Combination of 4-HPR and EGCG induces apoptosis and inhibits angiogenesis and tumorigenesis of neuroblastoma SH-SY5Y and SK-N-DZ cells

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Abstract

Neuroblastomas are extracranial, malignant solid tumors in childhood with a dismal prognosis. N-(4-Hydroxyphenyl) retinamide (4-HPR) is a synthetic retinoid that induces apoptosis in tumor cells. (-)-Epigallocatechin-3-gallate (EGCG) is a potent antioxidant abundant in green tea with anticancer properties. In the present investigation, we have studied the combined effect of 4-HPR and EGCG to induce apoptosis and to inhibit angiogenesis and tumor progression of human neuroblastoma malignant SH-SY5Y and SK-N-DZ cells in vivo. The cells were treated with 1 μM 4-HPR, 10 μM EGCG, or combination of both and subjected to MTT assay and TUNEL staining. Western blotting was performed for molecules involved in both receptor and mitochondria mediated apoptotic pathways. Angiogenesis and subcutaneous tumor growth studies were performed in immunocompromised mice. Treatment with combination of 4-HPR and EGCG resulted in more than 70% apoptosis and 80% inhibition in cell proliferation in both cell lines. Apoptosis was associated with induction of control and mitochondria mediated apoptotic pathways. Our in vivo angiogenesis studies demonstrated significant inhibition of neovascularization after treatment with either agent alone and complete inhibition after treatment with combination of both agents. Studies also demonstrated marked suppression of subcutaneous tumorigenesis and solid tumor formation after treatment with combination of 4-HPR and EGCG. In conclusion, our studies demonstrated that treatment with combination of 4-HPR and EGCG effectively induced apoptosis and inhibited cell proliferation, angiogenesis, and growth of neuroblastomas in vivo. Therefore, the combination of 4-HPR and EGCG could be used as a potential therapeutic regimen for effectively controlling the growth of human malignant neuroblastomas. This investigation was supported in part by the R01 grants (NS-57811 and CA-91460) from the National Institutes of Health (Bethesda, MD).

Introduction

Neuroblastomas are the most common extracranial, malignant solid tumors in childhood with a dismal prognosis. Neuroblastoma originates from immature neuroblast cells of the peripheral (sensory) nervous system and usually arises in a parasagittal position in the abdomen or chest. It generally occurs in infants and very young children and is rarely present in children older than 10 years of age. About 50% of neuroblastomas tumors occur in children younger than two years old and 75% occur in children less than 4 years old. The etiology of neuroblastoma is unknown, but it seems unlikely that environmental factors are involved. A subset of patients inherits a genetic predisposition to neuroblastoma, which is mapped to the short arm of chromosome 16, and these patients usually have multifocal primary tumors that arise at an early age. Neuroblastoma accounts for 8-10% of pediatric cancers and 15% of the deaths attributable to malignant tumors in children. In most cases, neuroblastomas have already metastasized outside of the original site at the time of diagnosis. Toxic and severe side effects are significant in the current treatment regimens, and there is little room to modify the present therapy. Alternative treatment strategies with no toxicity and minimal side effects are therefore necessary to improve the survival rate.

Epigallocatechin-3-gallate (EGCG) is a potent anti-oxidant abundant in green tea. EGCG blocks the activation of EGF and HER-2 receptors. EGCG also inhibits telomerase and DNA methylationtransferase, the enzymes involved in tumor cell immortality. EGCG has been shown to bind and inhibit Bcl-xL, the anti-apoptotic protein involved in tumor cell survival and promotes tumor cell invasion. There is increasing evidence to show the anti-tumor properties of EGCG against glioblastoma, prostate, cervical, and bladder cancers. However, the effects of EGCG on neuroblastoma have not been investigated.

N-(4-Hydroxyphenyl) retinamide (4-HPR) is a synthetic retinoid that induces apoptosis in tumor cells. It is observed that 4-HPR increases reactive oxygen species (ROS), decreases mitochondrial membrane potential and induces apoptosis in several neuroblastoma cell lines. In the present investigation, we examined the combination effect of EGCG and 4-HPR to induce apoptosis and to inhibit angiogenesis and tumor progression in SH-SY5Y and SK-N-DZ neuroblastoma cells.

Conclusions

• Combination treatment with EGCG and 4-HPR resulted in decreased cell viability of both SH-SY5Y and SK-N-DZ neuroblastoma cells.

• Combination treatment with EGCG and 4-HPR resulted in apoptosis in more than 80% SH-SY5Y and SK-N-DZ neuroblastoma cells.

• Combination treatment with EGCG and 4-HPR resulted in complete inhibition of neuroblastoma.

• Simultaneous administration of EGCG and 4-HPR resulted in significant reduction of subcutaneous tumorigenesis in nude mice.

• Combination treatment with EGCG and 4-HPR resulted in marked reduction of solid tumor development in the subcutaneous region of nude mice.

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