Mechanism of inhibition of peritoneal angiogenesis by Butyrate in EAT bearing mice is mediated by Sp1 transcription factor

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Introduction: Ehrlich ascites tumor cells, during their twelve day growth period in the peritoneum of mice produce ascites with extensive peritoneal angiogenesis. We have shown that butyrate represses VEGF/KDR gene expression system in EAT cells in vitro (1). Butyrate is currently being evaluated as an antineoplastic therapeutic agent (2). The inhibitory role of butyric acid against tumor growth is mediated through a direct effect on tumor cells that results in cell cycle arrest, differentiation or apoptosis. Butyrate induced apoptosis in EAT cells is shown to involve caspase –3 and activation of CAD (3). Repression of VEGF gene expression or in vivo antiangiogenesis by butyrate is shown to be mediated by dephosphorylation of Sp1 and thereby decreased capacity binding to VEGF promoter site.

Methods:
Butyrate induced expression of phosphoprotein phosphatase was verified by immunoflorescence and ELISA. Using specific primers for mouse tyrosine phosphatase, butyrate induced phosphatase was PCR cloned. Binding of transcription factor to proximal promoter region of VEGF gene was done by EMSA. Transient transfection by calcium phosphate precipitation method followed by VEGF promoter
luciferase reporter analysis was done to verify the regulation of VEGF gene expression by butyric acid.

**Results:**
The results presented show that butyrate induces expression of phosphoprotein phosphatase in EAT cells. The PCR cloned phosphatase from butyrate treated EAT cells showed sequence similarity with tyrosine phosphatase. In butyrate treated EAT cells there was no binding of Sp1 transcription factor to the proximal promoter region of VEGF gene. Sp1 in the nuclear extract of EAT cells treated with butyrate and inhibitor of tyrosine phosphatase did bind to the proximal promoter region of VEGF gene. Further, transient transfection assays using VEGF promoter luciferase reporter constructs indicated that the butyrate down regulates VEGF gene expression in EAT cells. Taken together, these results clearly indicate that the antiangiogenic effect of butyric acid *in vivo* involves dephosphorylation pathway and Sp1 transcription factor to down regulate VEGF gene expression.

**References:**