Phytopharmaceuticals in Cancer Chemoprevention

Debasis Bagchi, Ph.D., MACN, CNS, MAIChE
University of Houston College of Pharmacy, Houston, TX, USA
Iovate Health Sciences Research Center, Oakville, ON, Canada
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Phytopharmaceuticals

- In Western medicine, professionals dealing with cancer rely heavily on aggressive treatment through the use of pharmaceutical intervention.
- The use of phytopharmaceuticals for prevention, or as adjuncts to conventional therapy, is largely ignored.
- This presentation will cover the potential mechanistic roles of natural products in cancer prevention and therapy.
Cancer: Basic Facts

- Cancer is derived from the latin word for Crab.
- Like a crab, cancer cells attach themselves to tissues and organs and hang on.
- The study of cancer is called "oncology" named after oncos, which means tumor in Greek.
- Although cancer is often referred to as a single condition, it actually consists of more than 100 different diseases, all characterized by the uncontrolled growth and spread of abnormal cells causing masses of tissues called tumors.
Cancer cells are developed in three phases:

- **Phase I (Initiation):** When something (such as free radical, carcinogen or radiation) irreversibly alters a cell's genetic makeup, it causes the cell to divide more frequently than it should.

- **Phase II (Promotion):** When the damaged or genetically altered cell fails to fix its own damage, it goes into uncontrolled growth.

- **Phase III (Progression):** The growing tumor itself builds a blood supply network through angiogenesis and invades the surrounding tissue.
Chemoprevention

- Literally means a process of repeated exposure to an agent that completely prevents a lesion from occurring or overturns morphogenetically altered tissues and restores them to a normal state. This definition can have several interpretations.
  - Selected removal of altered or mutated cells or tissues
  - Selected blockage of further propagation of morphogenetically altered cells without harming pre-existing cells.
  - Specialized interference with intracellular metabolic events or pathways that temporarily or permanently alter cellular functions in abnormal cells.
  - Specific influence on the genomic machinery in favor of the host
  - Selective enhancement of carcinogen clearance by altering absorption and metabolism
  - Preparation of protection of normal cells from future episodes of such noxious attacks

These propositions are based on the concepts that cell proliferation in premalignant conditions can be slowed down or arrested and that new tissue can grow beneath lesions and displace initiated cells or tissues.
Curcuma longa or Turmeric

Â Curcumin is a diferuloylmethane present in extracts of the plant
Â Curcuminoids are responsible for the yellow color of turmeric
Â Recent phase 1 clinical trials indicate that people can tolerate a dose as high as 8 gms/day
Â In the US, curcumin is used as a coloring agent in cheese, spices, mustard, cereals, pickles, potato flakes, soups, ice-creams and yogurts (www.kalsec.com)
Â Numerous studies have demonstrated that curcumin has antioxidant, anti-angiogenic, chemopreventive, and anti-inflammatory properties. Furthermore, it downregulates cyclin D1 expression and this down regulation occurred at the transcriptional and posttranscriptional level. Curcumin suppresses the expression of MMP9 and iNOS, the key biomarkers of tumor metastasis.
The plant *Curcuma longa* (panel A) from which curcumin is derived, and its structure (panel B):
molecular targets shown to be regulated by curcumin.

Gene expression

Protein kinases

Curcumin

Transcription factors

Others

Enzymes

IKK ↓
EGFR ↓
HER2 ↓
AKT ↓
Src ↓
JAK2 ↓
TYK2 ↓
JNK ↓
PKA ↓
PKC ↓
NF-κB ↓
AP-1 ↓
Egr-1 ↓
STAT1 ↓
STAT3 ↓
STAT5 ↓
PPARγ ↑
EpRE ↓
CBP ↓
β-catenin ↑
Nrf2 ↑
VCAM-1 ↓
Bcl-2 ↓
Bcl-xL ↓
ICAM-1 ↓
FTPase ↓
GST ↑
GSH-px
TF ↓
AR/ARP ↓
P53 ↑
MDR ↓
ELAM-1 ↓
Hemeoxygenase ↑
Xanthine oxidase ↓
uPA ↓
Constitutive activation of transcription factors
- STAT3, AP-1 & NF-κB

Overexpression:
- Oncogenes
- HER2
- Growth factors (e.g., EGF, PDGF, FGF)
- Growth factor receptors
- Survival factors (e.g., Survivin, Bcl-2 and Bcl-xL)
- Cyclin D1
- Decoy receptor

Overexpression:
- Matrix metalloproteinases
- Cyclooxygenase-2
- Adhesion molecules
- Chemokines
- TNF

Transformation
Normal cells → Tumor cells
Proliferation
Tumor cells → Tumor growth
Invasion
Tumor growth → Tumor Metastasis
Curcumin
Berry Anthocyanins

- A broad spectrum of data demonstrate the novel antioxidant and antiangiogenic benefits of structurally diverse berry anthocyanins

- Our recent study demonstrated the potential for berry anthocyanins to eradicate the deadly bacteria *Helicobacter pylori*
Berries are well known to demonstrate a diverse, broad-spectrum of Health Benefits:

- **Antioxidant Properties**
- **Cardiovascular protection**
- **Blood sugar control**
- **Healthy brain function and mental clarity**
- **Anti-aging properties**
- **Urinary health**
- **Healthy vision**
- **Skin health**
In Vitro ANGIOGENESIS
ASSAY: MATRIGEL ASSAY

- **Matrigel is a matrix of a mouse basement membrane neoplasm**
- **Differentiation of endothelial cells to form capillary like structures on a basement membrane matrix, Matrigel, derived from the EHS tumor**
- **Matrigel represents a complex mixture of basement membrane proteins including type IV collagen, entactin/nitrogen, proteoheparan sulfate and other growth factors**
Matrigel induces endothelial cells to differentiate as evidenced by both the morphological changes and by the reduction in proliferation, and therefore offers a convenient model to study biochemical endothelial cells in vitro angiogenesis.

Human dermal microvascular endothelial cells were used for this assay that was performed using a kit, provided by CHEMICON International, Inc., where the conditions are optimized for maximal capillary-like structure formation. Under basal conditions as specified by the kit, we were able to obtain numerous long capillary-like structures.
Human dermal microvascular endothelial cells (0.5 x 10^5 cells/per well) were seeded onto 4-well plates precoated with Matrigel. After 48 hr of seeding, Novel Berry Formulation was added (50 μg/ml). Endothelial tube formation is observed and digitally photographed under an inverted light microscope at 20-100 x (20 x shown) magnification.
HEMANGIOMA

- Hemangiomas are the most common infancy tumors, which occur in approximately 1:100 normal newborns, however, in premature infants weighing less than 1000 grams the incidence rises to 1:5 live births. The hemangioma is characterized by rapid growth during the first year of life (proliferative phase).

- Proliferating hemangiomas are highly angiogenic and, thus, experimentally, hemangiomas represent a powerful model to study in vivo angiogenesis.

- The presence of macrophages is associated with proliferating hemangiomas. The CC chemokine, MCP-1 (monocyte chemotactic protein-1) has been shown to be responsible for recruiting macrophages to infection or inflammation sites. MCP-1 is acknowledged as a major accessory facilitating angiogenesis and a direct role of MCP-1 on angiogenesis has been recently demonstrated. Thus, antagonists to MCP-1 are considered to be anti-angiogenic.
EOMA Survival Curve

Days

# Mice
HEMANGIOMA IN CHILDREN
HEMANGIOMA (cont’d)

- Endothelioma (EOMA) cell line derived from the spontaneously arising hemangioma was used.
- The EOMA cell line has also been well characterized in the literature with regard to its derivation, expression of endothelial cell markers, production of endostatin and response to angiostatin.
- Following 24 hr of seeding of EOMA cells, the culture medium was replaced with fresh medium and the cells were treated with Berry Formulation for 12 or 24 hr. High baseline luciferase activity suggests elevated levels of basal MCP-1 transcription in these EOMA cells. Cells were activated with TNFα (400 IU/ml) for 12 hr.
- Pretreatment of the EOMA cells with Berry Formulation significantly inhibited basal MCP-1 transcription in EOMA cells. TNFα potently induced NF-κβ transcription. Inducible NF-κβ activity was also significantly lower in EOMA cells pretreated with this Berry Formulation.
MCP-1 Protein Expression

MCP-1 Reporter Assay
Cell Viability Assay. Flow cytometric propidium exclusion assay for viability

NF-κβ reporter assay. NF-κβ-Luc reporter construct was transiently transfected in EOMA cells.
HEMANGIOMA (cont’d)

- Eight week old 129P3/J mice were injected subcutaneously with 100 µl of EOMA cell suspension in PBS for a total dose of $5 \times 10^6$ cells.

- Injection of the EOMA cells pretreated with Berry Formulation did not result in hemangioma formation in all mice. Approximately, 100% of the control and 92% of the Berry-treated group tested positive for the presence of hemangioma. Although the Berry treated group did test positive for the presence of hemangioma, the average mass of such tumor growth was below 50% as compared to the untreated control group. Histological analysis demonstrated markedly decreased infiltration of macrophages in hemangioma of treated mice compared to placebo-treated controls.
Immunohistochemical localization of macrophages in hemangioma
Hemangioma mass, incidence and appearance. Data collected one week after subcutaneous injection of Berry Formulation treated or untreated (control) EOMA cells to 129P3/J mice. Mean ± SD. **Lower mass compared to control group. P<0.01. Photo: Top, Control; Bottom, Berry-treated. Bar graph represents the average mass of hemangioma. Scale = 1 inch.
Helicobacter pylori in vitro by various berry extracts, with enhanced susceptibility to clarithromycin

- Approx. 50% of World’s population is infected with H. pylori, a noxious bacteria responsible for chronic gastritis, peptic ulcer and gastric cancer

- Clarithromycin is a key component of many therapeutic regimens recommended for eradication of H. pylori. Antibiotic treatment for H. pylori infection is often accompanied by side effects including development of resistance to antimicrobial agents, including clarithromycin

- Evaluated the inhibitory effects of various berry extracts with and without clarithromycin, against a pathogenic strain of H. pylori (ATCC strain 49503)
trans-Resveratrol

- A novel antioxidant and cardioprotectant
- Diverse biochemical and physiological actions, including anti-inflammatory, anti-proliferation and promotion of differentiation, and chemopreventive effects.
- Recently, it is attracting increased attention due to its health benefits, especially in common age-related diseases such as cardiovascular disease, cancer, type 2 diabetes, and neurological conditions.
Role of Grape Seed Proanthocyanidins in Human Health and Cancer Chemoprevention
Grape Seed Proanthocyanidin Extract

- Oligomeric proanthocyanidins and catechins accumulate principally in the lignified portion of grapes, especially in the seeds.
- HPLC analysis in conjunction with GC-MS of GSPE demonstrated 54% dimeric, 18% trimeric, 7% tetrameric, and a small amount of monomeric and high molecular weight proanthocyanidins.
Structure of Procyanidin Monomers, Dimers, Trimers and Tetramers
GSPE & Free Radicals

- Exhibited concentration-dependent dramatic scavenging ability towards biochemically generated superoxide anion, hydroxyl and peroxyl radicals, and provided significantly better scavenging as compared to vitamins C and E.

- Provided dose-dependent protective ability against TPA-induced hepatic and brain lipid peroxidation, DNA fragmentation, peritoneal macrophage activation in mice, and provided better protection as compared to vitamin C, E and β-carotene.

- Demonstrated protection against smokeless tobacco-induced oxidative stress, DNA damage and apoptotic cell death in human oral keratinocytes, and provided better protection as compared to vitamin C and E, singly and in combination.
Flow Cytometric Analysis
Laser Scanning Confocal Microscopy
Chemopreventive Properties of GSPE

- Exhibited significant cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal cells.

- Protected against chemotherapeutic drug-induced cytotoxicity towards normal human liver cells by modulating apoptotic regulatory genes \( bcl-2, c-myc \) and \( p53 \).

- Provided excellent \textit{in vivo} protection against structurally diverse drug- and chemical-induced multiorgan toxicity.

- Provided protection against acetaminophen-induced hepatotoxicity by dramatically enhancing the expression of \( bcl-X_L \) gene in liver tissue.
DOX-induced Apoptotic Cell Death in Cardiac Tissue

Owl's Eye-Shaped Configuration - Condensed Nuclei - Condensed and Fragmented Apoptotic Nuclei
Inhibitory effect of GSPE on Cardioregulatory CD36 gene in cDNA Microarray Analysis

- 2,400 Genes were screened using the MICROMAX Human Full-Length cDNA Microarray (NEN Life Sciences, Boston, MA) to determine the regulatory effect of GSPE on cardioregulatory genes.

- GSPE exhibited potent inhibitory effect on CD36 gene, which has been identified as a potential Ox-LDL receptor and directly linked to foam cells and atherosclerosis.

- This inhibitory effect on CD36 correlates very well with the results of foam cell inhibition data in a hamster atherosclerosis model and clinical study conducted in hypercholesterolemic human subjects.
Novel Mechanistic Pathways of Cardioprotection by GSPE Includes:

- Free Radical Scavenging Ability
- Anti-Apoptotic, Anti-Necrotic and Anti-Endonucleolytic Potentials
- Modulatory Effects on Apoptotic Regulatory bcl-XL, p53 and c-myc Genes
- Cytochrome P450 2E1 Inhibitory Activity
- Inhibitory Effects on Adhesion Molecules
- Modulatory Effects on Proapoptotic and Cardioregulatory c-JUN, JNK-1 and CD36 Genes
Summary & Conclusions

Overall, a significant number of natural products demonstrated potential safety and their role in cancer chemoprevention.