学位論文内容の要旨

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学　位　論　文　題　名

Targeting tumor endothelial cells by EGCG causes anti-inflammatory and anti-thrombotic effects

（腫瘍血管内皮細胞を標的とした **EGCG** 投与による抗炎症・抗血栓効果）

キーワード（5つ）**Tumor endothelial cells，Angiogenesis，Tumor inflammation，ROS，Tumor thrombosis**

Angiogenesis is required for tumor progression and metastasis. Tumor cells secrete vascular endothelial growth factor (VEGF) to induce angiogenesis to provide oxygen and nutrients for tumor development. Dr. Judah Folkman proposed anti-angiogenic therapy in 1971 which has become a significant aspect of cancer treatment. Angiogenesis inhibitors (e.g., bevacizumab, a neutralizing antibody against VEGF) exert anti-tumor effects by disrupting tumor blood supply, normalizing vascular structural abnormalities caused by excess VEGF. However, VEGF targeting is non-specific as normal physiological angiogenesis in normal endothelial cells (NECs) also requires VEGF. We have reported that tumor endothelial cells (TECs) show different in terms of gene expression profile, pro-angiogenic properties, sensitivity to drugs and so on compared to NECs. It is crucial to explore novel approach to inhibit angiogenesis that specifically target TECs independently of VEGF signaling.

In recent years, it has been demonstrated that tumor-associated chronic inflammation promotes immunosuppression of the tumor microenvironment and tumor progression. The study indicates that TECs augment pro-inflammatory signaling and invasiveness in cancer cells. Leukocytes often aggregate around tumor blood vessels and stimulate angiogenesis and tumor metastasis. Numerous studies have confirmed that thrombosis is a common complication in cancer patients and is the second leading cause of cancer deaths. Inflammatory cytokines promote the procoagulant phenotype of ECs and promote platelet activation. This interrelationship between inflammation and thrombosis highlights the importance of studying both aspects in the context of cancer.

Epigallocatechin gallate (EGCG) is the most abundant polyphenolic compounds in green tea. EGCG has been reported as a natural antioxidant and anti-inflammatory agent in the treatment of cancer and multiple diseases. We previously reported that EGCG inhibited TEC proliferation and migration, but not NEC. However, the anti-inflammatory effect of EGCG on TEC is currently unclear. Since endothelial cells activation is one of trigger of thrombosis by upregulating procoagulant factors, we hypothesized that EGCG would exert an anti-inflammatory effect on TECs and act in anticoagulation.

First, we compared the expression of inflammatory cytokines (NF-α, IL-6 and IL-1β) between TECs and NECs and found a significant upregulation of these genes in TECs. To determine whether EGCG has anti-inflammatory effects on ECs, we performed qRT-PCR after EGCG treatment. The gene expression of TNF-α, IL-6 and IL-1β showed a significant decrease in TECs after EGCG treatment, whereas there were no significant changes in NECs. In addition, EGCG reduced the expression of those genes in LPS-stimulated NECs. These data suggested the anti-inflammatory effects of EGCG on inflammatory ECs including TECs.

ROS are key signaling molecules in the progression of inflammation. High levels of ROS are often associated with increased inflammatory signaling in tumors. Because EGCG has been previously demonstrated to exert its anti-inflammatory effects by scavenging ROS, we performed DHE staining to analyze ROS signals in TECs after EGCG treatment. The ROS levels were significantly decreased especially in TECs by EGCG treatment. In addition, the phosphorylation of NF-κB, a key molecule of

inflammatory genes, in TECs was inhibited by EGCG treatment. Moreover, the NF-κB inhibitor, BAY11-7082, decreased the expression of TNF-α, IL-1β, and IL-6 in TECs. These data suggested that EGCG, which acts as an antioxidant by scavenging ROS, leads to inhibit NF-κB activity, thereby downregulation of the inflammation-related genes in TECs.

To target TECs with EGCG in vivo, we established the nanodrug delivery system (DDS) with lipid nanoparticles of MEND (Multi-Enveloped Nanodevices: MEND). Binding of cyclo (Arg-Gly-Asp-D-Phe-Lys) (cRGD) to MEND resulted in the specific delivery of the content of the MEND to TEC, because the receptor for cRGD is an α V β 3 integrin, which is selectively highly expressed in TEC. We administered EGCG-MEND (10mg/kg/mice) via tail vein every three days in CT26 tumor-bearing mice after palpation of the tumors. Empty RGD-MEND with no EGCG was used as a control. Tumor growth was slightly inhibited in the EGCG-MEND group. Also, in comparison to the Ctr-MEND group, the EGCG-MEND group exhibited a significant reduction in angiogenesis. These data indicated that EGCG targeted delivery to tumor vasculature inhibit tumor angiogenesis. The anti-inflammatory effect of EGCG on TECs was analyzed by immunohistochemistry. The CD45-positive cells in CD31-positive perivascular areas were significantly reduced in the EGCG-MEND treatment group. Because it is reported that the inflammatory factors regulate extravasation of leukocytes to mediate their recruitment to sites of inflammation, the delivering EGCG to tumor vasculature may inhibited vascular inflammation with inhibition of CD45-positive leukocyte infiltration. Tumor inflammation is reported not only to induce a

procoagulant phenotype, but also stimulate neutrophils to release NETs, which is involved in thrombus formation. Because MPO is a representative component of NETs, we visualized MPO by immunohistochemistry. Interestingly, there was a trend towards a decrease in MPO-positive cells by EGCG treatment. Then, we next analyzed thrombus formation in the tumors with CD41 staining to visualize platelet aggregation. A significant reduction of CD41-positive area was observed in the EGCG-MEND group. These data suggest that targeting TECs by EGCG may inhibit thrombus formation by suppressing vascular inflammation.

Tumor immune escape is a major strategy for the survival and progression of cancer. Several studies have shown that in tumor tissues, the expression level of PD-L1, an immune checkpoint ligand, is closely related to the inflammatory state. We investigated the possible impact of EGCG on the regulation of the tumor immune microenvironment. The immunohistochemistry of PD-L1 showed that PD-L1 expression was significantly reduced in the EGCG-MEND group. In addition, the number of CD8-positive T cell in the tumors increased by EGCG treatment. These data suggest that EGCG may improves tumor immunity by suppressing inflammation.

Our data suggest that targeting tumor vasculature with EGCG may improve patient prognosis by providing anti-inflammatory and anti-thrombotic effects.

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