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Abstract. The cancer-preventive effects of green tea and its main constituent (-)-epigallocatechin gallate [(-)-EGCG] are widely supported by results from epidemiological, cell culture, animal and clinical studies although the molecular target has not been well defined. We previously reported that ester bond-containing tea polyphenols, e. g. (-)-EGCG, and their synthetic analogs potently and specifically inhibited the proteasomal activity. Subsequently, we further demonstrated that methylation on green tea polyphenols under physiological conditions decreased their proteasome-inhibitory activity, contributing to decreased cancer-preventive effects of tea consumption. Since (-)-EGCG is unstable under physiological conditions, we also developed the peracetate-protected or prodrug form of (-)-EGCG, Pro-EGCG (1), and shown that Pro-EGCG (1) increases the bioavailability, stability, and proteasome-inhibitory and anticancer activities of (-)-EGCG in human breast cancer cells and xenografts, suggesting its potential use for cancer prevention and treatment.

Key words: Tea polyphenols – Proteasome inhibitors – Molecular target – Methylation – Prodrug – Cancer prevention – Chemotherapy

Introduction

Annually, more than 5 million people are diagnosed with cancer and more than 3.5 million people die from cancer worldwide (Ferlay et al., 2001). When analysis of cancer incidence by racial group is performed for many types of cancers, Asian and islander populations have significantly reduced incidence and mortality due to cancer that seems to correlate with dietary intake of green tea (Fujiki, 1999; Gupta et al., 1999; Kazi et al., 2002). The attraction of green tea as a cancer chemopreventative and a chemotherapeutic agent is suggested. Tea consumption is not associated with toxic effects. Populations that practice extensive tea consumption have demonstrated reduced incidence and mortality due to cancer. The principle components of tea exhibit a wide array of cancer preventing activities (Fujiki, 1999; Gupta et al., 1999; Kazi et al., 2002).

The ubiquitin-proteasome pathway

In recent years, proteasome inhibition has become increasingly important in cancer and drug resistance research. The vast majority of regulated proteolysis in eukaryotic cells occurs through the actions of the ubiquitin-proteasome pathway (Ciechanover et al., 2000). Although it would seem disastrous to alter the activity of this crucial protein degradation system, proteasome inhibition has been well established as a rational strategy for multiple myeloma (Richardson et al.,
2005; Catley et al., 2005), non-Hodgkin lymphoma (Goy et al., 2005) and some other solid tumours (Dou and Goldfarb, 2002). Understanding the mechanisms of action has led to integration into combination regimens using both proteasome inhibitors and standard chemotherapeutics.

The ubiquitin-proteasome pathway involves two successive steps: conjugation of multiple ubiquitin molecules to the protein substrate, and degradation of the tagged protein by the 26S proteasome (Fig. 2, left). Ubiquitin is a highly conserved 76-amino acid protein that becomes covalently ligated to a target protein by a multi-enzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and the Ub-ligating (E3) enzymes, which act in a sequential manner. This is a three-stage process that starts with activation of ubiquitin by the E1 enzyme in an ATP-requiring reaction that generates a high-energy thiol ester intermediate, E1-S-ubiquitin. Activated ubiquitin is then transferred from E1, by one of several ubiquitin-conjugating enzymes, E2, via an additional high-energy thiol-ester intermediate, E2-S-ubiquitin. From E2 to the E3-bound substrate, the activated ubiquitin can be then transferred directly or via a third high-energy thiol ester intermediate, E3-S-ubiquitin (Ciechanover et al., 2000).

Ubiquitinated proteins are recognized by the 26S proteasome, a large multi-subunit protease complex that is localized in the nucleus and cytosol and selectively degrades intracellular proteins. In almost all of the cases, only proteins containing polyubiquitin chains on sequential lysine residues are recognized and degraded by the proteasome and the ubiquitin is released and recycled. The proteolytic core of this complex, the 20S proteasome, contains multiple peptidase activities and functions as the catalytic machine. This core is composed of 28 subunits arranged in four heptameric, tightly stacked rings (α7, β7, β7, α7) to form a cylindrical structure (Groll et al., 1999). The α-subunits make up the two outer, and the β-subunits the two inner, rings of the stack (Fig. 2, right). The entrance of substrate proteins to the active site of the complex is guarded by the α-subunits that allow access only to unfolded and extended polypeptides. The proteolytic activities are confined to the β-subunits conferring the unique and distinguishing proteasome feature of multiple peptidase activities that include chymotrypsin-like (cleavage after hydrophobic side chains, mediated by the β5 subunit), peptidylglutamyl peptide hydrolyzing-like or PGPH-like (cleavage after acidic side chains, mediated by the β1 subunit), and trypsin-like (cleavage after basic side chains, mediated by the β2 subunit) activities (Groll et al., 1999) (Fig. 2).

The ubiquitin-proteasome pathway is vital in the degradation of proteins involved in cell cycle progression, proliferation, apoptosis and a vast majority of abnormal proteins that result from oxidative damage and mutations. The proteasome could therefore contribute to the pathological state of several human diseases including cancer, in which some regulatory proteins are either stabilized due to decreased degradation or lost due to accelerated degradation (Ciechanover, 1998). Many important target proteins of the proteasome have been identified, including cyclins A, B, D and E, tumour suppressor protein p53, pro-apoptotic protein Bax (Li and Dou, 2000), cyclin-dependent kinase inhibitor p27 (Pagano et al., 1995; Sun et al., 2001), and the NFκB inhibitor, IκB-α (Perkins, 2000). Since inhibition of the ubiquitin-proteasome