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Current paradigms of cancer chemoprevention

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Abstract: Cancer chemoprevention has been continually evolving ever since the term was coined in the 70s. From the original approaches of identifying chemopreventive agents from the dietary constituents based on the epidemiological data to the current status of adapting them from the molecular targets, significant understanding of cancer as a disease and its possible prevention has been achieved. The identification of the chemopreventive agent from a complex mixture of natural product extracts involves numerous steps including bioassay guided fractionation; evaluation of the extracts, fractions and isolated new chemicals selectively using in vitro and in vivo experimental models; and understanding the mechanism of their action. This in turn provides identification of the molecular target for the parent drug and a basis for the synthesis of more effective, nontoxic analogs. A chemopreventive agent by definition needs to be nontoxic, since it is expected to be consumed by healthy people that may be at a higher risk of developing cancer. This makes a case for these agents to be evaluated as possible adjuvant chemotherapeutic agents. It is expected that more selective delivery methods to the target organs to eliminate toxicity and use of chemopreventive agents as adjuvant chemotherapeutic agent using personalized approaches will be the next steps for chemoprevention.

Key words: Chemoprevention, biomarkers, prevention trials, discovery of chemopreventive agents

1. Introduction
It is true that the enormous effort diverted to basic and clinical cancer research has resulted in identifying new approaches to cancer treatment. However, the interest in prevention of cancer has been evolving. As Benjamin Franklin said “an ounce of prevention is worth a pound of cure”. Over the past 30 years considerable progress has been made in the field of cancer prevention (Bilecová-Rabajdová et al., 2013). The word chemoprevention was coined by Michael Sporn and was defined as “the use of pharmacologic or natural agents that inhibit the development of invasive breast cancer either by blocking the DNA damage that initiates carcinogenesis, or by arresting or reversing the progression of premalignant cells in which such damage has already occurred.” (Sporn et al., 1976). Since then the progress in the field of chemoprevention has resulted in the development of the concept of molecular chemoprevention, which includes altered gene expression and signal transduction that in turn can prevent cell transformation or progression of the disease (Mehta et al., 2010). In order to understand chemoprevention, it is essential to differentiate between chemoprevention and chemotherapy. As shown in Figure 1, the major difference between these 2 groups is the fact that absolutely no toxicity is acceptable for chemopreventive agents, whereas most chemotherapeutic agents are more or less toxic (Mehta et al., 2012). This is based on the concept of risk versus benefit. As chemotherapeutic agents are employed for cancer patients some risk of toxicity is acceptable as compared to chemopreventive agents, which are expected to be consumed by a high risk group of people who are otherwise healthy. However, the demarcation between chemoprevention and chemotherapy is very cloudy. It is not clear where chemoprevention ends and chemotherapy begins. Since chemopreventive agents have been evaluated during all phases of carcinogenesis including initiation, promotion, and progression, they can be used in combination with chemotherapeutic agents in order to enhance the effectiveness of chemotherapeutic drugs and to reduce toxicity (Cabrespine-Faugeras et al., 2010). To this end it is interesting to note that most of the chemopreventive agents inhibit proliferation of cancer cells in vitro, providing a rationale for their use in combination with other drugs. Yet clinical trials for prevention of cancer have been less successful as they require large enrolment and the studies are cost prohibitive (Steward and Brown, 2010). Despite that, many clinical trials have been completed with some success (Visvanathan et al., 2013).

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In this review, progression of the field of chemoprevention is concisely summarized and some possible avenues of future research in this area are highlighted.

2. Discovery of new chemopreventive agents

Healthy dietary habits, which include consumption of fruits and vegetables and reduced intake of fatty food and red meat, as well as refraining from smoking, have been equated to reduced cancer incidence. The chemical composition that has been suggested as cancer preventive includes phytoestrogens (resveratrol), flavonoids (such as genistein, quercetin), vitamins (retinoids (A), deltanoloids (D), alpha tocoferol (E)), organosulfurs (brassinin, sulforaphane, isothiocyanates), minerals (such as calcium and selenium) etc. (Khuda-Bukhsh et al., 2014). There is significant epidemiological evidence that supports the fact that various phytochemicals can have a protective role against cancer development and its progression (Kocic et al., 2013). Many of these have been evaluated extensively in chemically induced carcinogenesis models in vivo (Naithani et al., 2008; Smith and Muller, 2013). Earlier the chemopreventive agents were broadly divided into blocking agents and suppressing agents (Wattenberg, 1992). Typically the tumor blocking agents or anti-initiating agents were effective in scavenging free radicals, suppressing inflammatory responses, enhancing phase II metabolizing enzymes, suppressing carcinogen uptake and metabolism, preventing alterations in the methylation to protect the cells from oncogenic expression or from inactivating tumor suppressor genes, and inducing DNA repair (Mehta et al., 2010). The suppressing agents on the other hand can mediate their effects in a variety of different ways including altered gene expression and signaling cascade, induction of cell senescence, inducing cell differentiation or apoptosis, and blocking cell cycle (William et al., 2009). Chemically the phytochemicals are divided into a variety of major chemical structures including flavonoids, chalcones, isoflavonoids, flavonones, anthocyndines, coumarines, carotinoids, organosulfurs, polyunsaturated fatty acids, and certain glycoproteins such as lactoferrin and selenium (Naithani et al., 2008). The biological activities of many of these chemopreventive agents are listed in Table 1.

During the past 20 years a major effort has been directed towards discovering new cancer preventive agents from natural products in systematic bioassay guided fractionation. Several laboratories throughout the world are engaged in such research. One such program was led by Dr John Pezzuto at the University of Illinois, Chicago. Several chemopreventive agents were identified as a part of this National Cancer Institute supported program (Mehta and Pezzuto, 2002; Gullett et al., 2010) and a few of them have been evaluated in clinical trials. The steps to discover a new chemopreventive agent are summarized in Figure 2. Briefly, the plants were rationally selected from various parts of the world based on the scientific literature and community-identified medicinal values (Kinghorn et al., 2010). The plant extracts were evaluated in in
Table 1. Summary of the cancer chemopreventive mechanism of natural chemopreventive agents.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Chemopreventive agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant effects</td>
<td>Ellagic acid, vitamin C, carotenoids, flavonoids, anthocyanins, phenolic compounds</td>
</tr>
<tr>
<td></td>
<td>polyphenols, curcumin, anthocyanin, conjugated fatty acids</td>
</tr>
<tr>
<td>Phase I enzyme induction</td>
<td>Diallyl sulfide, curcumin, indole-3 carbinol, catechins</td>
</tr>
<tr>
<td>Phase II enzyme induction</td>
<td>Carotenoids, flavonoids, anthocyanins, phenolic compounds, polyphenols, curcumin,</td>
</tr>
<tr>
<td></td>
<td>anthocyanin</td>
</tr>
<tr>
<td>Immunomodulatory effects</td>
<td>Carotenoids, flavonoids, lactoferrin</td>
</tr>
<tr>
<td>Modulation of the hormone</td>
<td>Genistein</td>
</tr>
<tr>
<td>Antimicrobial effect</td>
<td>Curcumin, sulfur compounds, sulfuraphane, isothiocyanates</td>
</tr>
<tr>
<td>Apoptosis induction</td>
<td>Limonene, isothiocyanates, diallyl sulfides, flavonoids, organo-selenium compounds</td>
</tr>
<tr>
<td>Anti-angiogenesis</td>
<td>Lactoferrin, flavonoids</td>
</tr>
<tr>
<td>Cell differentiation induction</td>
<td>Lariciresinol (II) and sitoindoside II</td>
</tr>
<tr>
<td>Molecular association with carcinogen</td>
<td>Flavonoids, anthocyanins, polyphenols, curcumin, anthocyanin</td>
</tr>
<tr>
<td>Cell cycle arrest</td>
<td>Genistein, DADS, DATS, and SAMC</td>
</tr>
<tr>
<td>Arachidonic acid cascade modification</td>
<td>PUFA, curcumin, flavonoids, resveratrol</td>
</tr>
<tr>
<td>Inhibition of DNA adduct formation</td>
<td>Organosulfur compounds, DAS and DADS, selenium compounds</td>
</tr>
<tr>
<td>Inhibition of polyamine metabolism</td>
<td>Organosulfur compounds, organoselenium compounds, DFMO</td>
</tr>
<tr>
<td>Inhibition of DNA synthesis</td>
<td>DHEA, fluasterone</td>
</tr>
<tr>
<td>Inhibition of oncogene activity</td>
<td>Perillyl alcohol, limonene, DHEA</td>
</tr>
<tr>
<td>Increase of intercellular communication</td>
<td>Carotenoids, retinoids</td>
</tr>
</tbody>
</table>

Rational selection of plants

Screening of plant extracts

Effective Extract (Primary screen)

Secondary screens (Active extract)

Fractionation: Selective assay(s)

Active Fraction (Confirmation)

Identification of novel CPA

In Vivo Carcinogenesis

Mechanism of action

GMP Synthesis

Preclinical Toxicity (FDA approval)

Clinical Trials

Figure 2. Discovery of a new phytochemical as a chemopreventative agent: from bench to bedside.
vitro bioassays to examine cell proliferation, apoptosis, differentiation, antioxidant property, and anti-estrogenic and anti-aminase activities. Any extract exhibiting positive response is then evaluated in a secondary assay, which includes a mouse mammary gland organ culture assay (MMOC) (Mehta et al., 2008) and aberrant crypt foci (ACF) in a mouse colon assay (Saleiro et al., 2010). In the MMOC assay it is determined if the test agent inhibits development of precancerous lesions induced by a carcinogen in organ culture. In the ACF assay it is investigated whether the test agent inhibits formation of azoxymethane induced ACF in mice. In contrast to cell cultures, here the organs are involved with multiple cell types. The extract is then fractionated into various fractions and the active fraction is identified and characterized by medicinal chemists. This new agent is then either isolated or synthesized and evaluated for its efficacy in chemically induced carcinogenesis model(s). Using this approach we identified brassinin and deguelin for colon and breast carcinogenesis, resveratrol for mammary carcinogenesis, sulforamate for mammary carcinogenesis, zaptatin for lung carcinogenesis, and 4-bromophenol for mammary carcinogenesis (Mehta et al., 2010). The synthetic chemistry group in the program has synthesized several analogs of the parent molecules with the intention of developing more potent and less toxic chemopreventive agents. One such analog of vitamin D, 1α-hydroxyvitamin D5, developed in our laboratory was chemically synthesized and has been approved for clinical trial by the FDA (US Food and Drug Administration).

3. Molecular targets and signaling molecules essential for chemoprevention

The transformation of a normal cell to a cancer cell involves multiple molecular steps (William et al., 2009; Lee et al., 2012). The molecular pathways regulating normal cell functioning apparently get deregulated during the process. Suppressors of these processes broadly, for example synthetic or natural chemicals that will inhibit carcinogen metabolism (Lee et al., 2012), increase DNA repair (Mahmoud et al., 2014), and regulate cell proliferation and programmed cell death (Wang and Zheng, 2013), would classify as chemopreventive agents. Over the years as the molecular mechanisms for each of these processes became clearer, the molecular targets started to emerge (William et al, 2009). Selectively identifying chemopreventive agents that would specifically block or enhance the target genes or proteins can serve as ideal chemopreventive agents. For example, in inflammation mediated carcinogenesis the inflammation stimulus releases TNFa, which can activate the NFκB pathway in epithelial cells (Aggarwal et al, 2013). The signaling cascade then would activate production of BcL-XL and IAP-1 (Szliszka and Krol, 2011) and cell cycle regulatory cyclins, such as cyclin D. During the inflammation induced carcinogenesis, expression of Cox-2 is also upregulated, resulting in increased prostaglandin E2, which is responsible for loss of contact inhibition, increased cell proliferation, and loss of E-cadherin. Therefore, compounds that would block TNFa, NFκB, or Cox-2 may serve as possible chemopreventive agents against inflammation induced cell transformation (Aggarwal et al, 2013; Wang et al, 2013). There are several chemopreventive agents that have targeted Cox-2 expression, which include resveratrol, indol-3-carbinol, epigallocatechin gallate (EGCG), curcumin, sulforaphan etc. (Temraz et al, 2013).

It has become increasingly clear that the stromal microenvironment plays a big role in the process of carcinogenesis (Labarge et al., 2014). Numerous reports have focused on targeting microenvironment regulators for cancer chemotherapy. However, this approach has not been extensively explored for chemoprevention. For example, several inhibitors of VEGF and FGF signaling such as anti-VEGFR or angiogenesis inhibitors such as endostatin or tumstatin have been evaluated experimentally as well as clinically for their efficacy (Funakoshi et al., 2014; Limaverde-Sousa et al., 2014). Moreover, many chemotherapeutic agents are targeted to suppress expression of EGFR, FGFR, PDGFR, and cMet. Inhibitors of extracellular matrix turnover such as suramin and dalteparin and matrix metalloproteinase (MMP) inhibitors have also been under investigation. It certainly is possible that some selective chemopreventive agents may have a similar function. For example, we recently reported the role of deguelin in suppression of cell invasion and metastasis of triple negative breast cancer cells (Mehta et al., 2013). It was also observed that the effect of deguelin was mediated by inhibiting cMet (Mehta et al., 2013). Deguelin was originally identified as a chemopreventive agent from an African plant and it inhibits skin, colon, and mammary carcinogenesis at nontoxic concentrations. An overview of many of these molecular mechanisms for cell transformation, and progression of transformed cells to achieve invasive and metastatic potential as well as the molecular targets that can be identified for cancer preventive and therapeutic agents are summarized in Figure 3 and Table 2.

Another major category where targeting therapy has become extremely successful in chemotherapy and to certain extent in chemoprevention is steroid receptors. Tamoxifen and raloxifene have been clinically used as a treatment for steroid receptor positive breast cancer patients (Jordan, 2014). A tamoxifen clinical trial was carried out for chemoprevention in women at high risk of developing breast cancer with some degree of success. Similarly, aromatase inhibitors such as letrozole are considered a
first line of therapy for ER+ postmenopausal patients (Van Asten et al., 2014). Like tamoxifen, aromatase inhibitors have also been evaluated as possible chemopreventive agents. More recently it was observed that antiprogestins suppress development of carcinogen induced mammary tumors in rats. This class of agents also provides a good lead for clinical intervention. Antiprogestins to target progesterone receptors for breast cancer patients are being developed for chemoprevention trials (Wiehle et al., 2011).

Similarly, considerable attention is also focused on the inducers of ERb in mammary and colon carcinogenesis (Saleiro et al., 2012).

More recently HIF-1 (hypoxia inducible factor 1) has been considered as a target for both cancer therapy and prevention (Wouters et al., 2013). This is based on the fact that tumor cells are less oxygenated as compared to normal cells. New cancer therapeutic agents are being developed targeting hypoxia-induced factor-1 (HIF-1). In addition to
several cancer types including breast and prostate cancers, HIF-1 expression is found to be elevated in premalignant colon ACF, ductal carcinoma in situ (DCIS), and prostate intraepithelial neoplasia (PIN). This would then lead to a possibility that HIF can be targeted for chemoprevention. The drugs that are being considered as HIF-1 inhibitors so far are relatively toxic (Wouters et al., 2013). The challenge would be to develop nontoxic HIF-1 targeting chemopreventive agents. There have been a few reports suggesting HIF-1 inhibitory activities of genistein and apigenin. However, these reports are very preliminary. We observed that deguelin also inhibits HIF-1 activity in breast cancer cells (Mehta et al., 2013). Deguelin has not been evaluated in cancer prevention protocols for HIF-1 activity.

4. MicroRNA as possible targets for chemoprevention
MicroRNAs are small, 19–24 nucleotides in length, noncoding RNAs that control gene expression by triggering translation repression and RNA degradation. There are less than 2000 miRNA regulating translational function of about 35% of the genome (Ye and Cao, 2014). One miRNA can regulate expression of numerous mRNAs. Since miRNAs are differentially expressed in normal and cancer cells they can be identified as molecular targets for cancer chemoprevention. The miRNAs have generally been classified as potential oncogenes or suppressors. For example, miRNA-82 is a potential oncogene, whereas let-7 is a suppressor that suppresses HMGA2 oncogene and regulates RAS oncogene through translation repression (Peng et al., 2008; Ouyang et al., 2014). We expect miRNAs as possible targets for chemoprevention since chemopreventive agents such as curcumin and folates have been reported to modulate miRNA expression (Sarkar et al., 2013). We reported earlier that induction of oxidative and chemical stress in MCF7 cells altered the expression of let-7 miRNA and the effects were reversed by vitamin D (Peng et al., 2010). Moreover, vitamin D induced cell differentiation was regulated by miR181 targeting p21 expression (Giangreco et al., 2013). The literature on MicroRNA regulatory functions is constantly evolving and will provide a logical molecular target for carcinogenesis and chemoprevention.

5. Nanochemoprevention
The fundamental requirement for chemoprevention is that the preventive agents have to be nontoxic. This limits the use of many potent chemopreventive agents. Another major issue has been bioavailability; many of the potential cancer preventive compounds are not readily bioavailable. To this end, recent advances in nanotechnology provide effective ways to deliver test agents to the target sites. In one of the reports it was observed that delivery of EGCG using nanoparticles retained its anticarcinogenic properties (Khan et al., 2014). Packaging and delivery of other chemopreventive agents including resveratrol and vitamin D (Almouazen et al., 2013) have been accomplished. More recently it was reported that treatment with a solid lipid nano-encapsulated combination of curcumin, aspirin, and sulforaphane resulted in enhanced reduction in the incidence of chemically induced pancreatic cancer in Syrian golden hamsters as compared to the unmodified combination of drugs at 10 times reduced concentrations (Grandhi et al., 2014). This study provides a strong lead for the nanotechnological approach to chemoprevention. Confirming such an approach for other chemoprevention models can provide a strong rationale for using nanotechnology for chemoprevention where bioavailability of the drugs is often a limiting factor. Similar drug-combination approaches can also identify interactive molecular signaling pathways for their action in chemoprevention.

6. Translational application of chemoprevention
The research efforts of many investigators in the field of chemoprevention for the past 3 decades have resulted in the application of chemoprevention approaches under clinical settings. Like clinical trials for chemotherapy, chemoprevention trials also need to identify safe dose and bioavailability (phase I and II) in humans. The randomized clinical trials (phase III) for chemoprevention require thousands of participants and have to be carried out over a long period. This can become cost ineffective. Therefore, more recently some studies have utilized phase 0 clinical trials (Steward and Brown, 2013). These studies require very low doses of the chemopreventive agent and new approaches to study pharmacokinetics and toxicity. Ideally the discovery of blood- or urine-based biomarkers that can be predictive of malignancy or response to chemopreventive agents would be extremely valuable. Currently only a few markers are routinely used in clinical practice (Uray and Brown, 2013). These include ACF of the colon, colon polyps and adenomas, breast density and mammograms, DCIS in women, PIN in men for prostate cancer etc. However, there is a clear need for developing new clinical noninvasive markers that can predict the disease more accurately. There have been more than 50 cancer prevention trials reported. However, the majority of the studies are incomplete or not supported by sufficient numbers of participants or appropriate statistics (Naithani et al., 2008). The most popular chemoprevention trials include the Breast Cancer Prevention Trial (BCPT) for high risk women. In this trial with tamoxifen more than 13,000 women participated and the results showed that there was a significant reduction in invasive and noninvasive breast cancer, indicating a positive outcome for tamoxifen as a
chemopreventive agent. However, tamoxifen treatment resulted in an increased incidence of endometrial cancers and thromboembolic events (Cuzick et al., 2013). Therefore, a second trial was carried out with raloxifene, an antiestrogen similar to tamoxifen but without the side effects of endometrial cancers. The results indicated that raloxifene was safer than tamoxifen and did not have any adverse effects on the endometrial cancers. However, the effectiveness was less as compared to tamoxifen. In addition, a few other robust clinical trials have also been completed. These include a clinical trial with finasteride, an inhibitor of 5α-reductase and an enzyme necessary for the production of dihydrotestosterone. Finasteride treatment for 7 years was effective in reducing prostate cancer incidence by 25% (Ankrest et al., 2013). Another chemoprevention trial was completed with aspirin, a nonsteroidal anti-inflammatory drug (NSAID), for colon cancers (Ishikawa et al., 2014). The results showed that 3-year treatment with a high dose of aspirin reduced colon cancer related deaths. To achieve similar effectiveness with a lower dose of aspirin (<300 mg daily) would require 5-year treatment. Contrary to this, a large clinical trial with selenium and α-tocopherol was carried out by recruiting 35,500 men for 4 arms. The men received a placebo, α-tocopherol, selenium, or both α-tocopherol and selenium. This trial had to be terminated since no positive results could be predicted (Dunn et al., 2010) and there was an increased risk of developing prostate cancer for people consuming α-tocopherol. These results collectively indicate that, unlike chemotherapeutic agents, there is a long way to go for chemoprevention trials, since they are very costly, need a large number of participants, and the trials have to be conducted for a long period.

7. Summary
In this review a broad brush picture of chemoprevention is provided. Briefly the field has evolved from determining the efficacy of dietary treatment of synthetic analogs of vitamin A to evaluation of hundreds of chemopreventive agents. More recently emphasis has been directed towards determining the efficacy of chemopreventive agents on selected molecular targets and employing newer approaches of safe targeted delivery that would require lower concentrations of the drugs and have reduced toxicity. Another major focus has been in identifying early endpoint markers that could be determined with less invasive procedures. To this end, some molecular marker present in blood, urine, sputum, or biological fluids would be ideal. Clinical trials for chemoprevention, while important and desirable, are cost prohibitive and require large number of volunteers who may be at high risk of developing cancer. Recent failures of chemoprevention trials also raise concerns for such long-term expensive clinical trials.

References


