

## Review

### Inflammatory breast cancer

# Vasculogenic mimicry and its hemodynamics of an inflammatory breast cancer xenograft model

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## Abstract

We recently established a new human inflammatory breast cancer (IBC) xenograft (WIBC-9) originating from a patient with IBC. The original tumor and WIBC-9 revealed invasive ductal carcinoma with a hypervascular structure of solid nests and marked lymphatic permeation in the overlying dermis. In the central part of the solid nests, vasculogenic mimicry, which showed an absence of endothelial cells, was observed. Comparison of WIBC-9 with an established non-IBC xenograft (MC-5), using time-course dynamic micro-magnetic resonance angiography analysis (with a newly developed intravascular macromolecular contrast agent for magnetic resonance imaging) demonstrated that the WIBC-9 tumor had blood flow and a vascular mimicry-angiogenesis junction.

**Keywords:** hemodynamics, inflammatory breast cancer, vasculogenic mimicry, xenograft model

## Introduction

Growth, proliferation, and metastasis of breast cancer, and of most other tumors, have been thought to be angiogenesis-dependent processes [1]. However, a non-angiogenesis-dependent pathway, in which tumors can feed themselves using alternative pathways, has also been reported [2–12]. Previously, we and others described the presence of vasculogenic mimicry (VM, a condition in which tumors [inflammatory breast cancer (IBC) and melanoma] feed themselves using alternative pathways without the participation of endothelial cells [ECs]) in the tumor-bearing state. In the present study, we established a new human IBC xenograft (WIBC-9) in BALB/c nude mice and investigated the hemodynamics of VM and angiogenesis of IBC, using WIBC-9 xenografts and dynamic micro-magnetic resonance angiography (micro-MRA) analysis. The unique patterns characteristic of VM and its hemodynamics provide a framework for the design

of non-invasive imaging techniques for detecting IBC and its metastases.

## Method

### Morphological and chromosomal analysis

The animal protocols for all experiments were approved by the Animal Use Committee of the National Cancer Center. Hematoxylin-eosin and Giemsa staining of paraffin-embedded specimens were performed, as were electron microscopic examinations following a conventional method. For karyotype studies of the xenograft, the Giemsa G banding method was performed after 6 and 12 passages.

### Dynamic micro-MRA with an intravascular contrast agent

We performed dynamic micro-MRA analysis, using our newly developed intravascular macromolecular contrast agent for magnetic resonance imaging, which consistently showed no significant leakage through the vascular wall

EC = endothelial cell; IBC = inflammatory breast cancer; MRA = magnetic resonance angiography; VEGF = vascular endothelial growth factor; VM = vasculogenic mimicry.

after remaining in circulation for more than 30 min, to evaluate the physiological properties of the vascular channels in the xenografted tumors [13]. We used female 8-week-old BALB/c nude mice bearing either WIBC-9 or MC-5 tumor xenografts. This procedure was performed with mice bearing WIBC-9 and MC-5 tumors ( $n=3$ , for each).

## Results

### Establishment of WIBC-9 tumors

The surgically resected tumors from 10 patients with IBC (Fig. 1A) were transplanted into BALB/c nude mice. The tumor from the ninth patient, referred to as WIBC-9, induced erythema in the overlying skin (Fig. 1B), thus showing the features of IBC. Histologically, WIBC-9 grew locally in an expansive manner, forming a solid nest structure and exhibiting marked lymphatic permeation. In the center of the solid nests, the tumor exhibited a lack of endothelial formation but without central necrosis (Fig. 1C,D). Transmission and phase-contrast electron microscopy clearly showed blood pooling without a lining of ECs in the center of the tumor nests (Fig. 1E,F). There was no vascular structure between the surrounding tumor cells and erythrocytes. Neither necrosis nor fibrosis was observed in the tumor nest. The VM surrounding tumor cells was positive for Flt-1 (vascular endothelial growth factor [VEGF] type 1 receptor) and Tie-2 (angiopoietin-1,2 receptor) (Fig. 1G,H). Our data on the clinical oncology of VM in IBC showed that these are key genes in expressing VM formation. This phenotype remained stable for more than 15 transplant generations. A karyotype analysis revealed chromosomal abnormalities in terms of structure and number. The median chromosome number was 75 (range 72–77) and there was aneuploidy ( $n=20$ ) (Fig. 1I).

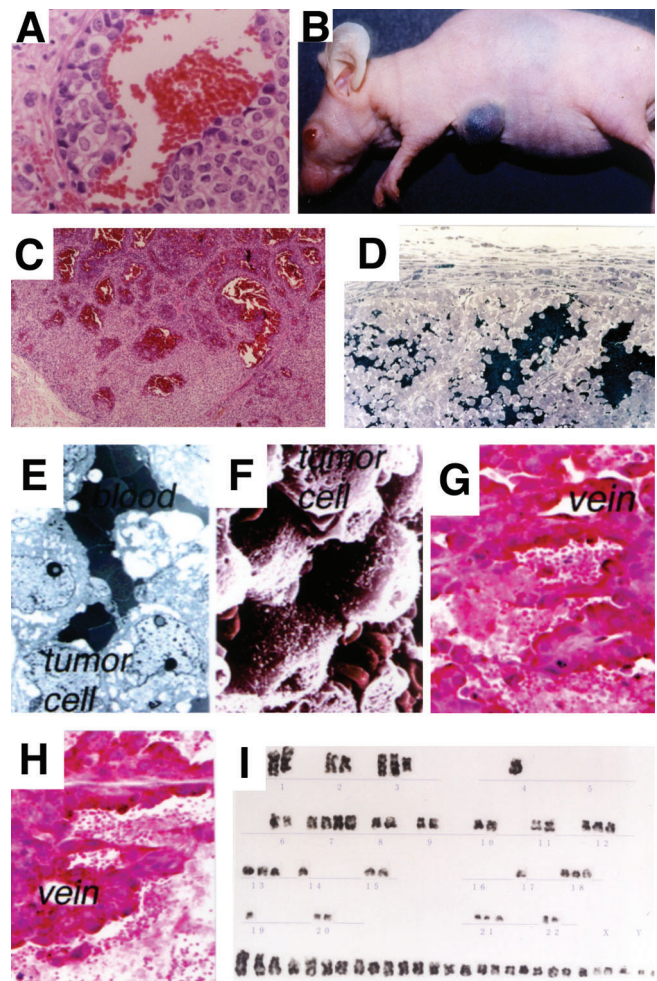
### Time-course MRA of WIBC-9 and MC-5 tumors

Time-course micro-MRA (Fig. 2) was performed to analyze hemodynamics in the VM (central tumor) and angiogenesis (marginal tumor) regions. The images were acquired before injection of the contrast agents and 1, 2, 3, 5, 10, 15, and 30 min after injection. The marginal region of WIBC-9 and MC-5 tumors exhibited a signal that gradually increased in intensity, a result somewhat consistent with the intensity recorded for lung and heart. This explained the connection between neovascular and preexisting vessels and their hemodynamics. In the tumor center, WIBC-9 tumors exhibited spots in which the signal gradually increased in intensity (which is consistent with the intensity observed at their tumor margin), whereas MC-5 tumors exhibited a lack of signal in association with central necrosis. This might explain the connection between VM and neovascular activity surrounding tumor cells.

### Hemodynamics of VM and angiogenesis of IBC and non-IBC xenografts

To analyze hemodynamics in VM and angiogenesis (blood flow through newly established, EC-lined blood vessels),

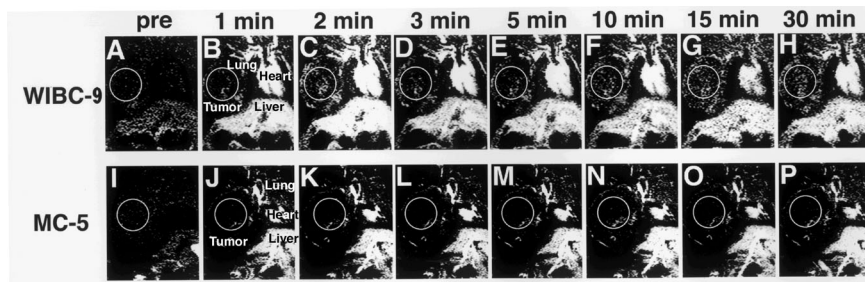
**Figure 1**



Morphological and chromosomal analysis. (A) Histological features of the human original tumor revealed invasive ductal carcinoma and blood pooling without a lining of endothelia. (B) Characteristic appearance of the tumor at the site of subcutaneous inoculation. (C,D) Microscopic analysis of WIBC-9 stained with hematoxylin-eosin (C) and Giemsa (D) revealed hypervascularity, blood pooling without a lining of EC and no central necrosis or fibrosis in the center of the tumor nest. (E) Transmission electron microscopy revealed the interface between tumor cells and erythrocytes. (F) Phase-contrast electron microscopy clearly visualized erythrocytes between tumor cells. Erythrocytes appeared black. (G,H) Vasculogenic mimicry surrounding tumor cells was positive for Flt-1 (G) and Tie-2 (H). (I) Karyotype analysis of WIBC-9 revealing aneuploidy and marked chromosomal abnormalities.

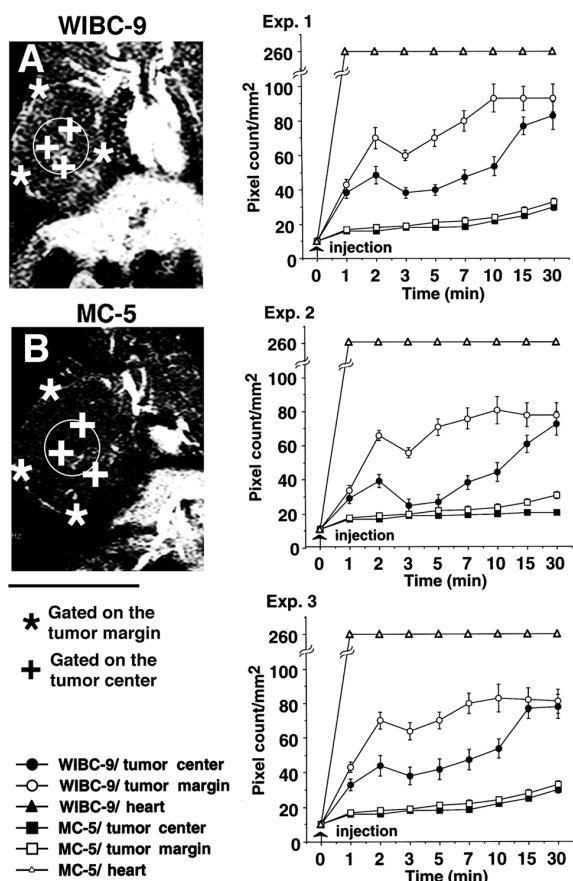
we focused on three regions of interest in the central area and the marginal area of the xenografted tumors, and measured the change in numbers of white pixels per  $\text{mm}^2$  (Fig. 3). The time course of intensity of the tumor center (corresponding to the hemodynamics of VM) was broadly consistent with the time course of intensity of the tumor margin (corresponding to the hemodynamics of angiogenesis). Examination of the hemodynamics of VM revealed blood flow with two peaks of intensity and a statistically

Figure 2



Time-course magnetic resonance angiography of WIBC-9 and MC-5 tumors. The images were acquired before the injection of the contrast agents (pre) and 1, 2, 3, 5, 10, 15, and 30 min after injection. The tumor marginal area of both WIBC-9 and MC-5 exhibited a signal that gradually increased in intensity. In the tumor center, WIBC-9 exhibited spots in which the signal gradually increased in intensity (consistent with the intensity recorded for the tumor margin), whereas the central region of MC-5 maintained a lack of signal.

Figure 3



Hemodynamics in vasculogenic mimicry of inflammatory breast cancer (IBC) xenografts and angiogenesis of IBC and non-IBC xenografts. All data are expressed as means  $\pm$  SD. The time course of intensity of the tumor center (corresponding to the hemodynamics in vasculogenic mimicry) was consistent with the time course of intensity of the tumor margin (corresponding to the hemodynamics of angiogenesis). The degree of tumor margin angiogenesis in WIBC-9 tumors was at least threefold that observed in MC-5.

significant time lag relative to the hemodynamics of angiogenesis. The rate of tumor margin angiogenesis in WIBC-9 xenografts was at least threefold that observed in MC-5.

### Discussion

The established xenograft WIBC-9 preserves the histological and biological characteristics of human IBC. The features of erythema in the overlying skin, marked lymphatic permeation and high rate of metastasis are commonly seen in both WIBC-9 and human IBC. WIBC-9 has two unique histological features: blood pooling without a lining of ECs. Electron microscopy revealed the interface of tumor cells and erythrocytes lacking both necrosis and fibrosis. These structures exhibited weak expression of the human activated EC marker, human integrin  $\alpha v \beta 3$ . These structures are lined externally with tumor cells and no ECs are seen. These results suggest the *de novo* formation of vascular channels by tumor cells in the central tumor under putative hypoxia and induced angiogenic factors. They also suggest that vessel regression has not been occurring in these tumors and that the blood from ruptured vessels has not filled tumor-lined lakes or channels. WIBC-9 exhibited no fibrosis, central necrosis, or lining of endothelia, whereas other breast cancer xenografts commonly exhibit fibrosis and central necrosis as the tumor grows. We believe these findings might be related to the expression of certain genes in WIBC-9 (i.e. *huFlt-1*, *huTie-2*, *huTie-1*, and *huIntegrin $\alpha v \beta 3$* ) [14]. This gene expression might result in the observed endothelial/vascular phenotype and the putative *de novo* formation of the vascular channel by tumor cells. In the tumor margin, WIBC-9 exhibited hypervascularity and also significantly more intense immunoreactivity of murine CD31 in the neovascular epithelia than did non-IBC xenografts. This might explain the endothelial sprouting of new vessels from pre-existing vessels as a result of overexpression of the angiogenic factors.

This article is the first in a review series on *Inflammatory breast cancer*, edited by Sofia Merajver.

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We have previously proposed an angiogenic pathway and a non-angiogenic pathway (VM), and focused on the relationship between the migration of ECs (including endothelial precursor cells) and *de novo* vascular channel formation associated with tumor cells [4]. In particular, we focused on the VEGF-Flt-1 and angiopoietin-1,2-Tie-2 pathway based on the clinical oncological data derived from a cDNA array analysis of VM cases and non-VM cases. When these pathways were blocked by the injection of adenovirus vectors encoding specific anti-angiogenic agents (sFlt-1 and sTie2) into WIBC-9 tumors, the formation of VM ceased [5]. The results of our recent studies demonstrated that six established human breast cancer lines, particularly the IBC line WIBC-9, induce postnatal endothelial precursor cell kinetics as well as EC kinetics [6,7].

In the present study we investigated the existence of VM and its hemodynamics in the IBC xenograft WIBC-9. In particular, we may have demonstrated the existence of a connection between VM and neovasculars.

### Competing interests

None declared.

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