EPITHEORESE KLINIKES FARMAKOLOGIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 89 (1999)

On the Mechanism of Tumor-Promoting Effect of Thrombin: Potentiation of Angiogenic and Metastatic Phenotype of Endothelial and Prostate Cancer Cells

N.E. Tsopanoglou, P. Andriopoulou and M.E. Maragoudakis

Department of Pharmacology, University of Patras, Patras, Greece

INTRODUCTION

A relation between blood coagulation and cancer growth and metastasis was first observed by Trousseau in 1872. Many investigators have verified that a systemic activation of blood coagulation is often in cancer patients. In addition, it has been shown that tumor cells interact with platelets, leucocytes and endothelial cells as well as thrombin and plasmin generating systems, all of which influence clot formation. In a recent clinical study it has been shown that primary thromboembolism increases 3-fold the risk of overt cancer diagnosis within the next 6-12 months after thrombosis. These clinical observations are in line with animal experiments, where thrombin treatment increases dramatically the number of lung metastasis in rats. More recently, it has been shown that the metastatic ability of human breast cancer cells is correlated with the number of thrombin receptors on these cells.

Although the involvement of thrombin in tumor growth and metastasis is well documented, the cellular and molecular mechanisms involved are unknown.

We have previously shown that thrombin and thrombin mimetic peptides (TRAP) promote angiogenesis, an essential step of tumor growth and metastasis, by activating several steps of the angiogenic cascade. In this report we explored the cellular and molecular mechanisms involved in the action of thrombin on endothelial and tumor cells from a metastatic prostate carcinoma (PC-3).

METHODS AND RESULTS

A) In endothelial cells thrombin and thrombin receptor activator peptide (TRAP) upregulates vascular endothelial growth factor (VEGF) receptors (KDR and flt-1) (7). This was shown by RT-PCR technique and Western blot analysis. In addition thrombin treatment of endothelial cells increases the secretion of gelatinase 72 KDa and the activated form of 64KDa (MMP-2) and decreases the adhesion of endothelial cells to extracellular matrix.

a) In PC-3 cancer cells thrombin treatment causes a time and dose-dependent upregulation of mRNA for VEGF as shown by RT-PCR technique. This increase in VEGF mRNA is accompanied by an increase in protein synthesis. Results obtained from ELISA and Western blot analysis show that the amount of VEGF protein released in culture medium is significantly increased in thrombin-treated PC-3 cells as compared to untreated cells.

b) Preliminary experiments indicate a significant upregulation of mRNA for β_3 subunit of $\alpha_v\beta_3$ integrin in PC-3 cells after exposure to thrombin for 4 hours. Similarly, the results from immunoprecipitation experiments and Western blot analysis show an increase in $\alpha_v\beta_3$ protein.

c) Zymography analysis of conditioned media from PC-3 cells on gelatin substrate gels show the presence of basal activity at 92 KDa corresponding to MMP-9. Exposure of PC-3 to thrombin increases the secretion of 92 KDa in a dose-dependent manner.

CONCLUSION

These findings provide further insight for the mechanisms by which thrombin promotes tumor progression and metastasis. VEGF is considered the key angiogenic factor which is secreted by cancer cells and is correlated with the metastatic potential. The $\alpha_v\beta_3$ integrin is only expressed in angiogenic endothelial cells and is present in metastatic tumor cells. MMPs are considered essential for the local dissolution of basement membrane and extracellural matrix, which is required for angiogenesis and metastasis.