, adding that they finally found a company in India that could supply the quantities they'd need for a clinical study. The scientists believe the drug helps obstruct tumors by inhibiting formation of strands of tubulin—proteins needed by cancer cells to grow.

"Glioblastoma is a fast-moving cancer. "Average survival is 15 to 20 months," says Riggins. "We're trying to improve therapy as quickly and strategically as we can." The Johns Hopkins team is at a critical juncture now—a phase I clinical trial with 24 patients to determine the drug's safety. "We are using the highest dose allowed by the review boards of Johns Hopkins and the Food and Drug Administration, and patients are tolerating it well," Riggins says of the study, which is about 50 percent complete.

"At best, the drug will slow tumor growth," says Riggins. He and his team are looking to pair it with other drugs. They expect that their formulation of mebendazole will also work in pediatric cases."

All I can say about all this is -

"惊人 - Jīngrén"

Amazing!

HK

Further Readings

Seventeen Reports on Curcumin vs. Cancers

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More Articles on Curcumin vs. Cancers:

16. And 17. The next two articles were derived from a web application which is a discussion tool between teachers and students at Medicine and Chirurgy department at the Turin University. No copyright marks were indicated, and this section of the course is not intended for commercial use, since no part of it will appear on any exams, since the reading materials in the course are free to read anyways, and since you only pay for the exam and receipt of CEU credits.

16. Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple targets

CURCUMIN AND PAPILLARY THYROID CANCER

Curcumin against Cancer elahe kharmandar 10/11/2015

Abstract: Phenolic compounds originated from one of the main class of secondary metabolites in plants, are natural phytochemicals derived mostly from phenylalanine and less often from tyrosine, and are widely present in food and nutraceuticals. They attract peoples' attention owing to their strong antioxidant activity and potentially protective effects against oxidative damage diseases including cancers. Recently, evidence suggesting phenolic compounds to possess an effective inhibitory effect on cancer invasion and metastasis is also increasingly reported in the scientific literature. Among these phenolic compounds, curcumin and its derivatives have been extensively studied and evaluated.

Introduction: Curcumin [bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione] is naturally a phenolic compound isolated as a yellow pigment from turmeric (Curcuma longa), which is commonly used as a spice, a food colourant, and also as a food preservative. Furthermore, curcumin is well documented for its medicinal properties in Chinese and Indian medicines and has been used in Ayurvedic medicine for over 6000 years. Several studies report that it is one agent that possesses a variety of biological and pharmacological activities including anti-proliferation, anti-apoptosis , anti-angiogenesis and inhibition of cell invasion and

metastasis. Previous studies demonstrated the potential antimetastatic activities of curcumin and its derivatives on a variety of cancers including lung cancer, breast cancer, colorectal cancer in vitro as well as in vivo. However, the exact molecular mechanisms by which curcumin exerts its effects is still to be determined.

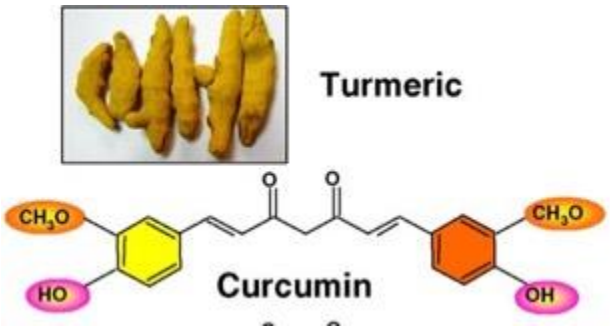


Fig. 1. Chemical structure of curcumin and its analogues derived from turmeric

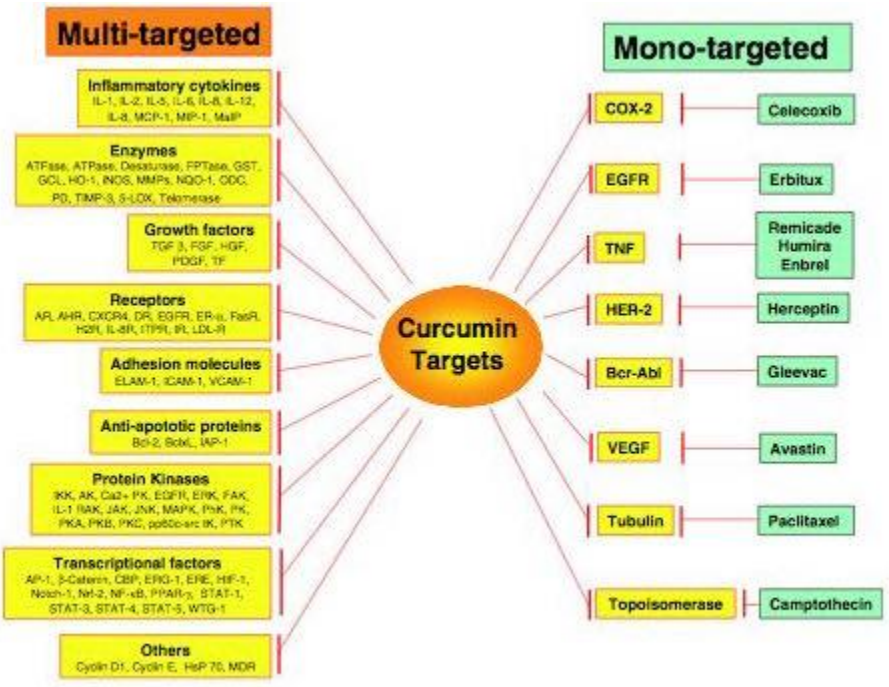


Fig. 2. Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple targets.

Thyroid cancer is the most common type of endocrine malignancy and its incidence is rapidly growing worldwide. The most prevalent thyroid cancer is that of papillary origin (PTC; account for about 60–80%) which differs from follicular (FTC; 15–25%) and anaplastic carcinomas (ATC; 2–5%). In addition, about 3–10% of thyroid tumours derived from parafollicular C-cells are termed as medullary thyroid carcinoma (MTC). However, well differentiated cancers such

as PTC may undergo dedifferentiation and progression to anaplastic carcinoma, a formidable disease characterized by widespread invasion, early distant metastasis.

Anti Metastasis Effects of Curcumin

Previous works strongly indicate that curcumin is a novel anti-metastasis agent used for treatment of thyroid cancer. curcumin could induce apoptosis in K1 papillary thyroid cancer cells via inducing intracellular formation of Reactive Oxygen Species followed by the collapse of MMP and the intracellular Ca2+ influx amount and affecting the expression of Bcl-2 and PARP. However, whether curcumin also plays a crucial role on metastasis of papillary thyroid cancer cells is still on question.
There Exist many investigations which have been shown that curcumin has inhibitory effects of metastasis toward a wide range of tumours, but its effect on thyroid cancer is not completely clarified. Curcumin dose-dependently suppressed cell viability of K1 as well as its cell attachment, spreading, migration and invasion abilities. Moreover, curcumin can also down-regulate the expression and activity of matrix metalloproteinase-9 (MMP-9).

Metastasis is a complex, multistep process made up of a cascade of interrelated, sequential steps including invasion, migration, adhesion, infiltration, colonization at a distant site, and the subsequent formation of new capillaries. To identify possible sites to prohibit cells treated with curcumin from metastasis.
It has been investigated the cytotoxicity of curcumin on papillary thyroid cancers. Curcumin dose-dependently inhibits the cell viability of K1 cells. (Fig. 3) then Among a series of crucial steps involved in initiating metastasis, invasion is the initial step and even the most essential one. An agent that could efficiently inhibit the ability of cancer cells to form secondary metastatic foci would be an ideal candidate to suppress cancer progression.

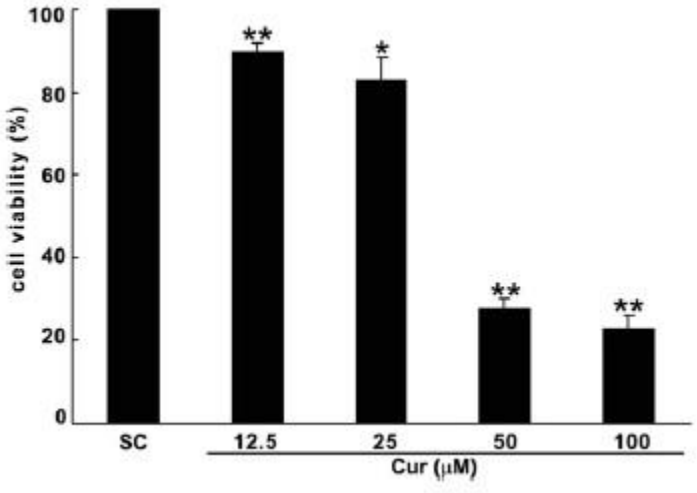


Fig 3.Effects of curcumin on K1 cell viability. Cells were incubated with or without curcumin (12.5, 25, 50 and 100 lM) for 24 h at 37 ————————————C. After

incubation, the effects ofcurcumin on cell viability were determined by MTT assay. All data represent as themeans ± SEM of five independent experiments. SC, solvent control.⁄P < 0.05 vs SC,⁄⁄P < 0.01 vs SC (Student's two tailed t-test).

During the process of invasion, the adhesion of cancer cells to their surrounding interstitium and basement membrane composition is the first step; in the process of invading to the blood vessels and then spilling out, the adhesion of cancer cells to vascular endothelialium and the basement membrane heterogeneity is the key step of metastasis. These all highlight the important role of adhesion in the cells attachment to tissue and finally spreading. The interaction and adhesion of cells with different biological surface is a dynamic process of invasion and metastasis of cancer cells. The adhesion process includes three steps: adhesion factors secret, cells attachment to tissue and finally spreading. The interaction and adhesion of cells with different biological surface is a dynamic process that requires specific cell surface receptors, structural proteins, signalling proteins and the intracellular cytoskeleton to participate in.

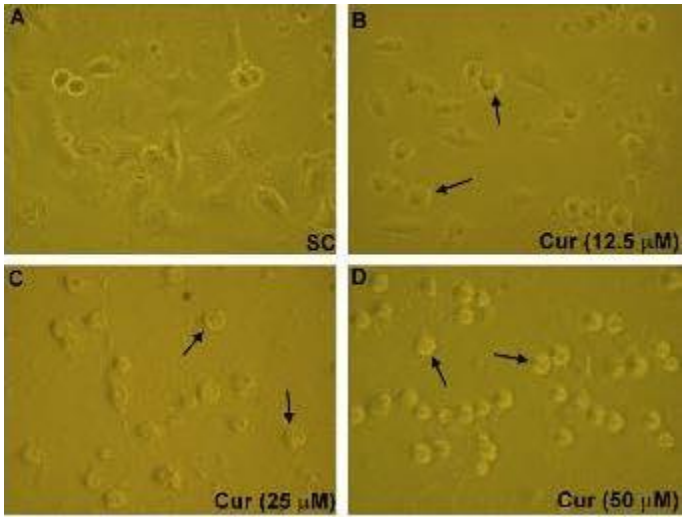


Fig. 4. Effects of curcumin on cell spreading.

As integrins are the principle cell surface adhesion receptors and Extracellular Cell Matrix is a complex mixture of matrix molecules including the glycoproteins, fibronectin, collagens, laminins, proteoglycans, and non-matrix proteins including growth factors . curcumin is able to inhibit metastasis via blocking the steps of integrin-mediated cell adhesion. Many researchers also reported curcumin dramatically inhibits cell adhesion ability in a great deal of cancers, such as breast cancer.

Afterwards invasive tumour cells must induce local degradation of ECM via proteolysis and then migrate through the modified matrix.Matrix metalloproteinases (MMPs), the major class of proteinases, play an important role in cell tumour invasion and metastasis.MMPs, a family of zinc dependent endopeptidases, are key enzymes involved in these processes. Two members of the MMP family, the 72-kDa type IV collagenase MMP-2 (gelatinase A) and the 92- kDa type IV collagenase MMP-9 (gelatinase B), have been shown to be highly expressed and to play a significant role in the pathogenesis of thyroid cancers. Moreover, it was reported that they have

been implicated in the invasion, metastasis, and tumour-mediated angiogenesis of many subtypes of thyroid cancers, including PTC, FTC and ATC. Expression of these extracellular matrix-degrading enzymes correlates positively with the presence of metastasis and disease progression in thyroid cancer patients. Thus, both gelatin zymography and Western blot assays were used to verify if curcumin could modulate the activity and expression of MMPs in thyroid cancer cells. the result show in figure 1 revealed that the activity and expression of MMP-9 were visibly downgraded by curcumin in a dose-dependent manner, while the same treatment of curcumin had no apparent effect on the activity of MMP-2 (data not shown). These results suggest that curcumin inhibits thyroid cancer cells metastasis at least in part through down-regulation of the expression and activity of MMP-9. It was reported that curcumin could also inhibit MMP-9 activity in osteosarcoma cells . While Lin et al. reported that curcumin suppressed invasion and metastasis of human lung cancer cells A549 in vitro by inhibition both of MMP-2 and -9 . In all of these cases, there may be a selective inhibitory effect of curcumin on the member of MMPs in different cell types.

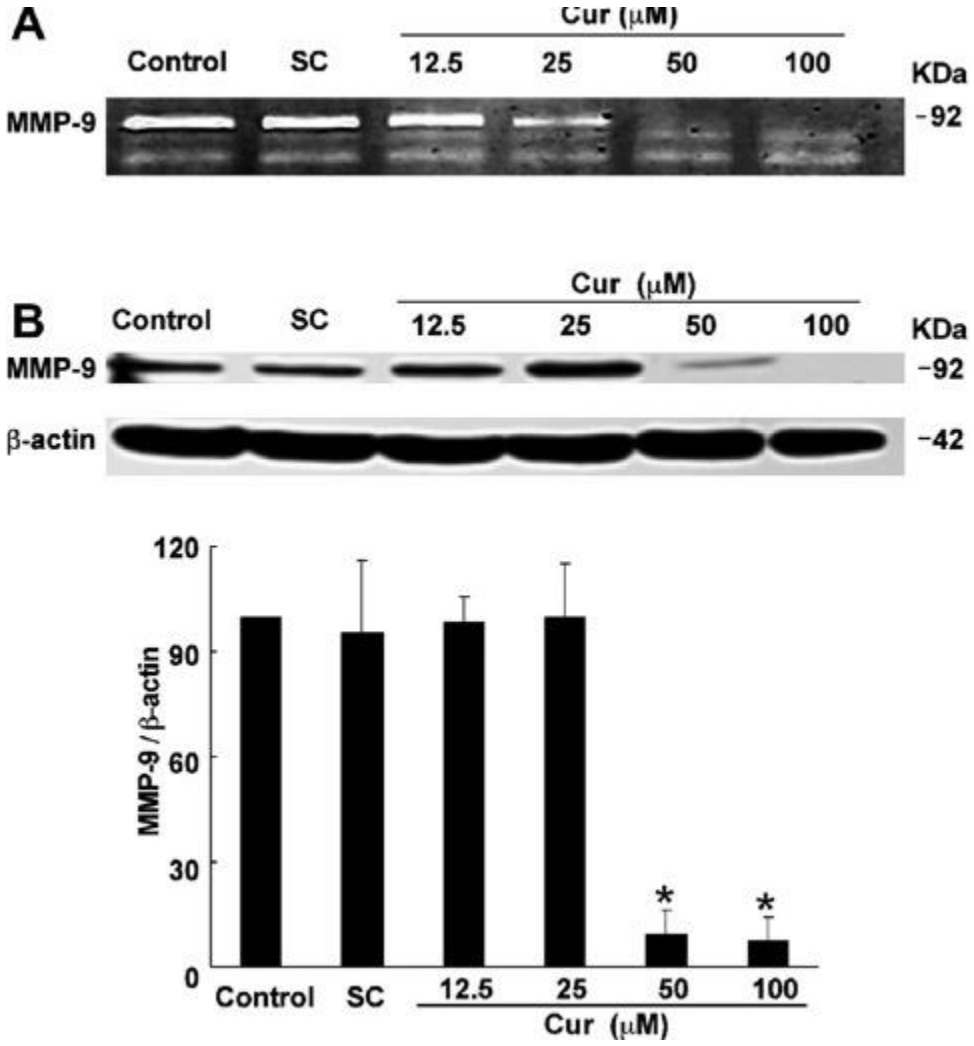


Fig. 5. Effects of curcumin on the activity and expression of MMP-9. (A) Gelatin zymography analysis of the activity of MMP-9 of cells pretreated with or without

curcumin (12.5, 25, 50 and 100 lM) for 24 h. (B) Western blot analysis of the expression level of MMP-9 of cells pretreated with or without curcumin (12.5, 25, 50 and 100 lM) for 24 h. b-actin was monitored as a loading control. All data represent as the means ± SEM of three independent experiments. SC, solvent control. ⁄P < 0.05 vs control, ⁄⁄P < 0.01 vs control (Student's two tailed t-test).

Conclusion

To successfully invade, cells must acquire the ability of migration. Migration of cells is based on cycles of lamellipodial extension, attachment, cell body translocation, and retraction of the cell. **The results showed that curcumin could reduce cell migration rate in a dose-dependent manner, and especially, up to 25 lM of curcumin treatment could completely suppress the migration of K1 cells. In other words, curcumin inhibits the ability**

of cell migration, consequently suppresses metastasis of thyroid cancer partially.

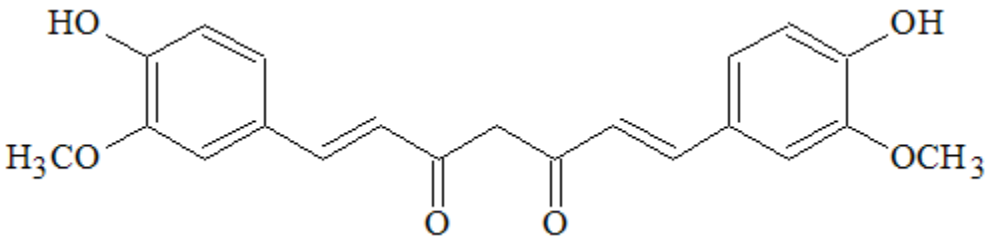
Cell migration is an important event that depends on efficient coordination between cell attachment and detachment on extracellular matrix. In summary, curcumin inhibits thyroid cancer cell attachment, spread, migration, invasion as well as the expression and activity of essential proteolytic enzymes, mainly MMP-9. Overall, these findings open a new avenue and clinical utility for curcumin that can be used for therapeutic and preventive purposes of thyroid proliferative diseases by not only suppressing the proliferation of thyroid cancer cells but also inhibiting metastasis-associated events.

17. Curcumin against Cancer [stefano sapelli]

01/03/2013

Introduction: Curcumin, commonly called diferuloyl methane, is a hydrophobic polyphenol derived from rhizome (Turmeric) of the herb Curcuma longa.

Turmeric is a member of the Curcuma botanical group, which is part of the ginger family of herbs, the Zingiberaceae. The root and rhizome stem of the Curcuma longa plant is crushed and powdered into ground Tumeric. Ground Tumeric is used worldwide as principal **curry powder's ingredient** and contains approximately **2%** of **Curcumin**, that is one of the most powerful phytochemicals whit biological effects including antioxidant, anti-inflammatory, inhibition of angiogenesis and anti-tumor activity.
Curcumin molecular formula is **$C_{21}H_{20}O_6$**



Plants provide health benefits

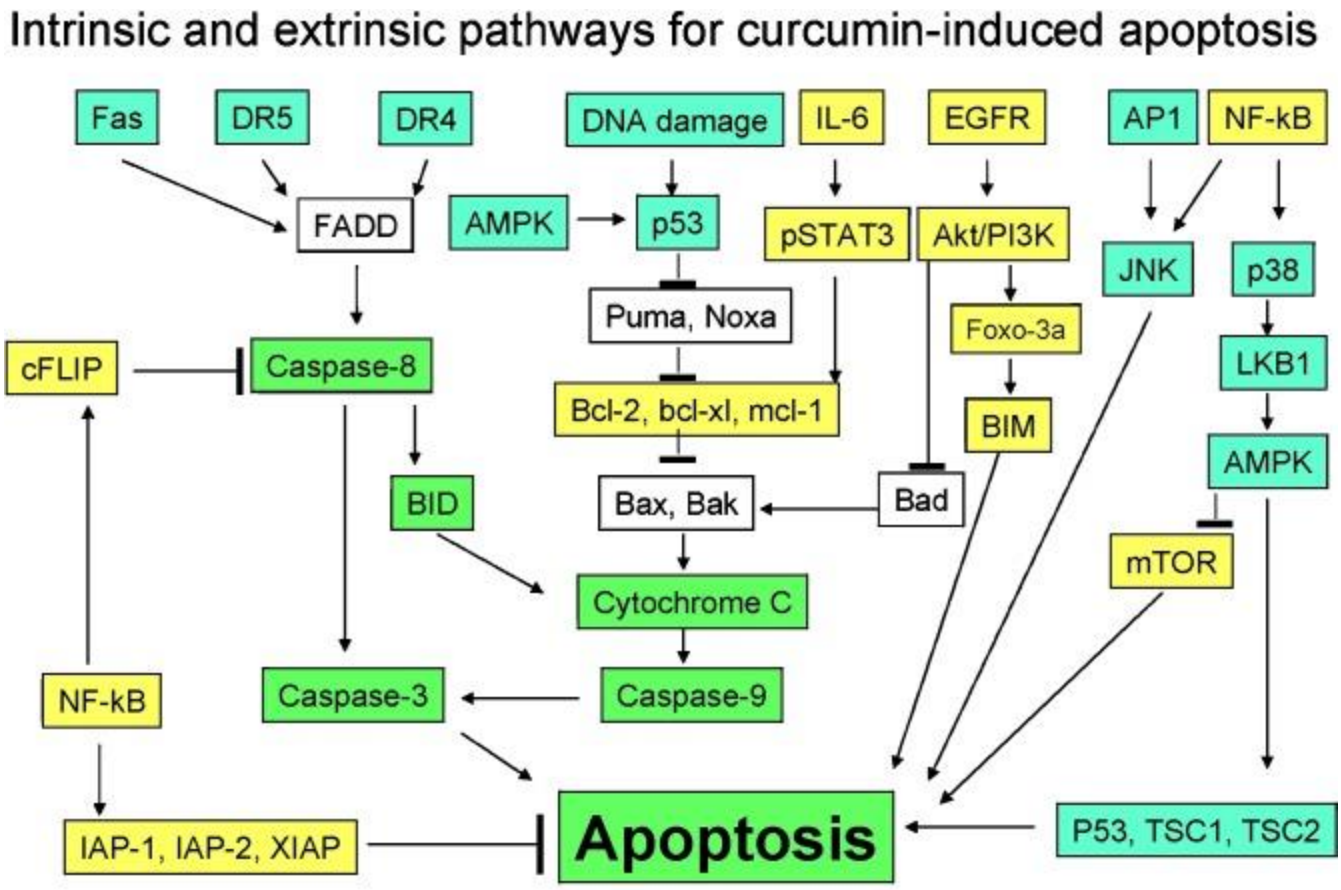
Certain plants manufacture chemicals that repel predators, parasites and diseases. Like most of these pharmacologically-active metabolites, Curcumin is involved in self-defense. Plants with higher levels of organic compounds that deter attackers become more successful, because of their advanced protection. In nature's never-ending interaction between predator and prey, insects evolve the ability to digest plant toxins, while plants evolve stronger chemicals to deter their enemies. This is a very important field of ecological research, scientists have discovered that

these phytochemicals are useful not only for plant's protection against insects, but also provide human health benefits. Curcumin's structure is very similar to other natural polyphenolics produced by plants in response to infectious attack.

Effects against cancer

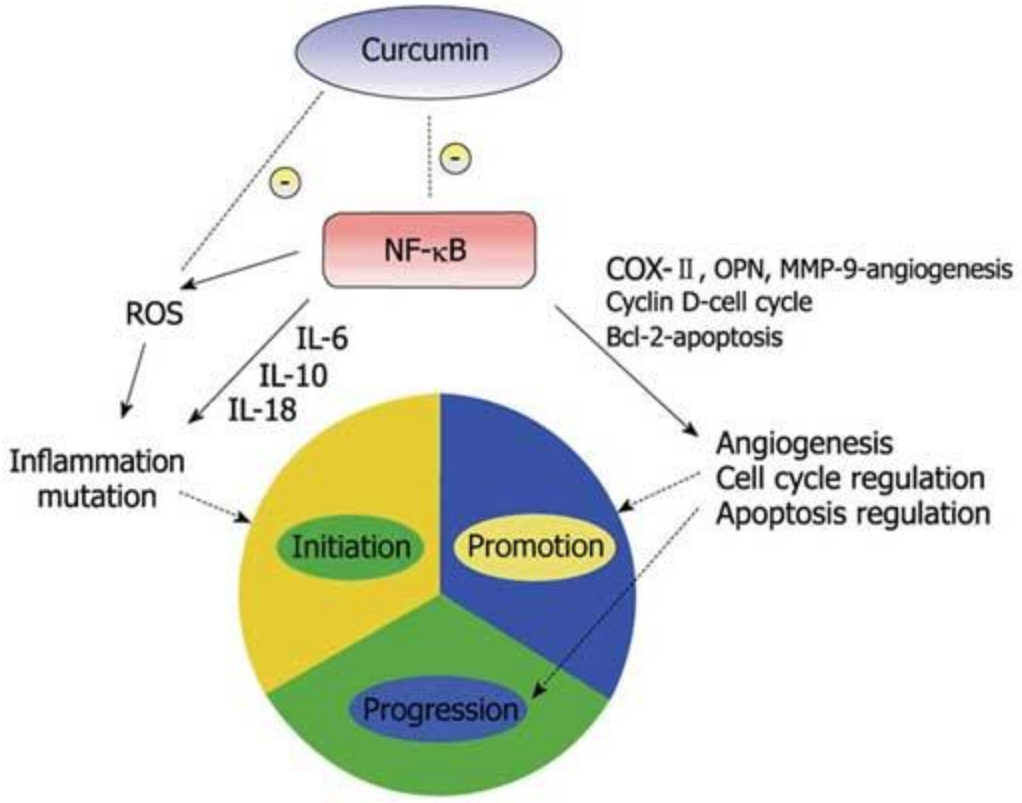
Extensive research over the last half century has revealed important functions of **Curcumin**. In vitro and in vivo research has shown various activities, such as anti-inflammatory, cytokines release, antioxidant, immunomodulatory, enhancing of the apoptotic process, and anti-angiogenic properties. Curcumin has also been shown to be a mediator of chemo-resistance and radio-resistance. The **anti-cancer effect** has been seen in a few clinical trials, mainly as a native chemoprevention agent in **colon** and **pancreatic cancer**, **cervical neoplasia** and **Barrets metaplasia**. Curcumin's potent anti-proliferative activity interacting with several intracellular signal transduction pathways may potentiate the anti-tumor effect of gemcitabine, a nucleoside analog used as chemotherapy. The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of tumor cells.
Curcumin down-regulates transcription factors **NF-kappa B** and **AP-1** and regulates trascription of **Egr-1**; down-regulate the expression of **COX2**, **Lox** (lipooxygenase), NOS, **MMP-9**, **TNF**, **chemokines**, **cell surface adhesion molecules** and **cyclin D1**.
It also down-regulate **growth factor receptors** such as EGF, HER2, FGF, VEGF, PDGF and insulin growth factor (IGF)-1, it results in suppression of tumor growth.
Curcumin also inhibit the activity of **c-Jun N-terminal kinase**, **protein tyrosine kinases** and **protein serine/threonine kinases**, signal transducers for cellular proliferation.
Curcumin also causes DNA damage and endoplasmic reticulum (ER) stress and mitochondrial-dependent-induced apoptosis through the activation of **caspase-3**, a cysteine protease that plays essential roles in apoptosis, necrosis, and inflammation.
Studies showed that curcumin works on **cell surface receptors**, in particular it inhibites the expression of **Bcl-2**, **Bcl-xL**, survivin, and **XIAP**, and induces the expressions **Bax, Bak, PUMA, Bim**, and **Noxa** and **death receptors** with induction of apoptosis.
It has been also reported that curcumin-induced apoptosis is mediated through the impairment of **ubiquitin proteasome system** (UPS), the direct inhibition of proteasome activity also causes an increase in half-life of IκBα that ultimately leads to the down-regulation of NF-κB activation, thus activating the apoptotic pathway.
Finally it induces tumor suppressors, such as **p53/p21 pathway**. The transcription factor p53 has been reported to play a very important role in apoptosis. As a tumor suppressor, p53 is responsible for protecting cells from tumorigenic alterations. The studies showed that curcumin selectively increases p53 expression at G2 phase of carcinoma cells and releases cytochrome c from mitochondria, which is an essential requirement for apoptosis.

Scientists have identified more than 40 biomolecules that are involved in cell death induced by curcumin. Curcumin and Cancer Cells: How Many Ways Can Curry Kill Tumor Cells Selectively? 2010



In this picture targets up-regulated by curcumin are in a **blue box**, those down-regulated are in a **yellow box**, and those unaffected are in a **white box**.

NF-kappaB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. It plays a key role in regulating the immune response to infection. Incorrect regulation of NF-kappaB has been linked to cancer, in fact active NF-kappaB turns on the expression of genes that keep the cell proliferating and protect the cell from conditions that would otherwise cause it to die via apoptosis.



In this picture you can see how Curcumin works aganist NF-kappa B with consequent inhibition of inflammation and proliferation.

AP-1 (the activator protein 1) is a transcription factor, that regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, and bacterial and viral infections. AP-1 in turn controls a number of cellular processes including differentiation, proliferation, and apoptosis. An excess of AP-1 activity is typical in cell's proliferation.

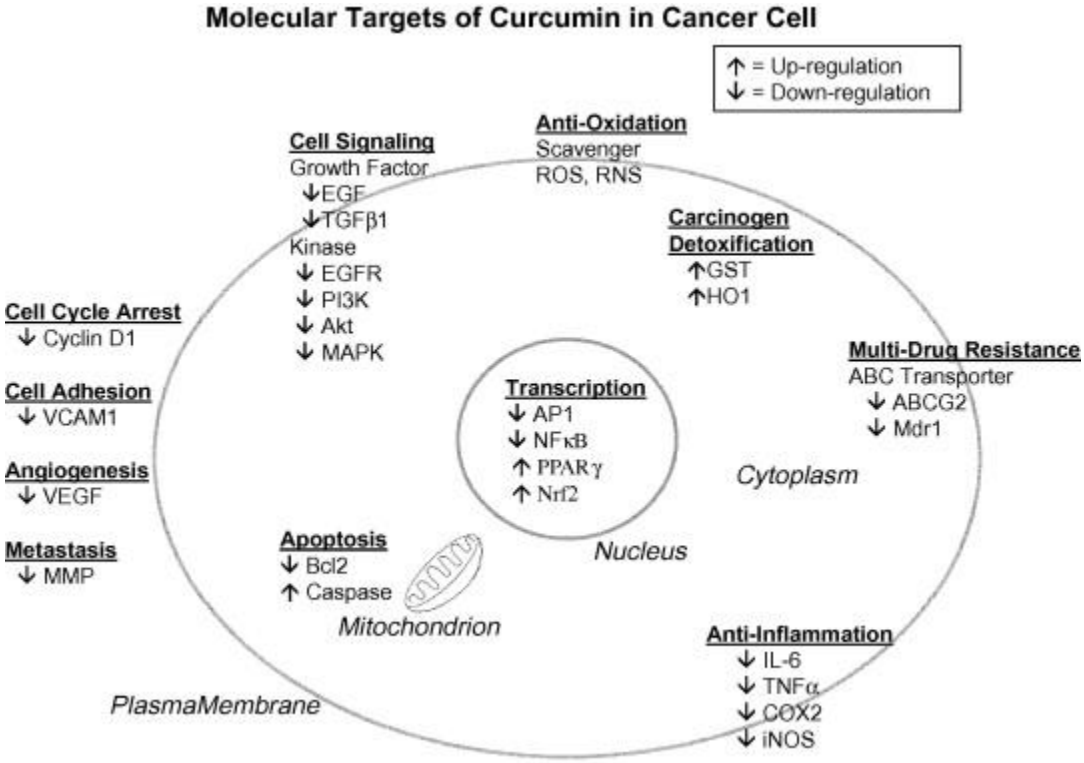
Egr-1 is a protein that in humans is encoded by the EGR1 gene. It is a nuclear protein and functions as a transcriptional regulator. The products of target genes it activates are required for differentiation and mitogenesis. Studies suggest this is a tumor suppressor gene that protects a cell from one step on the path to cancer. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes.

COX2 is an enzyme that is responsible for formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane, important during inflammation. It is demonstrated the expression of COX2 is higher in many neoplastic diseases. In fact one of its products, prostaglandin H2, is converted by enzyme PTGES2 into prostaglandin E2, that may trigger progression of cancer.

MMP-9 (matrix metallopeptidase 9) is an enzyme involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and

tissue remodeling. In disease processes it can be very dangerous because allows migration of neoplastic cells.

TNF (tumor necrosis factor) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages , although it can be produced by many other cell types as CD4+ lymphocytes, NK cells and neurons. The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, sepsis, cachexia, inflammation and to inhibit tumorigenesis and viral replication. Dysregulation of TNF production has been implicated in a variety of human diseases, in particular excessive production of TNF is associated with cancer.



A simple immage that summarizes Curcumin effects.

Bioavailability of Curcumin

Curcumin can be taken at high doses, but unfortunately exhibits poor bioavailability, with difficults caused by poor absorption, rapid metabolism, and rapid systemic elimination. To improve the bioavailability of curcumin, numerous approaches have been undertaken:
- use of adjuvant like **piperine** that interferes with hepatic glucuronidation, a way to eliminate drugs;
- use of **liposomal curcumin**;
- use of **curcumin nanoparticles**;
- use of **curcumin phospholipid complex**;

- use of **structural analogues of curcumin**, that has been reported to have a rapid absorption with a peak plasma half-life.

Bioavailability of Curcumin: Problems and Promises. 2007

Some clinical trials

Human studies of curcumin in cancer prevention and treatment are in the very early stages.
In scientific studies, curcumin does not absorb well from the intestine, so that big doses must be taken for even small amounts to get into the blood circulation. Large doses of curcumin would need to be taken in order to study any effects it might have in the body.

One study of 15 patients with colorectal cancer was done to find out how much curcumin they could safely take, and whether they could take a dose large enough to even be detected in the blood. The patients were able to take 3.6 grams of curcumin without noting ill effects. At this high dose, some curcumin and its products were found in the blood. Lower doses may be enough to directly affect the stomach and intestine. Even though it does not absorb well into the bloodstream, curcumin absorbs into the colon lining and into cancerous tissues in the colon. Small studies have found most people in study groups were able to take up to 10 grams of curcumin per day for a period of a few weeks without noticing problems other than the large volume of pills. There are also studies going on now that try different ways to formulate curcumin so that it absorbs well enough to be tested in humans.

A **2008 study** showed effects of Curcumin in Patients with **Advanced Pancreatic Cancer**. These patients received 8 g curcumin by mouth daily until disease progression, with restaging every 2 months. Twenty-five patients were enrolled, with 21 evaluable for response. Circulating curcumin was detectable as drug in glucuronide and sulfate conjugate forms, albeit at low steady-state levels, suggesting poor oral bioavailability. Two patients showed clinical biological activity. One had ongoing stable disease for 18 months; interestingly, one additional patient had a brief, but marked, tumor regression accompanied by significant increases in serum cytokine levels. Scientists found that Curcumin down-regulates expression of trascriptional factors that increase cellular proliferation. This brought to conclusions that oral curcumin is well tolerated and has biological activity in some patients with pancreatic cancer. Phase II Trial of Curcumin in Patients with Advanced Pancreatic Cancer. 2008

On **Wednesday 28 October 2009** was published an article in the British Journal of Cancer in which is said that molecules found in curry ingredients have been shown to kill **oesophageal cancer** cells in the laboratory. Scientists based at the Cork Cancer Research Centre treated oesophageal cancer cells with curcumin. They found that curcumin started to kill cancer cells within 24 hours. The cells also began to digest themselves.
The results additionally showed that curcumin kills cells by triggering lethal cell death signals. Dr Sharon McKenna, lead study author, based at the Cork Cancer Research Centre, University College Cork, said:
“These exciting results suggest scientists could develop curcumin as a potential anti-cancer drug to treat oesophageal cancer. Scientists have known for a long time that natural compounds have the potential to treat faulty cells that have become cancerous and we suspected that curcumin

might have therapeutic value. Dr Geraldine O'Sullivan-Coyne, a medical researcher in our lab had been looking for new ways of killing resistant oesophageal cancer cells. She tested curcumin on resistant cells and found that they started to die using an unexpected system of cell messages."
Curcumin induces apoptosis-independent death in oesophageal cancer cells

A **2011 study** took advantage of the fact that curcumin stays in the intestine rather than absorbing into the blood. Researchers tested it to find out if it could reduce the number of **cancer precursors in the colon and rectum**. They measured compounds that help promote cancer in rats, did colonoscopies to count abnormal crypt foci (a very early sign that colon cancer may be developing) in biopsy samples, then gave 2 to 4 grams of curcumin a day to 44 smokers. After a month on the curcumin, the researchers did second colonoscopies and biopsies to see if there was a lower concentration of pro-carcinogenic substances in the colon and rectum. The compounds were at the same level as they were before the study. But the smokers who took 4 grams of curcumin a day had fewer abnormal crypt foci after the study, while the smokers who took 2 grams a day had the same number as before. Researchers are still looking at whether curcumin might actually reduce the number of colon and rectum cancers.
Tumeric

On **September 2012** a clinica trial showed that Curcumin-cyclodextrin complexes potentiate gemcitabine effects in an orthotopic mouse model of **lung cancer**. Curcumin has low solubility, so scientists have used cyclodextrins (CD) as an excipient allowing a considerable increase of aqueous solubility and bioavailability of curcumin. The effects of solubilised curcumin have been evaluated in cell cultures as well as in an in vivo orthotopic lung tumour mouse model. They used in particular a combination of Curcumin and **Gemcitabine**, a nucleoside analog used as chemotherapy. They demonstrated that Curcumin, when given orally in a CD-solubilised form, reduces lung tumour size in vivo. In vitro experiments show impaired tumour cell proliferation and increased cell apoptosis. Moreover, their data underlined a potential additive effect of curcumin with gemcitabine thus providing an efficient therapeutic option for antilung cancer therapy. Curcumin-cyclodextrin complexes potentiate gemcitabine effects in an orthotopic mouse model of lung cancer. 2012

Further clinical trials are going on to find out what role, if any, turmeric and curcumin may play in the prevention or treatment of cancer.

Nutrition and Curiosities

Curcuma longa is one of the key ingredients in many **Asian dishes**. Indian traditional medicine, called Ayurveda, has recommended turmeric in food for its potential medicinal value, an active research topic. Its use as a coloring agent is not of primary value in South Asian cuisine. Curcuma longa is typically used in its dried, powdered form, that is one of ingredients for **Curry powder**, a mixture of spices of widely varying composition based on South Asian cuisine, that in north India is called garam masala. It is used in particular with meat and rice, but sometimes is also used as ingredient for drinks, yogurt and bisquits. Its health benefits have been described for thousands of years in traditional Indian and Chinese medicine largely because of its proven efficacy in treating conditions with inflammation. Marco Polo, writing of his travels in China,

described Tumeric in the 13th century, explaining its affinity with saffron and its large use in that land. In fact Curcuma is also called “indian saffron”.

Symbolic is Fauja Singh’s case. At the grand old age of 102, Fauja Singh is the world's oldest marathon runner. He is a world record holder in his age bracket. His current personal best time for the London Marathon (2003) is 6 hours 2 minutes, and his marathon record, for age 90-plus, is 5 hours 40 minutes, at the age of 92, at the 2003 Toronto Waterfront Marathon. The key, according to Singh, to conquering his daily 10-mile training regimen is drinking copious amounts of tea and in particular eating plenty of **ginger curry**, a simple sour, spicy and sweet curry.

[Here is a link to a copyrighted photo of Fauja Singh - inquire of the photofrappher Tom Maddick for its use. HK] - https://www.flickr.com/photos/tamphotographyuk/7250848038/

Here is the 102 years old Fauja Singh. May curry be the cause of his excellent health?

Conclusion

Evidence has also been presented to suggest that **Curcumin** can suppress tumor initiation, promotion and metastasis. Pharmacologically, Curcumin has been found to be safe, is considered GRAS (General Recognition And Safety) by the american FDA (Food and Drug Administration). All of these studies suggest that Curcumin has enormous potential in the **prevention** and **therapy** of cancer, it's important a continue science research about Curcumin's benefits, that may become very helpful in the future medicine. First of all we can improve the use of a spice such as **curry powder** for our meals.

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Curcumin induces apoptosis-independent death in oesophageal cancer cells

Web links for More Articles on Curcumin vs. Cancer

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