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Original Article

Endothelial cell transplantation in tumors restores normal vasculature, reduces tumor hypoxia, and suppresses tumor outgrowth

Shuji Kitahara [a,b,*,1], Aya Matsui [a,1], Asuka Yoshii [a,c,1], Yoshikazu Kuwahara [d], Miwako Nishio [e], Kumiko Saeki [e], Taichi Ezaki [a]

[a] Department of Anatomy and Developmental Biology, School of Medicine, Tokyo Women's Medical University, Tokyo, Japan
[b] Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[c] Departments of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, MA, USA
[d] Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[e] Department of Disease Control, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan

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ABSTRACT

Objectives: Vascular normalization, or restoration of the normal structure and function of blood vessels, using molecular-targeted therapy, has emerged as a potential strategy for treating malignant cancer and other vascular disorders. We hypothesized that restoring tumor blood vessels to their normal state would alleviate hypoxic conditions and potentially enhance the delivery of anticancer drugs. Our objective was to determine if transplanting normal endothelial cells into tumor-bearing mice could trigger vascular normalization.
Methods: Tumor cells were injected into the dorsal subcutis of severe combined immunodeficiency (SCID) mice (day 0). Tumor-bearing mice were injected intraperitoneally with cisplatin at day 14 to create scaffolds for blood vessel formation in the tumors. At day 28, human microvascular endothelial cells (HMVECs) or human embryonic stem-derived endothelial cells (ESECs) were transplanted into the necrotic regions of the tumor to induce normal angiogenesis.
Results: Microscopic observation revealed that the transplanted HMVECs or ESECs formed anastomoses with the host mouse vasculature. In addition, blood vessels with blood flow could be detected after 14 d. Blood vessels reconstituted by HMVECs or ESECs exhibited normal vasculature, and tumor growth was significantly inhibited upon treatment.
Conclusion: Reconstruction of tumor blood vessels to their normal state alleviated hypoxic conditions and improved the efficiency of drug delivery; the present approach provides a useful model for the development of new cancer therapies.

1. Introduction

Tumor blood vessels have been reported to be histologically different from normal blood vessels, having irregular shapes and abnormal branching patterns, and thus cannot be classified as arterioles, venules, or capillaries [1,2]. These endothelial cells are loosely interconnected and have intercellular openings and abnormal pericytes that are mainly responsible for vessel leakiness observed in tumor blood vessels [3,4]. When compared to normal blood vessels, tumor blood vessels appear immature, a phenotype that might be associated with structural aberrations in their basement membrane [5]. Vascular abnormalities associated with tumors may be attributed to abnormal microenvironments (characterized by interstitial hypertension, acidosis, and hypoxia); this can result in resistance to conventional chemo-, radio-, and immune-based-therapies [1,6].

Tumor hypoxia stimulates the release of angiogenic factors such as vascular endothelial growth factor (VEGF) from tumor cells and other cell types. In addition, tumor hypoxia stimulates angiogenesis and promotes a continuous cycle of abnormal vessel and tumor growth, resulting in increased hypoxic regions [7]. Although the main purpose of tumor angiogenesis is to maintain tumor blood supply, the process occurs in an unregulated fashion and results in a highly abnormal vascular network [8]. In addition, structural and functional abnormalities of blood vessels are a hallmark of solid tumors (i.e., those that contribute directly to the malignant properties of cancer) [9]. It has been suggested that tumor growth depends on angiogenesis; accordingly, molecular

* Corresponding author at: Department of Anatomy and Developmental Biology, Tokyo Women's Medical University, 8-1 Kawada-Cho, Shinjuku, Tokyo 162-8666, Japan. Tel.: +81 3 5269 7407; fax: +81 3 5269 7407.
E-mail address: skitahara@mgh.harvard.edu (S. Kitahara).
[1] These authors contributed equally to this manuscript.

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therapies that target angiogenesis have been extensively studied [10]. Such therapies have attracted considerable attention because inhibiting blood vessel formation and preventing an adequate supply of oxygen and nutrients is thought to inhibit the growth of cancer-causing tumors via starvation. However, some studies have suggested that anti-VEGF therapy alone cannot induce sufficient vascular regression to result in significant tumor shrinkage in a clinical setting. Therefore, vascular normalization has been proposed as an alternative method for treating malignant cancer and other vascular disorders [11].

The strategy underlying functional "normalization" of tumor vasculature aims to replace impaired blood vessels with functional vessels to control tumor proliferation [11]. Indeed, therapies that target VEGF or its cognate receptor, to "normalize" aberrant tumor vasculature, have shown clinical success in various human cancers [8]. The normalization hypothesis suggests that by correcting abnormalities in the structure and function of tumor vessels (rather than destroying the vessels completely), we can improve the tumor microenvironment, control tumor progression, and improve responses to other therapies. Normalized tumor vessels have decreased leakiness, dilation, and torsion, and exhibit a more normal basement membrane with greater pericyte coverage [7,12]. However, it is unknown if this process results in completely normal blood vessels. Moreover, the function and structure of normalized vessels have not been adequately characterized. In addition, anti-angiogenic therapy is not always effective, and intrinsic resistance to this novel therapy has been observed in some desmoplastic and hypovascular tumors [2]. Therefore, we hypothesized that normalization of tumor blood vessels would alleviate hypoxic conditions, potentially improving the delivery of anticancer drugs. To this end, we transplanted normal endothelial cells into tumor-bearing mice to determine if this could trigger vascular reconstruction and alleviate hypoxic conditions.

2. Materials and methods

2.1. Animals

Male SCID mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Mice were maintained in air-filtered clean

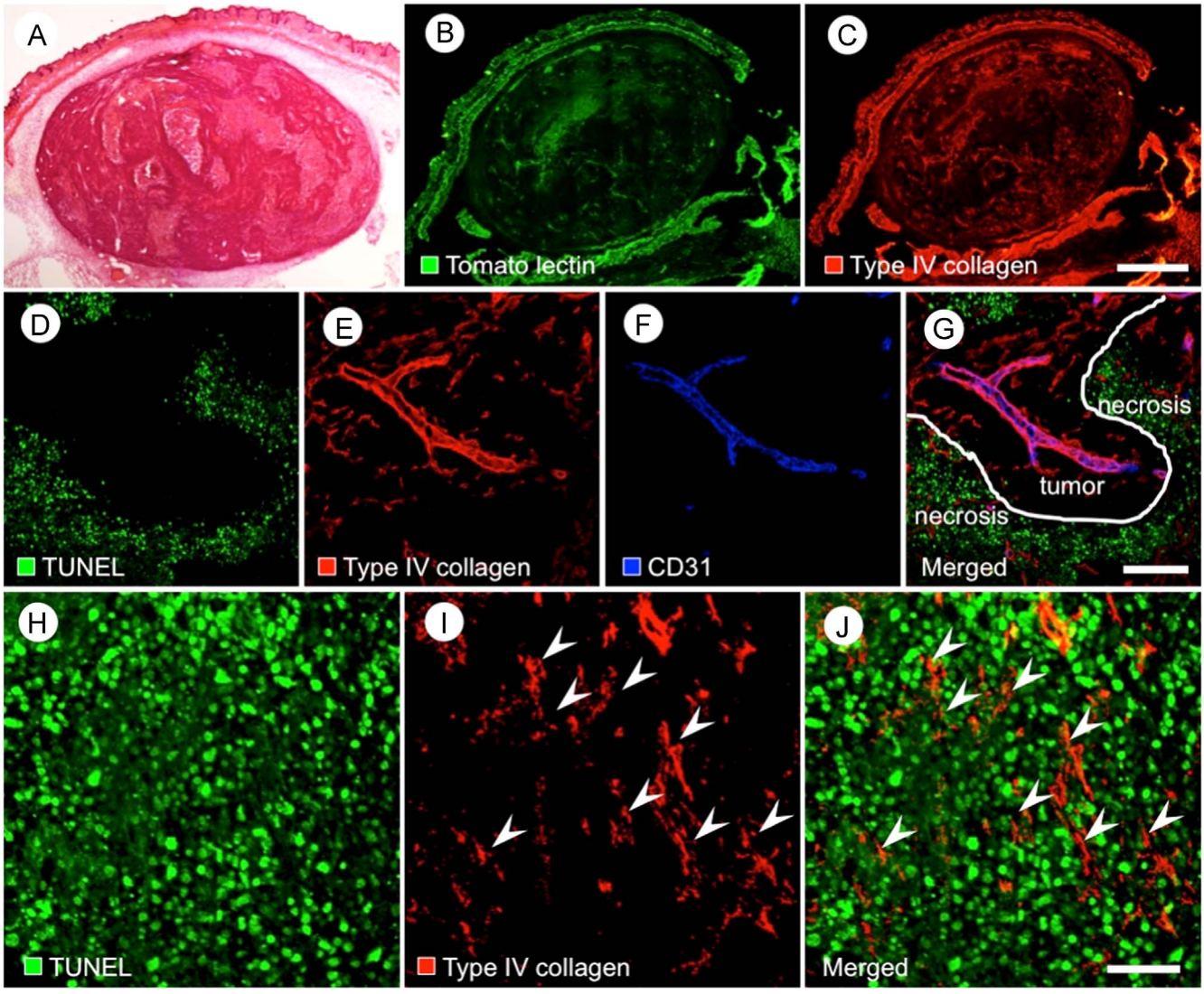

Fig. 1. Histopathological changes after the administration of cisplatin (CDDP). Necrotic tumor areas were observed (A–C); macroimage of a tumor. Hematoxylin and eosin staining (A). Tomato lectin (B), and type IV collagen (C) staining. (D–G) Triple immunofluorescence staining of a necrotic area using TUNEL (green), mouse CD31 (blue), and type IV collagen (red) was performed 14 d after the administration of CDDP. Conditions necessary for normal blood vessel reconstruction in tumor-necrotic regions. (H–J) Type IV collagen-positive basement membranes (red) were observed in TUNEL-positive necrotic areas (green). Type IV collagen-positive basement membranes (red) were abundant in TUNEL-positive tumor necrotic areas (green). Remnants of the basement membranes became scaffolds for the regeneration of blood vessels. Scale bars: 1 cm (A–C), 100 μm (D–H), and 50 μm (H–J).

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