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Review Article

Chemopreventive role of green tea in head and neck cancers

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ABSTRACT

In the era of personalized medicine, selecting the ideal treatment modality for head and neck cancer is becoming more complex. Also, despite the use of the newest agents, overall survival has not been improved notably over the past few decades. Currently, in accordance with the development of diagnostic tools, prevention and early detection of cancer are being emphasized more in obtaining better treatment outcomes. Among the various cancer preventative methods, the use of green tea is one of the most common approaches, and tea is known to be involved in multiple steps of carcinogenesis. Thus, in this short review, the protective roles of green tea components against the initiation, progression, and metastasis of head and neck malignancies will be discussed.

1. Introduction

Green tea, produced from the plant *Camellia sinensis*, is one of the most popular beverages in East Asian countries. Recently, due to multiple positive effects on various health conditions, the popularity of green tea has increased further. The components of green tea include proteins, carbohydrates, lipids, vitamins, caffeine, theophylline, carotenoids, and polyphenols. Among these, polyphenols are the most interesting constituent and have become the focus of much biomedical research.

The major polyphenols in green tea are catechins. The major catechins are epigallatocatechin-3-gallate (EGCG), (–)-epicatechin-3-gallate (ECG), (-)-epigallatocatechin (EGC), and (–)-epicatechin. Among these, EGCG is the key catechin, accounting for up to 80% of the total catechins; a cup of green tea contains ~300 mg.

A previous work has demonstrated diverse beneficial effects of EGCG in Parkinson's disease, Alzheimer's disease, diabetes, stroke, and even obesity.[1] In addition to these beneficial properties of EGCG, effects related to cancer prevention and treatment have been reported.

Because prevention and early detection improve the treatment outcome in every known cancer type, the concept of "chemoprevention" is becoming more significant in cancer research. Chemoprevention can be defined as the use of natural or synthetic substances to prevent or suppress the development, progression, and metastasis of cancer. A representative natural product used for chemoprevention of cancer is EGCG, which has been demonstrated to exert various positive effects on colon, breast, stomach, esophagus, lung, and prostate cancers and on melanoma.

Head and neck carcinoma, including thyroid cancer, is a common cancer that remains a significant cause of mortality

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and devastating morbidity. In the United States, over 48,000 new cases of this carcinoma are diagnosed annually, of whom more than 10,000 die. Most cases of head and neck cancer are treated by surgical intervention initially. Owing to the complexity of the anatomical structures in the head and neck area, complete and wide surgical extirpation of the disease can result in severe morbidity, in both functional and esthetical respects. Although medical and radiation therapies have been used widely to overcome and compensate for the weak points and morbidities related to surgery, these conventional treatment modalities have not improved the overall survival rate.

Thus, in accordance with the development of diagnostic technologies, early detection and prevention of carcinogenesis have been emphasized to improve the treatment outcome. That is, new approaches to cancer management, such as chemoprevention, should be considered and used to alter the overall survival rate. Several clinical applications and trials using natural products such as chemopreventive agents have demonstrated successful outcomes in terms of preventing cancer development in high-risk individuals. Here, we review recent research works on the effects of green tea and discuss future directions in head and neck cancer prevention.

2. Head and neck squamous cell carcinoma

Among the various cancers of the head and neck, squamous cell carcinoma (SCC) is the most common. As SCC development in this region is associated with different clinical courses and treatment outcomes according to the primary site, research outcomes regarding each site should be considered separately. In terms of the mechanisms of action of green tea components in head and neck SCC, induction of apoptosis and growth arrest and inhibition of metastasis are the two main research foci.

2.1. *Oral cavity and pharyngeal cancer*

Inducing the apoptotic process is a highly programmed protective mechanism that eliminates damaged cells from the body. Regarding carcinogenesis, cancer cells, which grow excessively regardless of homeostasis, should be eliminated by apoptosis. Thus, many previous studies have focused on enhancing apoptosis by treatment of the oral cavity cancer and pharyngeal SCC cell lines with green tea components.

The major catechin of green tea, EGCG, has been used in previous studies related to apoptosis induction.

The simplest work involves evaluation of cell viability and DNA synthesis levels in green-tea-component-treated oral squamous cancer cells (SCC-25).[2] Dose-dependent and direct cytotoxic effects of EGCG, ECG, and EGC have been shown. Inhibition of DNA replication was also detected after the treatment.

A cytotoxic effect of EGC in the human squamous cancer-2 cell line has been reported. The focus was on the intracellular reactive oxygen species, hydrogen peroxide, generation of which increased after EGC treatment compared with the levels in normal cells. This increased level of hydrogen peroxide caused degradation of nucleosomal DNA and induced caspase-3 activity, which accelerated cell death.[3,4]

With the exception of these two studies that focused on direct cytotoxic effects of green tea components, other research works on the relationship between green tea and oral cavity/pharyngeal SCC have focused on the modulation of cell signaling by green tea. Although cytotoxic effects of EGCG, EGC, and ECG have been reported, only EGCG has been found to exhibit signaling modulation activity.

Among signaling molecules, cyclin-dependent kinase and related molecules are the primary targets for EGCG. Expression of an apoptosis inhibitor and a cyclin-dependent kinase, p57, were elevated in normal keratinocytes in dose- and time-dependent manners, whereas the levels in the SCC25 and OSC2 oral carcinoma cell lines were consistent.[5] This expression pattern was correlated with the apoptosis status of treated cells, confirming the chemopreventive effect of EGCG. Although this report mentioned modulation of apoptosis by p57, the whole apoptotic cascade was not considered. Another molecule, p21WAF, which plays a role in cell growth, differentiation, and apoptosis, was upregulated in the oral carcinoma cell line, OSC2, after treatment with EGCG. Using a p21WAF siRNA, the EGCG-induced overexpression of this molecule was shown to enhance caspase 3-mediated apoptosis.[6]

Epidermal growth factor receptor (EGFR) is a plasma membrane protein that has an intrinsic tyrosine kinase activity. A cancer cell phenotype is known to result from the overexpression of this receptor. As in colon cancer cells, SCC of the head and neck frequently exhibits upregulation of EGFR expression.[7,8] EGCC treatment of SCC cells first results in the inhibition of EGFR phosphorylation. Afterward, downstream proteins, such as signal transducer and activator of transcription 3 (Stat3), and extracellular regulated kinase (ERK), are inhibited sequentially. Other proteins, such as basal and transforming growth factor-α-stimulated c-fos and cyclin D1, were also downregulated, with the consequent significant arrest of cell growth and proliferation.[8] Similarly, HER-2/neu receptor (HER-2), known to be associated with a poor prognosis in patients with breast carcinoma, was modulated by EGCG in head and neck SCC.[9] The working mechanism of action of EGCG against HER-2 was identical to that against EGFR. Accordingly, downstream proteins, such as Stat3, c-fos, cyclin D1, and Bcl-XL were inhibited in order. We also presented a novel target for chemoprevention using EGCG in head and neck SCC. Nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1) is a member of the transforming growth factor-β superfamily, known to be involved in inflammation, apoptosis, and carcinogenesis.[10] As the role and mechanism of NAG-1 in carcinogenesis are controversial, according to the primary site of the cancers, we focused on determining the role of this protein in SCC of the head and neck.[11–13] EGCG induced NAG-1 expression at the transcriptional level, which was directly related to EGCG-induced apoptosis in the KB cell line through caspase-3 activation. We also demonstrated that ataxia telangiectasia-mutated protein functions to activate NAG-1, p53, and p21, consequently facilitating apoptosis. Another strong point of our work was the *in vivo* result obtained using an immunocompetent syngeneic mouse model and intraperitoneal EGCG injection. The results being identical to those *in vitro* provided evidence for not only a new mechanism of head and neck SCC development, but also a promising target for new therapeutic agents.

In addition to the cytotoxic and proapoptotic effects of green tea in cancer chemoprevention, inhibition of invasion and metastasis is another research focus. Depending on the primary site of SCC in the head and neck, such as the oropharynx and hypopharynx, clinical manifestations and subjective symptoms in patients are frequently detected only in advanced stages of malignancy. Determination of the appropriate treatment strategy for advanced SCC, elimination of cancer cells, and protection against progression and metastasis should be performed in combination. Thus, the antimetastatic effects of EGCG may become an important area of future research. However, relatively few studies have addressed the antimetastatic or anti-invasive effects of EGCG in SCC of the head and neck.

Common targets for modulating metastasis and invasion in most cancers include matrix metalloproteinase (MMP) and urokinase-plasminogen activator.[14,15] Three previous reports on the role of EGCG in metastasis discuss MMP and urokinase-plasminogen activator. MMP-13, also known as collagenase-3, is known to degrade type IV collagen and various other extracellular matrix components. Owing to its proteolytic activity, MMP-13 has been assumed to play a role in cancer progression.[16] In the oral cavity cancer cell line, the expression level of MMP-13 was significantly higher than in noncancerous matched tissue and was downregulated by EGCG treatment. Additionally, the biological activity of MMP-13 was inhibited by EGCG treatment.[17]

Targets of EGCG were also identified upstream of MMP. The *RECK* gene, a tumor suppressor recognized as a negative regulator of MMP, was demethylated by EGCG treatment and consequently inhibited the expression of MMP-2 and MMP-9.[18] Due to this inhibition, both the number of invasion foci and the invasion depth were decreased in a three-dimensional collagen invasion model.[18] A different approach to determining the antimetastatic effect of EGCG in oral cavity cancer was reported by Chen et al.[19] They focused on the epithelial-to-mesenchymal transition, which is a critical process in epithelial-origin cancer metastasis. Expression of proteins related with epithelial-to-mesenchymal transition, such as p-focal adhesion kinase, p-Src, snail-1, and vimentin, were decreased markedly after treatment of the SCC-9 cell line with EGCG.

Our study of the antimetastatic effects of EGCG in the oral cavity cancer demonstrated that hepatocyte growth factor (HGF) and its receptor c-Met protein have a close relationship with cancer invasion.[20–22] Inhibiting HGF-induced phosphorylation of c-Met *in vitro* by EGCG decreased MMP-2 and MMP-9, which accounted for the antimetastatic effect. The downstream signal transduction pathway of c-Met was revealed, and the AKT and ERK proteins were found to be involved. Inhibition of these proteins was associated with activation of p38, JNK, caspase-3, and PARP. Translating this *in vitro* result into an *in vivo* system resulted in an identical outcome. Inhibition of HGF may be a therapeutic target for preventing oral cavity cancer cell metastasis and invasion.

Despite the evidence of the positive effects of green tea components, many are curious about the protective effect that can be achieved simply by drinking green tea. This was investigated in a large Japanese cohort study.[23] In total, 20,550 males and 29,671 females, aged 40–79 years, were included and observed for an average of 10.3 years. Prior to the analysis, age and other etiological factors associated with oral cavity and pharyngeal carcinogenesis were adjusted. Interestingly, only females who consumed green tea showed a reduced risk of cancer, whereas males apparently did not gain any advantage from its consumption. This result is inconsistent with much research data and reflects the need for further research, outcome accumulation, and clinical trials.

2.2. *Laryngeal cancers*

Compared with oral cavity and pharyngeal SCC, studies of laryngeal SCC are relatively rare. Specific studies on laryngeal cells were mentioned only in four articles, each of which had a different focus. Durgo et al[24] attempted to increase the sensitivity to cisplatin of the laryngeal cancer cell line, CK2, using EGCG and ECG. EGCG and ECG treatment caused DNA damage and lipid peroxidation in cancer cells.

Telomerase was another target in laryngeal cancer, which had not been investigated in oral cavity or pharyngeal cancers.[25] Most normal somatic cells have very low or no telomerase activity. Additionally, the telomerase holoenzyme, human telomerase reverse transcriptase (hTERT), is expressed in cancer cells, including laryngeal SCC. Inhibition of this enzyme protects against cancer cell proliferation and malignant transformation of normal cells.[26,27]

Treatment of a laryngeal SCC cell line with EGCG resulted in the inhibition of telomerase and hTERT activities, inducing apoptosis.[25] One common apoptotic pathway involves caspase-3 activation in cancer cells; this mechanism has been reported to be active in laryngeal SCC cells.[25] However, another article reported that EGCG-induced apoptosis in laryngeal SCC proceeded through a caspase-independent pathway, involving a p53-mediated mitochondrial pathway and nuclear translocation of apoptosis-inducing factor and endonuclease G.[28]

These seemingly contradictory results suggest the possibility of multiple effects of EGCG in cancer cell apoptosis; indeed, the mechanism may differ among cell types. As no single type of cancer is treated with a single therapeutic agent or modality, novel research on the combined usage of the natural products curcumin and catechin shows promise.[29] Treatment of HepG2 cells and laryngeal SCC with this combination exerted a greater cytotoxic effect than either agent alone.

2.3. *Thyroid cancers*

Thyroid cancer is a common malignancy of the head and neck area that can be treated successfully with surgery and radioiodine therapy. Most are well-differentiated carcinomas (papillary and follicular cancers), with favorable prognoses after initial treatment. The problem develops when they recur or metastasize. No relationship between green tea and well-differentiated thyroid cancer has been reported; in fact, the first *in vitro* research was reported only recently. This study used human thyroid carcinoma cell lines and focused on inhibition of metastasis and invasion.[30] EGCG inhibited the growth of papillary (FB-2) and follicular (WRO) cell lines by a mechanism involving cyclin D1, p21, and p53. Also, cellular migration of FB-2 was modulated by EGCG, in association with the downregulation of vimentin, E- and N-cadherin, and

alpha-5 integrin. That is, EGCG inhibited growth and metastasis of well-differentiated carcinoma cells. Anaplastic thyroid carcinoma is an uncommon pathology of the thyroid with a very poor prognosis, due in part to the lack of a definite treatment. Green tea may have an effect in this case, because EGCG was found to have proapoptotic effects in the report by Lim and Cha.[31] Apoptosis in anaplastic thyroid carcinoma cells was associated with inhibition of EGF, ERK1/2, JNK, and p38 phosphorylation; reduced cyclin B1/CDK1 expression; accumulation of sub-G1 cells; and caspase-3 pathway activation.

Similar to the oral cavity and pharyngeal SCC study mentioned above, a Japanese population-based cohort study reported the relation between green tea consumption and thyroid cancer risk.[32] In total, 48,802 males and 51,705 females, with ages ranging from 40 years to 69 years, were enrolled and observed for a median of 14.2 years. The development of thyroid malignancy was noted in 159 cases, but no significant association was found in general. However, a positive correlation was noted between premenopausal thyroid cancer risk and green tea consumption, whereas postmenopausal thyroid cancer risk showed an inverse association.

3. Future considerations and conclusions

Selecting the most appropriate treatment in head and neck SCC is a difficult task for clinicians because these tumors are usually detected at an advanced stage, resulting in poor outcomes. To overcome the limitations of current treatments, there is a continuing need for a therapeutic agent with high efficacy, safety, and minimal toxicity. Thus, natural dietary agents have become a focus in cancer research because they may hold particular promise. Among them, a green tea component, EGCG, may be the single most important agent, which has demonstrated positive effects in preventing and treating head and neck cancers. EGCC protects against diverse steps in cancer progression and invasion through multiple and complex signal transduction pathways. However, most of the research outcomes to date are derived from cell lines and so do not provide evidence of *in vivo* efficacy. Also, the differences between concentrations in plasma and in *in vitro* culture systems make the evaluation of the efficacy of EGCG problematic. Thus, progress is needed in the following three areas prior to considering the clinical application of green tea. First, further understanding of the signaling pathways and molecules is indispensable for the development of EGCG-derived therapeutic agents. Second, integration of *in vitro* outcomes, *in vivo* animal model studies, and human clinical trials is needed. As we have stated here, only a few animal studies and clinical trials on head and neck SCC treatment with EGCG have been carried out. Finally, after these two tasks are carried out, the formulation and contents of green tea components should be standardized prior to its clinical assessment.

Conflicts of interest

The authors declare no conflicts of interest.

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