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BIOLOGY CONTRIBUTION

VASCULATURES IN TUMORS GROWING FROM PREIRRADIATED TISSUES: FORMED BY VASCULOGENESIS AND RESISTANT TO RADIATION AND ANTIANGIOGENIC THERAPY

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Purpose: To investigate vasculatures and microenvironment in tumors growing from preirradiated tissues (pre-IR tumors) and study the vascular responses of pre-IR tumors to radiation and antiangiogenic therapy.
Methods and Materials: Transgenic adenocarcinoma of the mouse prostate C1 tumors were implanted into unirradiated or preirradiated tissues and examined for vascularity, hypoxia, and tumor-associated macrophage (TAM) infiltrates by immunohistochemistry. The origin of tumor endothelial cells was studied by green fluorescent protein–tagged bone marrow (GFP-BM) transplantation. The response of tumor endothelial cells to radiation and antiangiogenic agent was evaluated by apoptotic assay.
Results: The pre-IR tumors had obvious tumor bed effects (TBE), with slower growth rate, lower microvascular density (MVD), and more necrotic and hypoxic fraction compared with control tumors. The vessels were dilated, tightly adhered with pericytes, and incorporated with transplanted GFP-BM cells. In addition, hypoxic regions became aggregated with TAM. As pre-IR tumors developed, the TBE was overcome at the tumor edge where the MVD increased, TAM did not aggregate, and the GFP-BM cells did not incorporate into the vessels. The vessels at tumor edge were more sensitive to the following ionizing radiation and antiangiogenic agent than those in the central low MVD regions.
Conclusions: This study demonstrates that vasculatures in regions with TBE are mainly formed by vasculogenesis and resistant to radiation and antiangiogenic therapy. Tumor bed effects could be overcome at the edge of larger tumors, but where vasculatures are formed by angiogenesis and sensitive to both treatments. Vasculatures formed by vasculogenesis should be the crucial target for the treatment of recurrent tumors after radiotherapy.

Radiation, Tumor bed effects, Microvascular density, Green fluorescent protein–tagged bone marrow, Antiangiogenic therapy.

INTRODUCTION

The growth and therapeutic responses of tumors are influenced by tumor microenvironments. Clinically, recurrent tumors after radiotherapy are less responsive to salvage radiotherapy or chemotherapy and have higher risk of metastasis (1). Experimentally, tumor transplants in preirradiated (pre-IR) tissues have shown longer growth delay and greater metastatic propensity than in nonirradiated tissues (2), a phenomenon known as the tumor bed effect (TBE) (3). An inadequate vascular supply from the pre-IR tissue is considered to be responsible for TBE (4).

Tumor bed effect can occur at a dose as low as 5 Gy and has been linearly correlated with doses up to 20–30 Gy (3). However, not all tumors display TBE (5), and the inherited genetic background of tumor cells or the content of tumor-associated macrophages are involved. In addition, there are contradictory results regarding the relationship between TBE and radiosensitivity, and increases of hypoxic or necrotic fraction in tumors could be the major determinants (6, 7). To improve the management of recurrent tumors, anti–vascular endothelial growth factor receptor 2 treatment has been tried and seems effective (8), suggesting that vascular responses might be

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Conflicts of interest: none.

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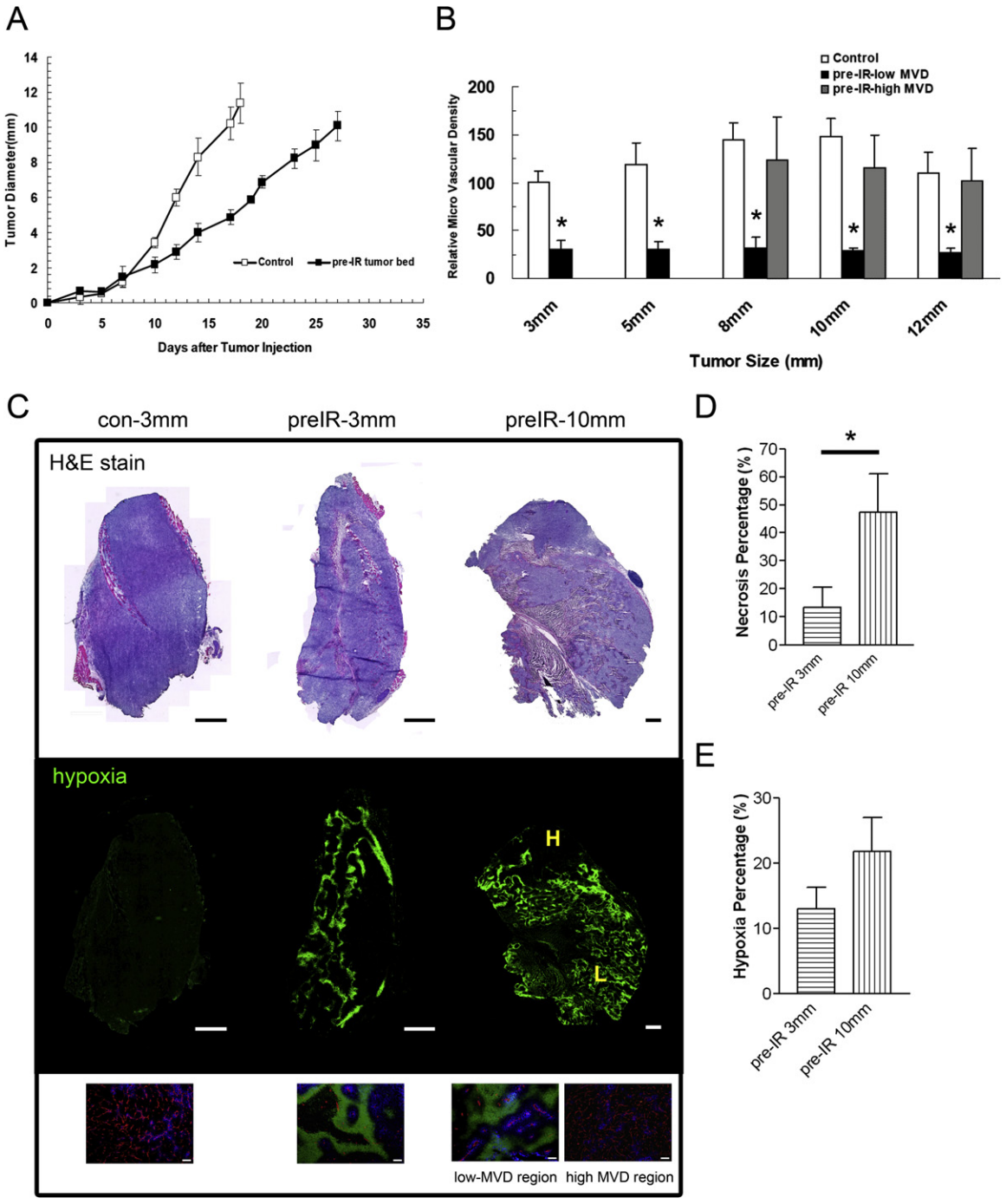

Fig. 1. Irradiation of tumor bed causes tumor growth delay, severe hypoxia, and reduces vascularity. (A) Growth curve of transgenic adenocarcinoma of the mouse prostate C1 tumors on unirradiated and preirradiated (pre-IR) tumor beds. (B) Quantification of tumor microvascular density (MVD). Tumors were harvested for control and pre-IR groups at sizes of 3 mm, 5 mm, 8 mm, 10 mm, and 12 mm. *$p < 0.0001$ vs. 3-mm control. (C) Irradiation of tumor beds induces necrosis and hypoxia and reduces MVD. Upper: Reconstructive histologic images of whole tumor. Scale bars, 1 mm. Middle: Reconstructive immunohistochemical images for hypoxia of whole tumor in serial section. Scale bars, 1 mm. Bottom: Representative images of immunohistochemical staining for hypoxia distribution (green), tumor vascularity (red), and vessel perfusion (blue) in control and pre-IR tumors at 3 mm and 10 mm (H = high-MVD region; L = low-MVD region). Scar bars, 100 μm (D, E) Quantification of necrotic and hypoxic percentage in pre-IR tumors. *$p < 0.05$ ($n = 5$ per group in each quantification).

a potential target for treating postirradiated recurrent tumors. Most recently, it was found that bone marrow–derived CD11b-positive myelomonocytic cells expressing matrix metalloproteinase 9 are required for vasculogenesis but not for angiogenesis in pre-IR tumors (9).

We recently demonstrated the association of microvascular density (MVD), hypoxia status, and tumor-associated macrophage (TAM) aggregation in tumors receiving radiation (IR tumors) (10, 11). In this study, we provide evidence to show that the microenvironment in pre-IR tumors was similar to