Summary
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During the past few decades our understanding of dietary agents as potential regulators of carcinogenic events has grown, providing opportunities for identifying new targets for therapeutic development. The momentum was based on the findings that as many as 35% of all cancers can be prevented by lifestyle changes including dietary modifications. Consistent with this observation are the epidemiological findings associating high consumption of fruits, vegetables and beverages such as red wine and green tea with lower incidence of cancer. Polyphenolic compounds are considered important bioactive components of these plant-derived human foods and attributed at least in part to the protective effect provided. In fact, numerous reports have documented that plant polyphenols are capable of inducing apoptosis and tumor regression in cancer cell lines and animal models, respectively. Of particular interest is the observation that a number of these polyphenols including EGCG, resveratrol and genistein induce apoptotic cell death in various cancer cell lines but not in normal cells. Moreover, it has also been realized that unlike conventional clinically used anticancer drugs, plant-derived polyphenolics have an extended margin of safety as they exhibit negligible toxicity even at relatively high concentrations. Therefore, various polyphenolic compounds such as gallicatehins, isoflavones, stilbenes, tannins and curcuminoids have been implicated as chemopreventive agents. However, the mechanism by which these compounds inhibit proliferation and induce apoptosis in cancer cells has been the subject of considerable interest. Thus studies related to identification of molecular targets and mechanism of action of plant polyphenols that are relevant to tumor microenvironment are critical steps in cancer chemoprevention.

Most of the pharmacological properties of plant polyphenols are considered to reflect their ability to scavenge endogenously generated oxygen radicals or
those free radicals formed by xenobiotics, radiations, etc. However, some data in literature suggests that antioxidant properties of the polyphenolic compounds may not fully account for their chemopreventive effects. The conventional wisdom holds that these dietary polyphenols act as antioxidants and this action is primarily involved in their role as cancer preventive agents. However, polyphenols have also shown cancer regression and such a therapeutic outcome remains unexplained by an antioxidant mechanism. Most of the plant polyphenols possess both antioxidant as well as prooxidant properties and it has been proposed that the endogenous copper dependent prooxidant cytotoxic action of polyphenols rather than antioxidant effect may be an important mechanism of their anticancer and apoptosis inducing properties. Previous work in our laboratory has provided evidence that these antioxidants may also behave as prooxidants to initiate reactive oxygen species (ROS) mediated cell death. Such a prooxidant mechanism is a result of redox-active microenvironment in cancer cells due to elevated levels of copper. Copper is an important redox active metal ion present in chromatin, closely associated with DNA bases and can be mobilized by metal chelating agents. Several reports in literature have established that both serum and tumor copper levels in cancer patients are significantly elevated. Therefore, cancer cells may be more subject to electron transfer between copper ions and polyphenols to generate ROS responsible for DNA cleavage and cell death.

In order to further explore the chemical basis of the chemopreventive activity of plant polyphenols, in this thesis I have attempted to elucidate one of the important putative anticancer mechanisms of action of dietary polyphenolic compounds that involves endogenous copper mobilization. In chapter I, using fluorescence and absorption studies, it has been shown that the flavones apigenin, luteolin and chrysin are able to bind both DNA as well as copper and are capable of reducing Cu(II) to Cu(I). These polyphenolic compounds are also capable of degrading supercoiled plasmid pBR322, calf thymus and cellular
DNA in the presence of copper ions. Further, using a system of permeabilized cells, it is demonstrated that nuclear copper is mobilized in such a cellular DNA cleavage reaction. The efficiency of DNA breakage induced by the flavones is found to be in the order - luteolin > apigenin > chrysin, which correlates with their relative copper reducing abilities. These results suggest that flavone-Cu(II) system for DNA breakage is physiologically feasible and could be of biological significance.

In chapter II, studies are done on rats by orally administering them with copper. Copper levels are found to be elevated in plasma and lymphocytes of such rats and upon treatment with different polyphenols, an increased degradation of cellular DNA is observed in such copper overloaded lymphocytes as compared to that in lymphocytes from control animals. Further, such DNA breakage is effectively inhibited by scavengers of ROS as well as copper chelator, but not by iron and zinc chelators, suggesting that the polyphenol-induced DNA breakage occurs through an oxidative process involving ROS generation and copper mobilization.

In chapter III, polyphenols have been shown to inhibit cell proliferation and induce apoptosis in different cancer cell lines and that such cell death is prevented to a significant extent by copper chelator neocuproine and various scavengers of ROS such as SOD, catalase and thiourea. Further, normal breast epithelial cells, cultured in a medium supplemented with copper, become sensitized to polyphenol-induced growth inhibition and are found to have an increased expression of copper transporter Ctr1 (as shown by Western blot analysis). Also, formation of ROS in cancer cells treated with polyphenol is detected by confocal microscopy. Copper chelator quenches such ROS production, confirming the conclusion that mobilization of intracellular copper by polyphenols causes ROS generation leading to prooxidant cell death.
Previous studies on anticancer mechanisms of polyphenols have mainly implicated antioxidant action or modulation of signal transduction pathways such as regulation of cellular proliferation, apoptosis, angiogenesis or metastasis. Based on the work presented in this thesis, I would like to conclude that mobilization of nuclear copper by plant polyphenols and the consequent prooxidant action could be one of the important mechanisms for their anticancer and chemopreventive properties. Indeed such a common mechanism better explains the anticancer effects of polyphenols with diverse chemical structures as also the preferential cytotoxicity towards cancer cells.