Pathogenesis of cancer-associated thrombosis

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Take Home Messages
- Risk assessment scores are used to stratify ambulatory cancer patients for their risk of VTE.
- Different cancer-types have different rates of VTE.
- Common and cancer-type specific mechanisms may contribute to cancer-associated thrombosis.

Introduction
Cancer patients have an increased incidence of both venous thromboembolism (VTE) (4-20%) and arterial thrombosis (2-5%) compared with the general population.1,2 This is commonly referred to as cancer-associated thrombosis (CAT). One study reported that thromboembolism and infection were the second leading causes of death (9.2% each) after cancer progression (70.9%).3 Cancer patients with VTE have reduced survival compared with patients without VTE.4 Risk factors include traditional risk factors for VTE as well as cancer-specific risk factors, such as chemotherapy. A better understanding of cancer-associated thrombosis may lead to improved therapies.

Risk factors and risk assessment scores for cancer patients
Risk factors for VTE in cancer patients can be divided in general risk factors (history of VTE, age, immobilization and obesity) and cancer-specific risk factors (tumor site and stage and treatment).5 Assessment scores are used to stratify ambulatory cancer patients in terms of their risk of VTE. The Khorana score was generated using the database from the “Awareness of Neutropenia in Chemotherapy Study Group” Registry.6 The score includes 5 variables: site of cancer, use of erythropoiesis stimulating agents, pre-chemotherapy levels of hemoglobin, platelet count and leukocyte count. It showed that cancer patients could be divided into low risk (0.3-0.8%), intermediate risk (1.8-2.0%) and high risk (6.7-7.1%) for VTE. The Vienna score added brain cancer as a high risk cancer and the biomarkers D-dimer and soluble P-selectin to the Khorana score.7 A recent study found that a combination of one clinical factor (tumor site) and one biomarker (D-dimer) predicts VTE in cancer patients.8 Importantly, it re-classified a significant number of patients that had been assessed using the Khorana score. In addition, due to the low accuracy of the Khorana score for patients with ovarian, lung and colon cancer patients, Gerotziafas and colleagues developed the COMPASS-CAT score for ovarian, lung, colon and breast cancer patients.9 This score contains 8 variables that can be divided into general risk factors (presence of cardiovascular risk factors [5 points], hospitalization for medical illness [5 points], history of VTE [1 points], platelet count [2 points]) and cancer-specific risk factors (anthracycline or anti-hormonal therapy [6 points], time since cancer diagnosis ≤6 months [4 points], central venous catheter [3 points], advanced stage of cancer [2 points]). Patients were divided into two groups; those with a score of 0-6 points had a low/intermediate risk of VTE (1.7%) whereas those with a score of ≥7 had a high risk of VTE (13.3%).

Cancer-type specific mechanisms of VTE
Rates of VTE vary amongst patients with different types of cancer.10 For instance, pancreatic, brain, ovarian, stomach, gynecologic and hematologic cancer patients have a high risk of VTE, colon and lung have an intermediate risk of VTE, and breast and prostate have a low risk of VTE. This suggests that there are cancer-specific mechanisms of VTE. To date, the majority of studies on cancer-associated thrombosis have used pooled patient populations with different cancer types. The advantage of this approach is that relatively large numbers of patients can be analyzed. However, the disadvantage of using pooled cancer patient groups is that it assumes that VTE in different cancer types is driven by the same or similar mechanisms. This might not be true. For instance, we have found that elevated levels of microvesicle (MV) tissue factor (TF) activity are associated with VTE in pancreatic cancer patients but is not associated with VTE in colorectal, lung, brain and ovarian cancer.11 Other studies have measure levels of podoplanin in brain cancer.12,13 Podoplanin is a ligand for the platelet receptor C-type lectin receptor.14 The first study found that brain cancer patients with low platelet counts and high plasma P-selectin levels had a high risk of VTE.12 A follow-up study showed that podoplanin expression by tumor cells activated platelets suggesting that tumor-derived podoplanin activated platelets and may contribute to VTE in these patients.13 Finally, neutrophil extracellular traps (NETs) may contribute to CAT.15 Indeed, a recent study showed that elevated plasma levels of the NETs biomarker citrullinated histone H3 (H3Citr) was predictive of VTE in lung and pancreatic cancer patients.16 These studies suggest that it is
better to study the mechanism of CAT in each cancer type independently.

**Mouse models of cancer-associated thrombosis**

Clinical studies can identify patient characteristics and biomarkers that are associated with VTE in cancer patients. However, these studies cannot directly analyze the role of a given factor in cancer-associated thrombosis. Mouse models have been used to investigate mechanisms of cancer-associated thrombosis. Different mouse strains, tumors, tumor sires and thrombosis models have been used. C57BL/6 and BALB/c mice are most commonly used immunocompetent strains for allograft models with murine cancer cells. Nude mice are the most common immunodeficient strain used for xenograft models with human cancer cells.

The majority of the studies using mouse models have used pancreatic cancer cells because pancreatic cancer is associated with high rates of VTE. The Panc02 cell line was derived from a tumor formed in the pancreas of C57BL/6 mice and expresses high levels of TF. We have observed an association between levels of MV TF activity and VTE in pancreatic cancer patients. Several studies have shown that human pancreatic tumors grown in mice release TF+ MVs into the circulation. Mice bearing subcutaneous Panc02 tumors exhibited increased thrombosis compared with controls. We used a human pancreatic cell line called BxPc-3. We found that nude mice with orthotopic BxPc-3 tumors have larger venous thrombi compared with controls. Importantly, inhibition of TF+ MV derived from the tumor decreased the size of the thrombus, indicating a direct role for TF in this model of cancer-associated thrombosis (Figure 1). NETs are released by activated neutrophils as part of the host defense to kill bacteria. NETs contain granule proteins and chromatin and have been found in both arterial and venous thrombi. The murine mammary cancer line 4T1 was used to determine the role of NETs in cancer-associated thrombosis. Mice bearing orthotopic tumors had elevated level of plasma granulocyte colony-stimulating factor, neutrophils and H3Cit and a prothrombotic state compared with controls. Another study found that the presence of 4T1 tumors increased arterial and venous thrombosis in mice bearing 4T1 tumors. We have observed increased levels of granulocyte colony-stimulating factor and neutrophils in mice bearing human pancreatic tumors (BxPc-3) and increased numbers of neutrophils in thrombi from tumor-bearing mice compared to thrombi from control mice (unpublished data). Taken together, these studies suggest that NETs may contribute to VTE in cancer patients.

**Figure 1. Mechanisms of venous thrombosis in a mouse model of pancreatic cancer.** Tumors release tissue factor (TF)-positive microvesicles (MV) into the circulation that trigger venous thrombosis. Tumors also increase levels of granulocyte colony-stimulating factor that increases levels of circulating neutrophils and neutrophil extracellular traps (NETs) that increase thrombosis.

**Future perspectives**

Further studies are needed to determine the common and cancer type-specific mechanism of CAT. These studies may identify cancer type-specific biomarkers that could be used to develop cancer type-specific risk assessment scores.

**Acknowledgments**

I would like to thank Dr. Yohei Hisada who helped preparing the manuscript.

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