Basic Investigation

EFFECT OF TEA POLYPHENOLS AND EGCG ON NASOPHARYNGEAL CARCINOMA CELL PROLIFERATION AND THE MECHANISMS INVOLVED

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ABSTRACT

Objective: Tea polyphenols present in green tea show cancer chemopreventive effects in many tumor models. Epidemiological studies have also suggested that green tea consumption might be effective in the prevention of certain human cancers. In the present study, we investigated the molecular mechanisms of the inhibition of cell proliferation by tea polyphenols in nasopharyngeal carcinoma (NPC) cell line CNE1-LMP1.

Methods: CNE1-LMP1 cells were treated with tea polyphenols at various doses (0, 25, 50, 100, 200 pg/ml) for 24 hours, the morphology of cells was observed by light microscopy, and cell survival rate was determined by MTT assay. At the same time, cell cycle of CNE1-LMP1 was analyzed by flow cytometry. Cyclin D1 transcription was analyzed by cyclin D1 promoterluciferase reporter system and expression of cyclin D1 protein by Western blot analysis. Transactivities of NF-κB and AP-1 was analyzed by Dual-fluorescence reporter gene system. Results: After treatment of CNE1-LMP1 cells with tea polyphenols, the number of proliferating cells was obviously decreased as determined by light microscopy and MTT assay (from 100% to 89.4%, 83.3%, 74.8% and 38.1%). With the increase of tea polyphenols concentrations, the number of cells in S-phase was obviously decreased, and the number of cells in G1-phase from 22.20% to 13.16%, and the number of cells in G0/G1 phase was elevated from 68.5% to 74.08%. It suggests that tea polyphenols could arrest the cell cycle at both of the two checkpoints. Furthermore, transcription and expression of cyclin D1 protein also decreased in a dose-dependent manner. Transactivities of NF-κB and AP-1 were obviously down-regulated in CNE1-LMP1 cells. Conclusion: Green tea polyphenols could inhibit cell proliferation, by suppressing the activity of NF-κB and AP-1, and by down-regulation of the transcription of cyclin D1.

Key words: Nasopharyngeal carcinoma (NPC), Tea polyphenols (TP), NF-κB, AP-1, Epstein-Barr virus, LMP1, Cyclin D1

Tea (Camellia Sinensis) is a promising agent for the chemoprevention of human cancers[1,2]. The inhibitory activity of tea against tumorigenesis has...
been demonstrated in many animal models and has been suggested by some epidemiological studies\(^{[1-3]}\), including those involving cancers of the lung, skin, esophagus and stomach\(^{[1,4,5]}\). Such activity has generally been attributed to tea catechins.

Tea consists of several components, but interest has been focused primarily on polyphenols, especially those found in green tea. These polyphenols include (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epicatechin (EC). Of these, EGCG accounts for > 40% of the total amount of tea components.

Green tea polyphenols and EGCG have been shown to inhibit the growth of human stomach cancer cell lines and several other human cancer cell lines\(^{[6,7]}\). However, the molecular mechanisms of growth inhibition are poorly understood. When added to tumor cells in culture, tea and tea polyphenols compounds display inhibiting effects on the activities of many enzymes including teleocidin-induced protein C, 12-O-tetradecanoylphorbol-13-acetate-induced epidermal ornithine decarboxylase, reverse transcriptase, DNA topoisomerase II and urokinase. Recent data showed that tea polyphenols down-regulate the transactivity of nuclear transcription factor AP-1 and so on\(^{[8-11]}\). However, among these possible biological activities, it is difficult to determine the most pertinent mechanism that links signal transduction to the cell cycle. This study was designed to investigate whether green tea polyphenols and major its constituent EGCG could perturb the cell cycle progression on nasopharyngeal carcinoma cells and the possible molecular mechanisms involved. The results suggest that green tea polyphenols and EGCG could inhibit nasopharyngeal carcinoma (NPC) cell proliferation, by suppressing the transactivities of transcription factors NF-κB and AP-1, and down-regulation of the transcription of cyclin D1.

MATERIALS AND METHODS

Tea Polyphenols

Purified green tea polyphenols (Sigma.) was a kind gift from Dr. Cao Jin (Tea and Health Lab. Xiang-Ya School of Medicine, Central-South University, China); EGCG was generously provided by Dr. Zigang Dong (The Hormel Institute, University of Minnesota, Austin, Minnesota, USA).

Plasmid

HIV-NF-xB-luciferase reporter was previously reported by Dr. Dong in 1994, this plasmid is driven by a 197 bp HIV-1 LTR fragment containing two NF-κB-binding sites (A kind gift from Dr. Jianjian Li, NIH, USA). pRLnull plasmid with a Renilla luciferase gene was purchased from Promega. An AP-1 reporter was constructed by inserting three tandem AP-1-binding sites in pRLnull plasmid (Promega), which can report the transactivity of AP-1 as previously reported (Luo Feijun et al., 2000) (12). Cyclin D1 promoter-luciferase reporter plasmid including promoter of the cyclin D1 gene was a kind gift from Dr. Strauss (Institute of Cancer, Biology Danish Cancer Society, Denmark).

Antibody

Rabbit-anti-human polyclonal antibody against cyclin D1 (CS1-4) was purchased from Santa Cruz Company. Mouse-anti-rabbit immunoglobulin G conjugated with horseradish peroxidase was purchased from Sino-American Biotech. Com., Beijing, China.

Cell Culture

Nasopharyngeal carcinoma cell line CNE1-LMP1 was established by Dr. Duan (Cancer Research Institute, Xiang-Ya School of Medicine) by introducing the expressing LMP1 plasmid of Epstein-Barr virus latent membrane protein 1 (LMP1) into nasopharyngeal carcinoma cell line CNE1. The cells were cultured in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FCS, 100U/ml penicillin, 100 μg/ml streptomycin, and 2 mmol/L L-glutamine and incubated at 37°C, 95% humidity, and 5% CO₂. All experiments were performed with exponentially growing cell cultures.

MTT Assay

The antiproliferative activity of tea polyphenols was determined by a colorimetric MTT assay. Briefly, serial concentrations (0, 25, 50, 100, 200 μg/mL) of tea polyphenols or EGCG were added to each well of the 96-well flat-bottomed plate containing 50ul of CNE1-LMP1 cells (10⁵cells/ml). Plates were incubated for 24h at 37°C, pulsed with 10 μl MTT (5mg/ml) and incubated for an additional 4h at 37°C. DMSO was added to all wells and mixed thoroughly to dissolve the dark blue crystal. The plates were read on a Dynatech EL309 Microlisa reader, using a test wavelength of 570nm and a reference wavelength of 450nm. Control well contained medium alone. Cell survival rate was calculated as...