学 位 論 文 の 要 旨

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学 位 論 文 名　Inhibition of Small‐cell Lung Cancer Angiogenesis by Irinotecan Metronomic Chemotherapy and Irinotecan Plus Everolimus

発 表 雑 誌 名　Anticancer Research
(45, 5287-5297, 2025)
(10.21873/anticanres.17868)

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論 文 内 容 の 要 旨

INTRODUCTION

Small-cell lung cancer (SCLC) accounts for approximately 10–15% of all lung cancers and is characterized by rapid growth, early dissemination, and poor prognosis. Despite recent advances in immunotherapy and molecular-targeted agents for non-small-cell lung cancer, therapeutic progress in SCLC has been limited. Although immune checkpoint inhibitors have modestly improved survival in extensive-stage disease, there are currently no established predictive biomarkers or effective targeted therapies for SCLC, particularly after second-line treatment failure. Therefore, the development of novel therapeutic strategies remains an unmet clinical need.

Angiogenesis plays a critical role in SCLC progression, and high expression of vascular endothelial growth factor (VEGF) has been associated with poor outcomes. However, anti-VEGF strategies such as bevacizumab have failed to demonstrate convincing benefit in SCLC, suggesting the involvement of VEGF-independent angiogenic pathways. Metronomic chemotherapy, defined as the frequent administration of low-dose cytotoxic agents without prolonged drug-free intervals, has emerged as a strategy to suppress tumor angiogenesis while minimizing systemic toxicity. This approach is thought to modulate the tumor microenvironment through anti-angiogenic, immunomodulatory, and stromal effects rather than direct tumor cell cytotoxicity alone.

Irinotecan (CPT-11), a topoisomerase I inhibitor widely used in SCLC, has shown potential anti-angiogenic effects when administered in metronomic schedules in other malignancies. Everolimus, an mTOR inhibitor, is known to suppress angiogenesis by inhibiting the STAT3/HIF-1α/VEGF signaling pathway. Although everolimus alone has demonstrated limited efficacy in SCLC, preclinical studies suggest possible synergistic effects when combined with other agents. In this study, we investigated the anti-angiogenic and antitumor effects of metronomic irinotecan and irinotecan combined with everolimus using in vitro assays and an orthotopic mouse model of SCLC.

MATERIALS AND METHODS

Human SCLC cell lines (N417, H82, and H187) were used to evaluate cytotoxicity and angiogenic signaling. Cell viability was assessed using a WST-8 assay following exposure to SN38 (the active metabolite of irinotecan) and everolimus. To assess angiogenic signaling, VEGFA mRNA expression levels were quantified by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

For in vivo analysis, an orthotopic human SCLC xenograft model was established by injecting N417 cells into the left lung of immunodeficient nude mice. Two experimental protocols were employed. The first assessed tumor-reducing effects of weekly irinotecan, daily low-dose (metronomic) irinotecan, and everolimus monotherapy. The second focused on early effects on tumor angiogenesis and apoptosis, comparing weekly irinotecan, daily irinotecan, and weekly irinotecan combined with everolimus, while maintaining equivalent cumulative dose intensity.

Tumor tissues were analyzed by immunohistochemistry for CD31 to quantify microvessel density as an indicator of angiogenesis. Apoptosis was assessed using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining combined with immunofluorescence imaging. Statistical analyses were performed using unpaired t-tests or one-way analysis of variance, with statistical significance defined as $p<0.05$.

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULT AND DISCUSSION

In vitro experiments demonstrated that SN38 induced concentration-dependent cytotoxicity in all SCLC cell lines, whereas everolimus showed limited direct cytotoxic effects. Combined treatment with SN38 and everolimus did not enhance or inhibit cytotoxicity relative to SN38 alone,

suggesting minimal synergistic effects on tumor cell viability. Analysis of VEGFA mRNA expression revealed no significant induction or suppression following treatment with irinotecan or everolimus, indicating that observed anti-angiogenic effects may occur independently of VEGF transcriptional regulation.

In the orthotopic mouse model, daily administration of low-dose irinotecan resulted in the most pronounced reduction in tumor burden compared with weekly irinotecan or everolimus monotherapy, despite equivalent cumulative doses. This finding highlights the importance of treatment scheduling in SCLC. In the second in vivo study, metronomic irinotecan demonstrated a consistent reduction in tumor neovascularization, as assessed by CD31 staining, compared with controls. Importantly, the addition of everolimus did not further suppress angiogenesis, suggesting that irinotecan-mediated anti-angiogenic effects are largely independent of mTOR inhibition.

Interestingly, TUNEL staining revealed increased tumor apoptosis only in the combination group treated with irinotecan plus everolimus. This suggests that everolimus may contribute primarily through enhancement of apoptotic signaling rather than direct inhibition of angiogenesis. Notably, vascular endothelial cells did not show increased apoptosis, supporting the concept that anti-angiogenic effects preceded overt tumor cell death.

Collectively, these findings suggest that metronomic irinotecan suppresses tumor growth primarily through early inhibition of angiogenesis, potentially via VEGF-independent mechanisms such as modulation of endothelial progenitor cells or alterations in the tumor microenvironment. The temporal dissociation between angiogenesis suppression and tumor apoptosis supports the hypothesis that vascular targeting is a critical initial step in the therapeutic effect of metronomic chemotherapy.

CONCLUSION

This study demonstrates that metronomic low-dose irinotecan exerts significant antitumor effects in SCLC, largely through suppression of tumor angiogenesis independent of VEGF signaling. While everolimus did not enhance anti-angiogenic efficacy, its combination with irinotecan promoted tumor apoptosis, suggesting complementary but distinct mechanisms of action. These results underscore the importance of treatment scheduling and support further investigation of metronomic irinotecan as a potential second-line therapeutic strategy for SCLC. Importantly, this approach may provide clinically meaningful benefits with reduced toxicity, offering a biologically rational option for a disease with limited therapeutic alternatives.