Published in final edited form as:
Cancer Prev Res (Phila). 2013 October ; 6(10): 1007–1010. doi:10.1158/1940-6207.CAPR-13-0311.

Big Punches Come in Nanosizes for Chemoprevention

Dipali Sharma and **Saraswati Sukumar**
Breast Cancer Program, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

Abstract

Literature to support the chemopreventive potential of several bioactive molecules has been prolific and convincing, but the clinical development of these agents has been slow. Major hurdles for development of bioactive chemoprevention approaches include low potency, lack of reliable formulations with high bioavailability that are suitable for oral administration, and relevant preclinical primary prevention models that use meaningful doses that can be translated to humans. The paper presented in this issue (Grandhi and colleagues) is an important step forward in this direction. It shows the efficacy of an oral, low dose, solid-lipid nanoparticles encapsulated curcumin and aspirin combined with free sulforaphane for long-term chemoprevention of pancreatic cancer in a carcinogen-induced hamster model. Reproducing this benefit in multiple cancer models, accompanied by development of intermediate markers of response will allow rapid translation of these findings. It will constitute the first successful multipronged attack at key pathways known to initiate and promote carcinogenesis.

Carcinogenesis, in many of the major organs, is a long process spanning 10 to 30 years starting from initiated cells and advancing to invasive cancer, theoretically providing a long "intervention window." However, once diagnosed, cancer represents a heavy cost both through disruption of normal life and suffering to the individual and financial burden to the family and society in general. Effective chemoprevention strategies are invaluable in all phases of carcinogenesis, including tumor progression of locally controlled cancer to distant metastasis. Thus, chemoprevention can be geared toward defined groups of people including healthy individuals at normal risk, people with intermediate risk due to genetic predisposition, environmental and life-style factors including obesity, or former cancer patients at high risk of developing secondary primary cancers or recurrence. An ideal chemopreventive agent is safe, high efficacious, and lacks toxicity through long-term use. Cancer risk reduction using pharmacologic agents, although proven to work, are not generally accepted (1), and consideration of natural bioactive compounds from dietary sources has emerged as an attractive chemopreventive strategy as these sources have played

Corresponding Authors: Saraswati Sukumar, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21231. Phone: 410-614-2479; Fax: 410-614-4073; saras@jhmi.edu; and Dipali Sharma, dsharma7@jhmi.edu.

Authors' Contribution
Writing, review, and/or revision of the manuscript: D. Sharma, S. Sukumar

Disclosure of Potential Conflicts of Interest
S. Sukumar is a consultant/advisory board member of CBCRP. No potential conflict of interest was disclosed by the other author.

an important role in the discovery and development of novel chemopreventive agents (2, 3). A review of epidemiologic and preclinical studies strongly supports the suitability and efficacy of several bioactive compounds for future clinical development. Two classes of the most abundant dietary chemopreventive compounds are polyphenols and isothiocyanates (ITC). Among the major polyphenols known for their chemopreventive potential are curcumin from the spice turmeric and epigallocatechin-3-gallate (EGCG) from green tea, and among the important ITCs studied for chemoprevention are allyl ITC, benzyl ITC, phenethyl ITC, and sulforaphane (SFN) derived from garlic, broccoli, and similar cruciferous vegetables.

Research over the past few years has showed that curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), a natural polyphenolic compound isolated from the rhizome of *Curcuma longa* (4), can inhibit cancer in a wide range of tissues including breast, prostate, brain, ovary, skin, and pancreas (5). Curcumin targets cancer cells by inducing apoptosis and inhibiting proliferation. Importantly, National Cancer Institute (NCI), NIH, has listed curcumin as the third generation of cancer chemotherapeutic drugs. Multiple *in vitro* and *in vivo* studies have shown that curcumin exerts anticancer activities in many cancer cell lines and tumor models targeting multiple signaling pathways including Notch1 (6), COX-2 (7), chk1 (8), NF-κB (9, 10), SP1 (9), and Stat3 (11). Many animal models and recent human clinical studies have shown that very high doses of curcumin can be safely administered orally. Patients with colorectal carcinoma were given curcumin capsules (3,600, 1,800, or 450 mg daily) for 7 days, and tissue and plasma samples were measured for curcumin and its metabolites. In patients receiving 3,600 mg of curcumin, the concentration of curcumin in normal and malignant colorectal tissue was 12.7 ± 5.7 and 7.7 ± 1.8 nmol/g, respectively (12). This finding focuses attention on the fact that, despite its very low toxicity, orally ingested, hydrophobic curcumin has very low systemic availability and rapid systemic elimination (13, 14). This feature of low bioavailability and the need to consume mega-doses (up to 8 g) has severely limited the rapid clinical development of curcumin.

Various labs using *in vivo* models, including 9,10-dimethyl-1,2-benzanthracene–induced mammary cancer in rats (15), azoxymethane-induced colonic aberrant crypt foci in rats (16), and benzo[a]pyrene-induced forestomach cancer in mice (17) have shown that SFN prevents carcinogen-induced cancer in rodents. Oral administration of SFN inhibits malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice (18). SFN suppresses cancer cell proliferation, elicits G_2-M phase cell-cycle arrest, and induces apoptosis. The molecular mechanisms underlying its effects include inhibition of signal transducer and activator of transcription (Stat3), NF-κB, Akt, mitogen-activated protein kinase, p53, COX-2, NF-E2-related factor-2 (19). Pharmacokinetic studies have shown that dietary-absorbed SFN has wide distribution in the body, reaches target tissues in its active form, and attains μmol/L levels in the blood (20, 21). In a study led by Visvanathan at Johns Hopkins, an oral dose of broccoli sprout preparation (containing 200 μmol SFN) formulated as a fruit drink consumed by women 1 hour before the reduction mammoplasty resulted in a mean accumulation of SFN metabolites of 1.45 ± 1.12 pmol/mg breast tissue from the right breast and 2.00 ± 1.95 pmol/mg from the left breast (21). In its bioavailability and effects in

preclinical models, SFN definitely shows promise for further development as a chemopreventive agent.

More than other chemopreventive agents, nonsteroidal anti-inflammatory drugs (NSAID), such as aspirin, have garnered the attention of a large number of epidemiologists and clinicians. Studies investigating the association between long-term use of aspirin and cancer risk and mortality have shown that daily intake of aspirin for 5 years or longer reduces mortality from several cancers (22). Meta-analysis of randomized studies showed positive effects of both low dose (75–300 mg daily) and high-dose (≥500 mg daily) aspirin intake (22). At low-doses, aspirin acts mainly by an irreversible inactivation of platelet COX-1 activity, thus affecting thromboxane A2-dependent platelet activation. However, Cox-independent mechanisms for the anticancer effects of aspirin have also been shown, including suppression of Wnt/catenin pathway, Raf/Ras/ERK pathway, and NF-κB that lead to an inhibition of cell proliferation and induction of apoptotic stimuli (23, 24), suggesting a role in chemoprevention.

Because chemopreventive agents are expected to be taken for a long time, there is an increased interest in developing “combination chemopreventive strategies” using low doses of chemopreventive and pharmacologic agents with different mechanisms of action to simultaneously target multiple pathways. In addition to increasing efficacy and limiting toxicity, these combination strategies could limit the development of therapeutic resistance often associated with drugs that target single molecules. In this issue of the journal, Prabhu and colleagues report dramatic effects of a combinatorial nanotechnology-based oral chemoprevention regimen in a preclinical N-nitrosobis (2-oxopropyl) amine (BOP)-treated-Syrian golden hamster model in suppressing the progression of pancreatic intraepithelial neoplasms (PanINs) to pancreatic cancer (25). This study has several strengths: (i) a novel chemopreventive regimen that combines the triple punch of aspirin, curcumin, and SFN (ACS); (ii) solid-lipid nanoparticles (SLN) encapsulation to improve efficacy of the ACS combination; (iii) oral administration; and (iv) the low doses needed for optimal effects. The nanoencapsulated AC and free SFN were able to reduce tumor burden more effectively than the free forms of the drugs. Oral administration of nanoencapsulated ACS regimens reduced tumor incidence by 75% at doses that were 10 times lower than the free drug combinations. The fact that all 3 drugs in the ACS combination have different mechanisms of action ensures not only higher efficacy via simultaneous targeting of multiple pathways but also, potentially, minimizes chances of developing resistance.

Although preclinical studies over the last few decades have shown convincing evidence to support the chemopreventive potential of several bioactive molecules, the clinical development of these agents has been disappointing. Major hurdles for development of bioactive chemoprevention approaches include low potency, lack of suitable formulations for oral administration, low bioavailability, and limited availability of suitable preclinical primary prevention models for the different cancer types and subtypes. Primary prevention clinical trials require a large number of subjects, many decades of follow-up due to long latency of most cancers, and incur heavy costs. To ensure their success, it is important to establish a constant supply of bioactive compounds and standardize methods for production, storage, and formulation to guarantee delivery of a given concentration of bioactive

component. Another pitfall is that preclinical studies often use higher doses of bioactive reagents for shorter duration to show the efficacy of a bioactive compound. It is very important to use meaningful doses in preclinical studies that can be achieved in the clinical setting and can be safely administered for longer duration. This study led by Prabhu takes an important first step forward and shows the efficacy of an oral, low dose, nanotechnology-based combination treatment regimen for long-term chemoprevention.

So what is the innovation that rendered the ACS combination of chemopreventive compounds so potent? In recent years, nanotechnology has been used to solve several limitations associated with bioactive compounds. Most bioactive compounds are to a certain degree lipophilic, a property which is oftentimes required and appreciated because of the phospholipidic nature of cell membranes, thus aiding absorption through the intestinal wall following oral administration and subsequent pharmacologic action in the target tissue. High lipophilicity, on the one hand, improves permeability of the compound but on the other hand translates into poor aqueous solubility. Poor aqueous solubility is the major hurdle in development of orally delivered bioactive compounds, because the first step in the oral absorption is the dissolution of the compound in the gastrointestinal tract. Nanoparticles have shown great potential for improving the bioavailability of bioactive compounds and were first developed to deliver low water-soluble cancer medications in solid or liquid formulations. These have been formulated with biodegradable and biocompatible polymers such as polylactic acid, starch, chitosan, and so on. The term “nanochemoprevention” was coined by Mukhtar and colleagues who showed the superior performance of EGCG in a pegylated polylactic acid polymer in a prostate cancer xenograft model (26). Curcumin has been incorporated into nanoparticles for therapeutic and preventive effects for example, into polymeric amphiphile, mPEG-PA (27), or a water-soluble polyethylene glycol (PEG) conjugate (28), which have shown cancer-cell proliferation inhibitory effects at much lower concentrations than free curcumin. In our center, polymer nanoparticles composed of N-isopropylacrylamide, vinylpyrrolidone, and acrylic acid were used to encapsulate curcumin by Maitra and colleagues, who showed that these particles could be administered intraductally at 20-fold lower doses than oral curcumin to attain comparable chemopreventive effects in a carcinogen-induced mammary tumor model (29). In an earlier publication in this journal, Prabhu and colleagues showed the efficacy of an oral regimen of polylactide-co-glycolide biodegradable copolymer nanoparticles encapsulating aspirin and folic acid with free calcium to significantly decrease aberrant crypt formation in a colon tumor model(30). Among the agents used to make nano-particles, lipids have emerged as an attractive candidate because of their ability to enhance the bioavailability of compounds with poor aqueous solubility.

Solid lipid is one of the physical forms of lipid that is used to formulate nanoparticles known as SLNs. SLNs are colloidal carriers that remain solid at room temperature and body temperature and are a great alternative to emulsions, liposomes, and polymeric micro- and nano-particles. SLNs offer multiple advantages such as small size (50–500 nm), possibility of large-scale production, smooth and spherical shape, and lower toxicity than polymeric nanoparticle (5). SLNs are especially suitable for the development of formulation for lipophilic and, in general, poor water-soluble drugs made from either natural or artificial solid lipids (31, 32). Some of the concerns associated with SLNs are low payload for a

number of drugs, complexity of the physical state of the lipid, and high water content which during storage and administration compromises stability. Stability, in fact, is the major issue with SLNs that needs to be standardized, because physicochemical stability of SLNs is a fine balance between long-term storage stability and its destabilization that is necessary for release of the bioactive compound in its biological environment (33). Despite its few shortcomings, SLNs have the potential to improve the pharmaceutical properties of bioactive compounds and aid their clinical development, opening up new avenues for research in chemoprevention.

Because multistep development of human cancer includes sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, induction of angio-genesis, and activation of invasion and metastasis, an ideal chemoprevention strategy would be to develop a multi-pronged approach to simultaneously target and tame these processes. Bioactive compounds often lack a specific target and instead modulate multiple oncogenic pathways in a multitude of cancer types. Far from being a drawback, we believe that this intrinsic property can potentially enable them to target multiple oncogenic pathways concurrently impacting various stages of cancer progression. Bioactive compounds do have inherent problems related to aqueous solubility and bioavailability, but as illustrated by this very promising study of Grandhi and colleagues, optimization of dose, route, and innovation using nanotechnology-based approaches will be the key to advance the chemoprevention field in the near future where small, sustainable doses of novel combination-cocktails will redefine the coveted “silver bullet” for chemoprevention.

Rapid extension of this work to test efficacy *in vivo* in model systems of multiple cancer types made possible by ready availability of uniform, quality controlled preparations of ACS, combined with the development of intermediate markers of response in serum and target tissue will establish the facile translational of these findings to the clinic.

Acknowledgments

Grant Support

This work is supported by Breast Cancer Research Foundation (BCRF) 90047965 to D. Sharma, and the Rubenstein Family Fund to S. Sukumar.

References

1. Visvanathan K, Hurley P, Bantug E, Brown P, Col NF, Cuzick J, et al. Use of pharmacologic interventions for breast cancer risk reduction: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2013; 31:2942–62. [PubMed: 23835710]
2. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, et al. Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. J Nutr. 2000; 130:467S–71S. [PubMed: 10721931]
3. Singh SV, Singh K. Cancer chemoprevention with dietary isothiocyanates mature for clinical translational research. Carcinogenesis. 2012; 33:1833–42. [PubMed: 22739026]
4. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007; 595:1–75. [PubMed: 17569205]
5. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, et al. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol. 2008; 76:1590–611. [PubMed: 18775680]

6. Wang Z, Zhang Y, Banerjee S, Li Y, Sarkar FH. Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. Cancer. 2006; 106:2503–13. [PubMed: 16628653]
7. Padhye S, Banerjee S, Chavan D, Pandye S, Swamy KV, Ali S, et al. Fluorocurcumins as cyclooxygenase-2 inhibitor: molecular docking, pharmacokinetics and tissue distribution in mice. Pharm Res. 2009; 26:2438–45. [PubMed: 19714451]
8. Sahu RP, Batra S, Srivastava SK. Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. Br J Cancer. 2009; 100:1425–33. [PubMed: 19401701]
9. Jutooru I, Chadalapaka G, Lei P, Safe S. Inhibition of NF-kB and pancreatic cancer cell and tumor growth by curcumin is dependent on specificity protein down-regulation. J Biol Chem. 2010; 285:25332–44. [PubMed: 20538607]
10. Li L, Aggarwal BB, Shishodia S, Abbruzzese J, Kurzrock R. Nuclear factor-kB and IkB kinase are constitutively active in human pancreatic cells, and their down-regulation by curcumin (diferuloylmethane) is associated with the suppression of proliferation and the induction of apoptosis. Cancer. 2004; 101:2351–62. [PubMed: 15476283]
11. Glienke W, Maute L, Wicht J, Bergmann L. Curcumin inhibits constitutive STAT3 phosphorylation in human pancreatic cancer cell lines and downregulation of survivin/BIRC5 gene expression. Cancer Invest. 2010; 28:166–71. [PubMed: 20121547]
12. Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, et al. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. Cancer Epidemiol Biomarkers Prev. 2005; 14:120–5. [PubMed: 15668484]
13. Garcea G, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, et al. Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. Br J Cancer. 2004; 90:1011–5. [PubMed: 14997198]
14. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 2001; 21:2895–900. [PubMed: 11712783]
15. Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci U S A. 1994; 91:3147–50. [PubMed: 8159717]
16. Chung FL, Conaway CC, Rao CV, Reddy BS. Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. Carcinogenesis. 2000; 21:2287–91. [PubMed: 11133820]
17. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci U S A. 2002; 99:7610–5. [PubMed: 12032331]
18. Conaway CC, Wang CX, Pittman B, Yang YM, Schwartz JE, Tian D, et al. Phenethyl isothiocyanate and sulforaphane and their N-acetylcysteine conjugates inhibit malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice. Cancer Res. 2005; 65:8548–57. [PubMed: 16166336]
19. Kaminski BM, Steinhilber D, Stein JM, Ulrich S. Phytochemicals resveratrol and sulforaphane as potential agents for enhancing the anti-tumor activities of conventional cancer therapies. Curr Pharm Biotechnol. 2012; 13:137–46. [PubMed: 21466425]
20. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 2008; 269:291–304. [PubMed: 18504070]
21. Cornblatt BS, Ye L, Dinkova-Kostova AT, Erb M, Fahey JW, Singh NK, et al. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. Carcinogenesis. 2007; 28:1485–90. [PubMed: 17347138]
22. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet. 2012; 379:1591–601. [PubMed: 22440947]

23. Bruno A, Dovizio M, Tacconelli S, Patrignani P. Mechanisms of the antitumoural effects of aspirin in the gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2012; 26:e1–e13. [PubMed: 23199511]
24. Dovizio M, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. Recent Results Cancer Res. 2013; 191:39–65. [PubMed: 22893199]
25. Grandhi BK TA, Wang J, Prabhu S. A novel combinatorial nanotechnology-based oral chemopreventive regimen demonstrates significant suppression of pancreatic cancer neoplastic lesions. Cancer Prev Res. 2013; 6:1015–25.
26. Siddiqui IA, Adhami VM, Bharali DJ, Hafeez BB, Asim M, Khwaja SI, et al. Introducing nanochemoprevention as a novel approach for cancer control: proof of principle with green tea polyphenol epigallocatechin-3-gallate. Cancer Res. 2009; 69:1712–6. [PubMed: 19223530]
27. Sahu A, Bora U, Kasoju N, Goswami P. Synthesis of novel biodegradable and self-assembling methoxy poly(ethylene glycol)-palmitate nanocarrier for curcumin delivery to cancer cells. Acta Biomater. 2008; 4:1752–61. [PubMed: 18524701]
28. Li J, Wang Y, Yang C, Wang P, Oelschlager DK, Zheng Y, et al. Polyethylene glycosylated curcumin conjugate inhibits pancreatic cancer cell growth through inactivation of Jab1. Mol Pharmacol. 2009; 76:81–90. [PubMed: 19395473]
29. Chun YS, Bisht S, Chenna V, Pramanik D, Yoshida T, Hong SM, et al. Intraductal administration of a polymeric nanoparticle formulation of curcumin (NanoCurc) significantly attenuates incidence of mammary tumors in a rodent chemical carcinogenesis model: implications for breast cancer chemoprevention in at-risk populations. Carcinogenesis. 2012; 33:2242–9. [PubMed: 22831956]
30. Chaudhary A, Sutaria D, Huang Y, Wang J, Prabhu S. Chemoprevention of colon cancer in a rat carcinogenesis model using a novel nanotechnology-based combined treatment system. Cancer Prev Res. 2011; 4:1655–64.
31. Parhi R, Suresh P. Preparation and characterization of solid lipid nanoparticles-a review. Curr Drug Discov Technol. 2012; 9:2–16. [PubMed: 22235925]
32. Mattheolabakis G, Rigas B, Constantinides PP. Nanodelivery strategies in cancer chemotherapy: biological rationale and pharmaceutical perspectives. Nanomedicine (Lond). 2012; 7:1577–90. [PubMed: 23148540]
33. Parveen R, Ahmad FJ, Iqbal Z, Samim M, Ahmad S. Solid lipid nanoparticles of anticancer drug andrographolide: formulation, *in vitro* and *in vivo* studies. Drug Dev Ind Pharm. Jul 4.2013 [Epub ahead of print.].