Cellular targets for the beneficial actions of tea polyphenols

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ABSTRACT
Green and black teas contain different biologically active polyphenolic compounds that might offer protection against a variety of human diseases. Although promising experimental and clinical data have shown protective effects, limited information is available on how these beneficial effects of tea polyphenols are mediated at the cellular level. Evidence is accumulating that catechins in green tea as well as theaflavins and thearubigins from black tea are the substances responsible for the physiologic effects of tea in vitro. The green tea catechin epigallocatechin-3-gallate (EGCG) is generally considered to be the biologically most active compound in vitro. The changes in the activities of various protein kinases, growth factors, and transcription factors represent a common mechanism involved in cellular effects of tea polyphenols. In addition to modification of intracellular signaling by activation of cellular receptors, it was shown that, at least for EGCG, tea polyphenols can enter the cells and directly interact with their molecular targets within cells. There, they frequently result in opposite effects in primary compared with tumor cells. Although tea polyphenols were long regarded as antioxidants, research in recent years has uncovered their prooxidant properties. The use of high nonphysiologic concentrations in many cell culture studies raises questions about the biological relevance of the observed effects for the in vivo situation. Efforts to attribute functional effects in vivo to specific molecular targets at the cellular level are still ongoing.

INTRODUCTION
Tea polyphenols have attracted much attention as potential beneficial agents in a variety of human diseases. Human intervention studies using tea polyphenols as beverages, tea extracts, or as isolated individual compounds are ongoing. A broad spectrum of experimental, animal, and clinical data have shown beneficial effects of tea polyphenols for various medical conditions (1–3). However, there is as yet limited information available on how these beneficial effects are mediated at the cellular level. Elucidation of the molecular targets of tea polyphenols at the cellular level is essential for the improvement of their potential in vivo relevance. The extent of biological activity of tea polyphenols depends on their chemical structure. There is strong evidence that catechins in green tea as well as theaflavins and thearubigins from black tea are the substances responsible for the physiologic effects of tea in vitro. The green tea catechin epigallocatechin-3-gallate (EGCG) appears to be the biologically most active polyphenol at the cellular level (7). Overall, catechins and theaflavins containing a galloyl group at their 3′ position proved to be the most potent compounds physiologically. The contribution of various other tea ingredients, such as theobromine or caffeine, is less well understood.

Because of the beneficial properties of tea in cancer prevention, neurological disorders, cardiovascular disease, and other human diseases, a diverse spectrum of different cell types is involved in the molecular actions of tea polyphenols. These comprise cells from different tissues, organs, and tumor stages for cancer; neurons, astrocytes, and microglia for neurodegenerative diseases; and cardiomyocytes and endothelial cells for cardiovascular pathologies. All of these cells are characterized by an

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5 Abbreviations used: EGCG, epigallocatechin-3-gallate; eNOS, endothelial nitric oxide synthase; ERα, estrogen receptor α; FITC, fluorescein isothiocyanate; miRNA, microRNA; mRNA, messenger RNA; NO, nitric oxide; ROS, reactive oxygen species; TF3, theaflavin-3,3′-digallate; 67LR, 67-kDa laminin receptor.

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array of differences in signaling cascades, gene expression levels, types of intracellular and membrane receptors, redox status, and overall cell morphology (Figure 1). In addition to the diversity of cell types, there is the complexity of tea polyphenols used to treat the cells. Although impressive progress has been made in recent years in the elucidation of molecular targets of tea polyphenols in diverse cell types, many questions still remain open. Attempts to attribute functional effects in vivo to specific molecular signal transduction pathways in vitro are still at the beginning stages.

However, it should be kept in mind that many cell culture studies used much higher concentrations of tea polyphenols than can be achieved after tea consumption in vivo. Whereas this approach is able to detect many molecular targets at the cellular level, it also raises questions about the biological relevance of the observed effects in vivo. Maximum plasma concentrations for catechins (especially for EGCG) are only in the low μmol/L range (8, 9). In addition, tea polyphenols undergo extensive conjugation in vivo (4). A comprehensive overview on absorption/metabolism of tea polyphenols in humans is provided in another article in this supplement issue (10).

GENERAL TARGETS OF TEA POLYPHENOLS AT THE CELLULAR LEVEL

A common mechanism involved in the molecular effects of tea polyphenols is the modification of the activity of various transcription factors, growth factors, and protein kinases. By affecting the activity of intracellular signal transduction pathways, the major ingredients of green and black tea exert a variety of beneficial impacts in diverse cell types. In addition to activation or inhibition of protein kinases, tea polyphenols can modify protein acetylation (11) and glycation (12), further contributing to changes in enzyme activities. On the transcriptional level, tea polyphenols induce or suppress gene transcription, interfering at different levels of transcriptional regulation. It has been shown that EGCG affects alternative splicing of a signal-transducing membrane protein (gp130) in fibroblasts, subsequently resulting in suppressed IL-6 signaling and reduced inflammation (13). Tea polyphenols can also target and regulate levels of microRNAs (miRNAs). miRNAs are small, noncoding RNA species that regulate the levels of target messenger RNAs (mRNAs) through mRNA degradation and suppression of protein translation. EGCG has been shown to decrease lung cancer cell proliferation through upregulation of mir-210 expression (14). The involvement of miRNAs was also shown by microarray analysis in EGCG-mediated protection against UVB radiation in dermal fibroblasts (15).

Recently, epigenetic changes induced by tea polyphenols became a focus of research. EGCG was shown to restore estrogen receptor α (ERα) expression in breast cancer cells through epigenetic changes in the ERα promoter region. ERα-negative breast cancer is clinically more aggressive and has a poor prognosis because of the lack of hormone-targeted therapies (16). In prostate cancer cells, treatment with green tea polyphenols resulted in re-expression of silenced glutathione-S-transferase pi (GSTP1) by demethylation in the GSTP1 promoter region (17). In addition to transcriptional changes, tea polyphenols can influence intracellular levels of reactive oxygen species (ROS), concentrations of second messengers, ie, 3′,5′-cyclic AMP and 3′,5′-cyclic guanosine 5′-monophosphate, as well as amounts of secreted soluble cellular factors. All of these cellular actions of tea polyphenols can occur either short term (within minutes or being transient) or long term (persisting for hours or days). An overview of the general actions of tea polyphenols at the cellular level is shown in Figure 2. Because cellular processes are in constant interaction, changes in one parameter result in modifications of interacting partners. This makes the elucidation of the primary target for a particular action of tea polyphenols demanding. In addition, whether an enzyme, transcription factor, or signaling pathway is either activated or inhibited by tea polyphenols depends largely on the cell type investigated. Surprisingly, tea polyphenols frequently result in opposite effects. Whereas in fast-proliferating, activated cells (eg, tumor cells) tea polyphenols inhibit the activity of intracellular signaling cascades (leading to cell cycle arrest and apoptosis), in primary resting cells these pathways are often activated. These apparently contradictory

**FIGURE 1.** Interaction of many different cell types with structurally different tea polyphenols. Multiple cell types are involved in the elucidation of molecular targets for anticancer, cardiovascular, and neuroprotective effects. Tumor and primary cells originating from different tissues and organs show distinct heterogeneity in cellular morphology and molecular responses.
cellular actions provide the rationale for the potential use of tea polyphenols both against cancer and against diseases without uncontrolled cell proliferation. In cancer cells, the activities of mitogen-activated protein kinases and the prosurvival phosphatidylinositide-3-kinase–Akt pathway are frequently inhibited by tea polyphenols (18–20), whereas in primary cells the same pathways get activated (21, 22).

ROLE OF GREEN AND BLACK TEA POLYPHENOLS

Green and black teas are consumed in different geographical regions. The question of whether green and black tea can contribute equally to beneficial effects at the cellular level (as well as in vivo) therefore represents an important health issue. Although the overall content of polyphenols in green and black tea is comparable, the compositions of individual compounds differ. As a result of the extensive research on EGCG (the main catechin in green tea), it is frequently thought that green tea might be superior to black tea in terms of beneficial health effects. However, research in recent years indicates that compounds from black tea are not inferior to green tea catechins. The antioxidant activities of a number of theaflavins were compared with EGCG. The theaflavins proved to be more effective than EGCG in scavenging of superoxide, hydrogen peroxide, and the hydroxyl radical. With the exception of superoxide, the digallate theaflavin-3,3′-digallate (TF3) was most effective, followed by the 2 theaflavin monogallates and the nongallated form (23). A similar order of potencies was previously found for the antioxidant properties of theaflavins (24).

We compared the cardiovascular beneficial effects of green tea catechins and various black tea compounds in terms of vascular nitric oxide (NO) production, produced by endothelial NO synthase (eNOS) in endothelial cells. In agreement with the results for antioxidant effects, the theaflavin digallate was most effective in stimulation of eNOS activity and NO production, followed by the monogallates. The theaflavins were more potent than equimolar doses of EGCG. In addition, the black tea thearubigins were effective inducers of eNOS activity and NO production in endothelial cells (25). Another study showed that theaflavin (TF1) prevents LPS-induced proinflammatory signaling in bone marrow–derived macrophages (26). TF3 was more effective than EGCG in protecting cardiomyocytes from oxidative damage (27). In human leukemic cells, 3 different fractions of thearubigins induced apoptosis but did not show any toxic effects in normal peripheral blood mononuclear cells (28). Black tea polyphenols (both theaflavins and thearubigins) induced cell cycle arrest in human leukemic cells by affecting multiple pathways involved in cell cycle control (20). The above examples show that catechins from green tea as well as ingredients from black tea exert beneficial effects at the cellular level in vitro that are of potential interest for a variety of human diseases. It remains to be proven, however, whether the high-molecular-weight compounds from black tea are absorbed in vivo and can contribute to beneficial effects of black tea in the body. The thearubigins are probably not absorbed at all, and only minute amounts of theaflavins (1 ng/mL) entered the circulation after intervention with 700 mg of mixed theaflavins (29).

CELLULAR SITES FOR MOLECULAR ACTIONS OF TEA POLYPHENOLS

The common cellular effects of tea polyphenols involve binding to cell surface receptors and incorporation into cell
surface membranes. As a result, intracellular signaling pathways are either activated or inhibited depending on the cell type. This is frequently followed by more or less pronounced transcriptional changes (30). For a long time, the effects on the cell membrane were thought to be the main mode of action for tea compounds at the cellular level. However, lately the intracellular localization of tea polyphenols, especially of EGCG, was also shown. By using fluorescein isothiocyanate (FITC)-conjugated EGCG, a time-dependent incorporation of EGCG into the cytoplasm and subsequent nuclear translocation were shown through confocal microscopy in a murine suspension fibroblast cell line. After treatment of cells with 65 μmol/L FITC-EGCG, accumulation of EGCG in the cell membrane was seen within 30 min, and a concentration around the nucleus was observed after 1 h. With a longer incubation time of 4 h, EGCG could be found at all regions within the cells, including the cytoplasm and the nuclei (31). In human fibrosarcoma cells, intracellular uptake of FITC-EGCG started at 2 h, and cytoplasmic internalization and nuclear concentration were observed after 12 h. Between 24 and 48 h, EGCG was distributed throughout the cells (32). On the other hand, in normal human dermal fibroblasts, cellular uptake of 100 μmol/L FITC-EGCG started only at ~4 h. EGCG was located in the cytosol and the nuclei after 24 h. When cells were treated with 50 μmol/L FITC-EGCG for 2 d, EGCG was present in cell membranes and the cytoplasm for an additional 10 d, despite the removal of EGCG from the cell culture medium at day 2 (33). The authors suggested a different time pattern for the cellular and nuclear uptake of EGCG between primary and immortal cells. These results imply that EGCG can, in addition to its known effects on cell surface receptors, be directly incorporated into cells.

By using another approach with 3H-labeled EGCG, the cytosolic and nuclear incorporation of EGCG in lung cancer cells (34) as well as cytoplasmic and membrane-associated EGCG in human colon adenocarcinoma cells have been reported (35). In addition to cell membranes, cytoplasm, and nuclei, the accumulation of 3H-labeled EGCG in mitochondria of rat cerebellar granule neurons was recently shown, where it protected neurons from apoptosis triggered by mitochondrial oxidative stress (36). These findings suggest that tea polyphenols may potentially enter all cell compartments. However, it should be noted that not all of the above approaches (FITC-conjugated and 3H-labeled EGCG) assessed the intracellular localization of the label, not the parent compound itself. This may be of potential relevance because cells in culture might be able to metabolize tea polyphenols.

How can tea polyphenols enter the cells? Structural simulations suggest a strong affinity of the 4 main green tea catechins as well as of different black tea theaflavins for lipid bilayers (37, 38). Indeed, both EGCG and epicatechin-3-gallate can interact with phospholipid membranes, as shown by nuclear magnetic resonance spectroscopy (39). The contribution of the galloyl group of tea catechins in their interaction with phospholipid bilayers was recently shown (40). Tea polyphenols can subsequently be incorporated into the plasma membrane. A detailed model of the intracellular uptake of EGCG was provided by Patra et al (41). According to this model, EGCG may be transported into lipid rafts, membrane-associated cholesterol-enriched microdomains that harbor many signaling proteins, including receptors. EGCG may then bind to its target receptor (miscellaneous, depending on the cell type) and be transported into the cytosol by its receptor or receptors or by lipid rafts in a manner similar to endocytosis (41). The exact mechanism for the nuclear translocation of EGCG, or of tea polyphenols in general, is unknown. Once within the cell, tea polyphenols can bind to cytosolic target proteins (42). This indicates that both receptor-mediated transduction of signals (through the interaction of tea polyphenols with membrane receptors without entering the cells) as well as direct binding to intracellular molecules (after intracellular trafficking of tea polyphenols) can apparently contribute to tea polyphenol–mediated changes in intracellular signal transduction.

Tea polyphenols can also bind to DNA and RNA (43, 44), suggesting 2 alternatives for tea polyphenol–induced changes in gene transcription: (1) by nuclear translocation of transcription factors or (2) by direct binding of tea polyphenols to regulatory DNA sequences in the nucleus, thus activating or suppressing gene transcription. Furthermore, tea polyphenols may also interact with transcriptional activators/repressors in the nucleus. Potential modes of action for tea polyphenols are summarized in Figure 3. Due to the complex chemical structures and especially the high molecular size of black tea polyphenols, it can be hypothesized that they most likely are not able to cross the cell membranes and enter the cells. Their favored mode of action might therefore be limited to interactions with cell membrane receptors and initiation of intracellular signal transduction pathways but finally resulting in the same molecular actions as their green tea counterparts.

ANTIOXIDANT COMPARED WITH PROOXIDANT PROPERTIES OF TEA POLYPHENOLS

Many human diseases are characterized by sustained inflammatory processes. Therefore, antioxidant properties of tea polyphenols are very much in the foreground of scientific interest. Tea polyphenols possess direct and indirect antioxidant effects at the cellular level. These include direct scavenging of free radicals (45), chelating of metal ions (46), inhibition of cellular ROS-generating enzymes (47), and reduction in levels of inflammatory cytokines (48). In addition, tea polyphenols can upregulate expression of intracellular free radical scavenging enzymes. In human fibroblasts, EGCG induced the expression of catalase, superoxide dismutases 1 and 2, and glutathione peroxidase and protected cells against H2O2-induced oxidative damage (49). As a result of the number and arrangement of their phenolic hydroxyl groups, catechins and theaflavins are efficient scavengers of free radicals. Conversely, they may also contribute to ROS generation (50). Autoxidation of EGCG was shown in Tris-HCl buffer. Limited stability of EGCG in buffer is a result of the formation of EGCG quinones and EGCG dimer quinones, which are autooxidation products of EGCG (51). However, after high doses of intraperitoneal EGCG in mice, no oxidation products of EGCG could be detected in plasma (51). In cell culture systems, EGCG frequently produces ROS. EGCG-induced apoptosis in cancer cells involved and relied on generation of superoxide and H2O2 and was prevented by several antioxidants (52). The type and amounts of free radicals generated by tea polyphenols in vitro are highly dependent on the cell type and the cell culture medium (53, 54). This can easily cause misinterpretations of in vitro results and erroneously assigned putative targets of tea polyphenols. It has been suggested that the prooxidant activities
of tea polyphenols are mainly confined to cancer cells (where they contribute to induction of apoptosis), whereas in primary cells no generation of ROS is observed (55). However, there is accumulating evidence that the production of free radicals also mediates the protective effects of tea polyphenols in primary cells. EGCG-stimulated production of the antiatherogenic molecule NO in endothelial cells involved intracellular generation of H$_2$O$_2$ (56). Low-dose H$_2$O$_2$ production by EGCG contributed to protection against oxidative stress in primary human keratinocytes (57). Moderate oxidative stress may induce cellular resistance to subsequent exposure to higher ROS levels or other exogenous cellular stress.

Does EGCG itself produce ROS? Or are free radicals released from cells after induction of apoptosis? At least for tumor cells, it can be speculated that tea polyphenols themselves generate free radicals initially that are later perpetuated by apoptotic or dying cells. In summary, a complex picture of the pro- and antioxidative effects of tea polyphenols has emerged in recent years. Both the reduction in and generation of free radicals in tumor as well as in primary cells are involved in the cellular actions of tea polyphenols.

CELLULAR TARGETS FOR ANTICANCER, CARDIOVASCULAR, AND NEUROPROTECTIVE EFFECTS OF TEA POLYPHENOLS

Anticancer effects

Because cancer represents one of the most prevalent diseases worldwide, much research has been undertaken to study the anticancer effects of tea polyphenols. A multitude of different targets have been proposed for the anticancer activities of tea polyphenols (58, 59). One of the most promising is the 67-kDa laminin receptor (67LR), first described by Tachibana et al (60). 67LR is a cell surface receptor that is expressed in many cancer cells and was shown to mediate the anticancer activities of EGCG. In multiple myeloma cells, EGCG resulted in clustering of lipid rafts via 67LR and induction of apoptotic cell death (61). Silencing of the laminin receptor in cancer cells prevented EGCG-induced tumor growth inhibition in mice (62), showing the in vivo relevance. Both studies dissected intracellular signaling pathways downstream of the laminin receptor that are involved in EGCG-mediated cancer cell apoptosis (61, 62). The high expression of 67LR in prostate tumor cells was recently exploited in an innovative approach to treat prostate cancer in an animal model (63). Radioactive nanoparticles are used in molecular imaging and cancer therapy. Radioactive gold nanoparticles were coated with EGCG to specifically target prostate tumor cells that express 67LR and injected in tumor-bearing mice. Internalization of the EGCG-Au nanoparticles in prostate cancer cells was mediated via the 67LR. Intracellular localization was observed in vacuoles and the cytoplasm. Blocking of the 67LR with laminin or with anti-67LR antibody prevented cellular uptake of the EGCG-Au nanoparticles in cancer cells. Most important, treatment of mice with EGCG-Au nanoparticles resulted in an 80% reduction in tumor volumes after 28 d, compared with control animals (63). Even if human data are still lacking, these results provide a successful example of the translation of in vitro–derived cellular targets of tea polyphenols into in vivo applications. The selective expression of surface molecules in cancer cells (and in other cell types involved in human diseases) might in the future represent a promising approach for the specific delivery of EGCG to target tissues or cells.

Another promising target for the anticancer effects of tea polyphenols is human telomerase (64). Telomerases elongate and stabilize telomers, the end of chromosomes. The expression of telomerase is crucial for unlimited proliferation of cancer cells.
and thus tumor progression. The inhibition of telomerase by EGCG was described some years ago (65). But more recently, the involvement of epigenetic mechanisms in EGCG-mediated telomerase inhibition has been elucidated (66). EGCG suppressed the transcription of telomerase in human breast cancer cells by epigenetic histone modifications in the human telomerase reverse transcriptase promoter region (67). In addition, it inhibited DNA methyltransferase and histone acetyltransferase resulting in human telomerase reverse transcriptase promoter hypomethylation and histone deacetylation. Both processes contribute to the transcriptional repression of telomerase (67). On the other hand, epigenetic modifications in many tumor suppressor genes are involved in anticancer properties of tea polyphenols.

The elucidation of the primary cellular targets of tea polyphenols in cancer cells may be hampered by the suppression of intracellular signaling cascades after the induction of cell cycle arrest or apoptosis. Most cellular processes are energy dependent and rely on ATP as an intracellular energy source. The induction of apoptosis (a process that itself requires ATP) and cell cycle arrest are accompanied by a decline in cellular energy expenditure. Thus, the inhibition of protein kinases or of large energy-dependent protein complexes such as the proteasome (68) by tea polyphenols in tumor cells might at least partially involve the shortage of intracellular energy resources. In addition, high nonphysiologic concentrations of tea polyphenols are often applied in studying their anticancer effects.

Cardiovascular effects

Cardiovascular diseases are a leading cause of death throughout the world. Underlying pathogenic mechanisms are multifactorial. Hence, tea polyphenols act on multiple cellular targets in endothelial cells, cardiomyocytes, and monocytes and macrophages (69–71). Cytokine-stimulated expression of proatherogenic C-reactive protein in macrophages was inhibited by EGCG (72). Similarly, EGCG has also been shown to suppress LPS-induced proinflammatory nuclear transcription factor κB signaling (73) and expression of inducible NO synthase and cyclooxygenase-2 (74) in macrophages.

In murine cardiomyocytes, positive inotropic effects by enhancing the sarcoplasmic reticular Ca²⁺ content and an increase in ryanodine receptor type 2–mediated Ca²⁺ release and through inhibition of the Na⁺/Ca²⁺ exchanger were observed with nanomolar concentrations of EGCG (75). The contractility of perfused rat hearts and fractional shortening in isolated cardiomyocytes were improved by EGCG in the low-μmol/L range via activation of the Na⁺/H⁺ exchanger and the reverse mode of the Na⁺/Ca²⁺ exchanger (76). In addition to positive inotropic effects, tea polyphenols protect cardiomyocytes from exogenous damage, such as ischemia-reperfusion injury (77), doxorubicin (78), and H₂O₂ (27).

Endothelial cells are the main focus for the elucidation of cellular targets for cardiovascular diseases. These cells mediate vascular homeostasis and play an important role in atherosclerotic processes. Endothelial cells produce NO, one of the most important antiatherogenic molecules that also induces vasodilation in blood vessels. The stimulation of endothelial NO production by tea polyphenols and the mechanisms thereof have been intensively investigated (79). Green and black tea polyphenols stimulate NO production in endothelial cells via phosphorylation of eNOS (22, 56, 80). In addition to stimulating eNOS activity, low concentrations of EGCG increased expression of eNOS mRNA in human umbilical vein endothelial cells (81). Furthermore, caveolin-1, a negative regulator of eNOS, was downregulated by tea polyphenols (82). These data show that tea polyphenols can increase concentrations of NO in endothelial cells by multiple mechanisms and may thus contribute to improved vasorelaxation of blood vessels. Although epidemiologic data suggest beneficial effects of tea polyphenols for cardiovascular health, including risk factors such as obesity and diabetes (83–86), little is known about how these effects are mediated at the cellular level.

Neuroprotective effects

Neurodegenerative disorders are increasing as a result of the aging population. Neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and Huntington disease share some common features at the cellular level. These include increased inflammation/oxidative stress, cellular degeneration, elevated concentrations of free iron metals, and aggregation of proteins (fibrillogensis) (87). All of these detrimental processes are favorably influenced by tea polyphenols. EGCG prevented neuroinflammation (88), attenuated 6-hydroxydopamine–induced neuronal cell death (89), and protected neurons from damage by mitochondrial toxins (90). Recently, the impact of tea polyphenols on neuronal protein misfolding and aggregation has been studied in detail. Accumulation of amyloid fibrils results in cellular toxicity. EGCG was shown to inhibit amyloid-β (involved in Alzheimer disease) and α-synuclein (involved in Parkinson disease) fibrillogensis by direct binding to unfolded polypeptides and formation of nontoxic oligomers (91). Preformed amyloid-β and α-synuclein fibrils were converted into smaller, nontoxic protein aggregates by EGCG. Comparisons of different catechins showed that only compounds with a gallate group were able to remodel preformed fibrils. Mechanistically, tea polyphenols bind to β-sheet–rich aggregates and induce conformational changes (92). Prevention of amyloid-β and α-synuclein fibrillogensis as well as remodeling of amyloid-β fibrils into nontoxic aggregates have also been shown with different theaflavins (93). The structural interaction of EGCG with β-amylod peptides was elucidated by nuclear magnetic resonance (94). Recently, it has been suggested that EGCG-mediated inhibition of amyloid formation might be less efficient in phospholipid bilayers (95).

A number of promising targets for tea polyphenol–mediated beneficial effects against different aspects of neurodegenerative disorders exist at the cellular level. Whether these approaches can in the future contribute to the prevention or treatment of neurological diseases remains open.

CONCLUSIONS AND FUTURE DIRECTIONS

A plethora of molecular targets in different cell types are involved in the anticancer, cardiovascular, and neuroprotective effects of tea polyphenols. The modification of multiple cellular processes may have far-reaching consequences in the prevention and/or treatment of human diseases. The intracellular uptake of individual tea compounds, mainly from green tea, offers a potential for direct interactions of tea polyphenols with their...
intracellular targets. Due to the poor absorption of the high-
molecular-weight compounds from black tea (4, 29), the nature of the
metabolites that could contribute to beneficial effects of black tea in vivo remains unknown. Although EGCG represents the most active and best-characterized tea polyphenol in vitro, its contribution to the biological effects of green tea in vivo is still not proven and may vary between physiologic endpoints.

The original view that tea polyphenols act mainly as antioxid-
andant molecules can no longer be supported. One major challenge is to identify cellular targets in vitro with physiologic concentra-
tions of tea polyphenols. Many questions still remain open: Are common molecular targets shared by different tea poly-
phenols? What is the contribution of less well investigated tea compounds, e.g., theobromine or caffeine, to the putative benefits of tea consumption? What are the primary cellular targets and what are secondary effects due to changes in the cell cycle or induction of apoptosis? And the major question is, How can these identified cellular targets be translated into clinical studies? With ongoing research, both in vitro and in vivo, there is some hope of obtaining answers to these questions in the near future.

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