The inhibition of lung metastasis by inactivation of coagulation activity may lead to dissemination of tumor cells.

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INTRODUCTION:
Many tumor cells such as lung cancer cells, melanoma cells and osteosarcoma cells elicit procoagulant activity by transmembrane tissue factor, which activates factor X with factor VII, leading to the generation of thrombin and fibrin. It is well documented thrombin stimulated tumor cell mitogenesis [1] and further tumor cell adhesion to platelets [2, 3] endothelial cells [4] and extracellular matrix proteins [3]. The cooperation of these thrombin functions contributes to the experimental finding that thrombin-pretreated B16 tumor cells showed up to 156-fold increase in metastases in vivo [2]. Furthermore, the development of lung metastasis was strongly diminished in fibrinogen-deficient mice [5]. As shown above, there are considerable evidences that coagulation factors such as thrombin and fibrin play a critical role in tumor metastasis.

Warfarin, a major oral anticoagulant agent, inactivates blood coagulation activity by interfering with the hepatic synthesis of vitamin K dependent clotting factors II, VII, IX, and X. We previously reported that warfarin treatment significantly reduced lung metastases in vivo. However, the anti-metastatic effect of anticoagulant agent on various organs has been still unknown.

The purpose of this study was to survey the number and location of tumor metastases in various organs and to clarify the relation of metastasis between lung and other organs in vivo.

METHODS:
Warfarin administration: Warfarin potassium was provided from Eisai Co., Ltd. Six-week-old female C57BL/6 mice were given warfarin solution (0, 200, 250 or 300μg of warfarin/100ml of water).

Measurement of PT (prothrombin time) and INR (international normalized ratio): Four days after warfarin administration, 360μl of blood was taken from the heart with a syringe containing 40μl of 3.8% sodium citrate under diethyl ether anesthesia, and plasma was separated by centrifugation. PT and INR were measured using Thromboplastin C Plus and CA-50 (SYSMEX).

Tumor Cell injection into tail vein: Four days after warfarin treatment, B16F10 (5.5x10⁶) was injected into tail vein. Three weeks after warfarin administration, mice were sacrificed. The number of metastatic foci was counted in lung or whole body.

Statistical Analysis: ANOVA and Fisher’s PLSD as a post hoc test, or the Mann-Whitney test.

RESULTS:
Lung metastasis: Warfarin administration resulted in a significant prolongation of PT and INR in a dose-dependent manner (INR: 0.85 at control, 3.04 at 200μg/100ml, 5.81 at 250 μg/100ml and 9.52 at 300 μg/100ml of warfarin) (data not shown). Warfarin reduced lung metastasis in a dose dependent manner (-58.8% at 200μg/100ml, -89.3% at 250μg/100ml, -98.6% at 300 μg/100ml vs. control, P<0.001) (Fig1). The treatment of 300μg/100ml of warfarin significantly reduced lung metastases. However, metastases were seen in various organs except lung in warfarin treated group (Wa, Wa+2D) (Fig2). The main location of metastases was subcutaneous, fat, retroperitoneum, mesentery and ovary (data not shown). Compared with lung and various organs such as subcutaneous, fat, retroperitoneum, mesentery and ovary, the number of metastases in various organs except lung was increased in warfarin treated group without significant difference (Fig3).

Survey of the location of metastases
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DISCUSSION:
In the previous study, we reported that it was succeeded to make a continuous low coagulation activity model by warfarin in mice. In this study, lung metastasis was significantly decreased by warfarin in a dose dependent manner. At the highest dose of warfarin (300μg/100ml), lung metastasis was drastically inhibited (-98.6% vs. control). These results indicated that coagulation factors were critical and integrant factors for tumor cells to metastasize to lung in this model. Additionally, Palumbo reported that the number of tumor cells in the lung in the fibrinogen-deficient mice was decreasing from 4 hours after tumor cell injection and at 24 hours the number of tumor cells in those mice was 4 times less than in control mice. These denoted that fibrin plays an important role in sustained adhesion of tumor cells within the lung [5]. Therefore, it is speculated that tumor cells go through lung without coagulation activity.

In this study, we demonstrated that metastases in various organs except lung were increased while lung metastases were decreased in warfarin treated group. Inhibition of lung metastases may disseminate tumor cells to whole body and increase metastases in various organs. However, the involvement of organs as a soil in tumor grown is still unknown.

The treatment of lung metastases by anticoagulant agent has a risk of dissemination of tumor cells. Any agents to inhibit adhesion of tumor cells are thought to have a same risk. A wide spectral inhibition of adhesion mechanism is needed. This needs further study.

REFERENCES: