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Full Length Article

## Mouse models of cancer-associated thrombosis

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## ABSTRACT

Cancer patients have an increased risk of venous thromboembolism (VTE) compared with the general population. Mouse models are used to better understand the mechanisms of cancer-associated thrombosis. Several mouse models of cancer-associated thrombosis have been developed that use different mouse strains, tumors, tumor sites and thrombosis models. In this review, we summarize these different models. These models have been used to determine the role of different pathways in cancer-associated thrombosis. For instance, they have revealed roles for tumor-derived tissue factor-positive microvesicles and neutrophil extracellular traps in thrombosis in tumor-bearing mice. A better understanding of the mechanisms of cancer-associated thrombosis may allow the development of new therapies to reduce thrombosis in cancer patients.

### 1. Introduction

Cancer patients have an increased risk of venous thromboembolism (VTE) compared with the general population. Biomarkers studies have identified a number of distinct pro-coagulant pathways activated in cancer patients that may contribute to VTE [1]. Importantly, the incidence of VTE varies in different types of cancer [2–5]. This suggests that there may be cancer site-specific mechanisms of VTE [6]. However, the underlying mechanisms leading to VTE in each type of cancer have not been elucidated.

Mouse models are used to study mechanisms of cancer-associated thrombosis. A number of different models have been developed that vary by mouse strains, cancer-types and thrombosis models employed. This makes direct comparisons difficult. Here, we review the different models and the studies on the role of various pathways in cancer-associated thrombosis.

### 2. Choice of mouse strain and cancer cell

The strains of mice used to study cancer-associated thrombosis are largely dictated by the type of cancer-cells used. Immunocompetent mouse strains, most commonly C57BL/6 and BALB/c, are used to generate allograft models with murine cancer cells. It is important that the murine cancer cell line is compatible with the strain of mice. The major strength of immunocompetent models is that the host is able to mount a full immune response to the tumor that may contribute to thrombosis. In addition, one can analyze the role of a given host protein

using knockout mice. However, there are a limited number of murine cancer cell lines available. The three most commonly used murine cell lines for studies on cancer-associated thrombosis are 4T1, Lewis lung carcinoma (LLC) and Panc02. The 4T1 cell line is a thioguanine-resistant derivative of the 410.4 line that was isolated from a spontaneous mammary tumor in BALB/cf3H mice [7]. The LLC cell line was isolated from a spontaneous carcinoma in the lung of a C57BL/6 mouse [8]. The Panc02 cell line was derived from a tumor formed in the pancreas of a C57BL/6 mouse treated with 3-methyl-chloronothrene [9]. Genetically engineered mice, such as the *LSL-Kras<sup>G12D/+</sup>*; *LSL-Trp53<sup>R172H/+</sup>*; *Pdx-1-Cre* (KPC) model, develop primary tumors within the pancreas that look and behave like human pancreatic cancer [10]. The KPC model was developed using C57BL/6 mice. Three cell lines (KPC 4684, KPC 4112 and KPC 4580P) have been established from KPC mouse tumors that have been engineered to express luciferase under a *Pdx-1-Cre* promoter (Dr. J-J Yeh, unpublished data). We are currently comparing these cell lines to Panc02.

Immunodeficient mice are used to generate xenograft models. Immunodeficient mice include nude mice that lack T-cells, severe combined immunodeficient (SCID) mice that lack T and B cells, and non-obese diabetic (NOD)/SCID mice that lack T and B-cells, complement and have reduced NK cell activity. Nude mice are most often used. In these models, tumors are generated using human cancer cell lines or patient-derived xenografts (PDXs). The strength of using immunodeficient mice is the large variety of human cancer cell lines available. We and others have evaluated tissue factor (TF) expression in a variety of human colorectal and pancreatic cells lines. Interestingly,

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colorectal cancer cell lines with mutations in both *K-ras* and *p53*, such as the HCT116 subline 379.2, express higher levels of TF than those with mutations in only *K-ras*, such as HCT116 [11]. There is also a wide range of TF expression in the different human pancreatic cell lines; HPAF-II, HPAC and BxPc-3 express high levels of TF, L3.6pl express medium levels, PANC-1 expresses low levels and MIAPaCa-2 do not express TF [12–14]. The A549 epithelial-like lung cancer cell line was isolated from human cancerous lung tissue [15,16]. A weakness of using immunodeficient mice is that they are not able to mount a full immune response to the tumor.

Cancer cell lines may have a high or low metastatic potential in mice. It is simpler to use cancer cell lines that have a low metastatic potential for cancer-associated thrombosis studies because the tumor-burden is proportional to the size of the primary tumor and is not affected by secondary metastasis. Highly metastatic tumors may also increase the death of mice (Hisada Y unpublished data).

Human and mouse cancer cell lines are easy to grow in culture. However, the gene expression pattern of these lines may be altered by maintaining the cells in culture. PDXs are considered superior to cancer cell lines because they maintain the pathology [17,18], gene expression pattern [19] and single nucleotide polymorphisms [20] of primary tumors. PDXs are established by transferring them directly from the patient into mice and then maintained in immunodeficient mice [21]. Success rates of PDXs are variable (23–75%) and it can take 2–12 months to establish the different lines depending on the type of tumor [22]. The fundamental genotypic features of PDXs do not change over several passages in mice [23]. However, PDXs lose the gene expression profiles of primary tumors once the cells are cultured in vitro and these changes are irreversible [24]. The disadvantages of PDXs are that they must be maintained in mice, and it is difficult to transfer them between institutions [22].

### 3. Tumor site

There are two choices of site: subcutaneous or orthotopic (Tables 1 and 2). Subcutaneous tumors are typically implanted into the back or the right flank of mice between the dermis and underlying muscle. Matrigel is often used to support the establishment of the tumor. The subcutaneous tumor models are widely used because of the ease with

which cells can be implanted and tumor size can be measured using calipers. The weakness of this model is that it does not reproduce the tumor microenvironment in patients [25]. In addition, tumors grown subcutaneously rarely metastasize even if the original tumor is metastatic [25].

Orthotopic tumors are more clinically relevant because the tumor microenvironment more closely resembles that found in patients [25]. Importantly, orthotopic tumors retain their capacity to metastasize. A disadvantage of this model is that it is more difficult to monitor tumor growth. This is important when performing thrombosis experiments because a defined size of tumor is required to generate consistent results. To monitor tumor growth reporter genes, such as luciferase or green fluorescence protein (GFP), are introduced into the cell lines to allow non-invasive assessment of tumor size using luminescence or fluorescence molecular tomography. However, we have found that mice bearing orthotopic tumors are more prone to spontaneous or surgery-related death compared with mice bearing subcutaneous tumors (Hisada Y and Cooley BC, unpublished data).

As mentioned above, genetically engineered mice form spontaneous orthotopic tumors and are commercially available for most types of cancer. These models are more clinically relevant than xenograft models because they contain oncogenic mutations that occur in tumors in patients. They also mimic many features of tumor progression, such as angiogenesis, acquisition of secondary mutations and metastasis [26]. The disadvantage of genetically engineered mouse models is that tumor development is variable (typically 3 months or more), which means that it not practical to compare the effect of similarly sized tumors on thrombosis.

### 4. Thrombosis models

There are several thrombosis models that have been used to study cancer-associated thrombosis in mice (Tables 1 and 2). The most popular are those involving the infrarenal vena cava (IVC). An advantage of the IVC is that high frequency ultrasonography can be used for non-invasive longitudinal monitoring of thrombus formation [14,27,28]. Clot formation is typically analyzed between 0 and 48 h.

The IVC stasis model involves complete ligation of the IVC and side branches using non-reactive sutures [29]. Dorsal branches can also be

**Table 1**  
Summary of allograft models of cancer-associated thrombosis.

Mouse	Cancer cell	Tumor site	Thrombosis model	Blood vessel	Observation	Ref
C57BL/6	Panc02, LLC	s.c.	FeCl <sub>3</sub>	Mesenteric venules	Reduced time to occlusion	[47]
C57BL/6	Panc02	s.c.	Laser-induced injury FeCl <sub>3</sub>	Mesenteric venules	Tumor-derived MVs accumulated at the site of injury	[47]
C57BL/6	Panc02	s.c.	Laser-induced injury	Cremaster muscle microvessels	Tumor derived MV accumulation at the site of injury	[54]
C57BL/6	Panc02	s.c.	IVC stenosis	IVC	Increased incidence and thrombus weight	[55]
C57BL/6	M27	Orthotopic	FeCl <sub>3</sub>	IVC	Gas6 deficient mice had reduced thrombus size compared with controls	[65]
BALB/c	4T1	Orthotopic	FeCl <sub>3</sub>	Carotid artery	Reduced time to occlusion. DNase I abolished thrombosis.	[63]
BALB/c	4T1	Orthotopic	Rose Bengal	Jugular vein	Reduced time to occlusion. DNase I reduced thrombus size	[63]

IVC: infrarenal vena cava, s.c.: subcutaneous.

**Table 2**  
Summary of xenograft models of cancer-associated thrombosis.

Mouse	Cancer cell	Tumor site	Thrombosis model	Blood vessel	Observation	Ref
Nude	HPAF II	Orthotopic	IVC stenosis	IVC	No enhancement of thrombosis	[13]
Nude	HPAF II	Orthotopic	FeCl <sub>3</sub>	Saphenous vein	Shortened time to occlusion	[13]
Nude	BxPc-3	Orthotopic	IVC stenosis	IVC	Increased thrombus area but no increase in incidence of thrombosis	[14]
Nude	BxPc-3	Orthotopic	IVC stasis	IVC	Increased thrombus area and weight	[28]
Nude	A549	Orthotopic	IVC stenosis	IVC	Increased thrombus weight	[64]
Nude	A549	Orthotopic	FeCl <sub>3</sub>	Saphenous vein	Reduced time to occlusion	[64]

IVC: infrarenal vena cava.

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