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Original Research

Inhibition of lymphangiogenesis impairs antitumour effects of photodynamic therapy and checkpoint inhibitors in mice

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Abstract Photodynamic therapy (PDT) has been shown to destroy tumour-associated lymphatic vessels. Therefore, we sought to investigate the functional outcomes of PDT-mediated damage to the lymphatic vessels. We observed that PDT with verteporfin, completely but transiently, blocks the functional lymphatic drainage in the orthotopic mammary tumour models. Sustained inhibition of lymphatic vessels regeneration induced by lenalidomide or the soluble form of vascular endothelial growth factor receptor 3 (sVEGFR3) that neutralises lymphangiogenic vascular endothelial growth factor C (VEGF-C), significantly impaired antitumour efficacy of PDT. Antilymphangiogenic compounds also significantly inhibited the ability of intratumourally inoculated dendritic cells (DCs) to translocate to local lymph nodes and diminished the number of tumour-infiltrating interferon-γ-secreting or

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tumour antigen-specific $CD8^+$ T cells. Lenalidomide also abrogated antitumour effects of the combination immunotherapy with PDT and anti-programmed death-ligand 1 (PD-L1) antibodies. Altogether, these findings indicate that PDT-mediated damage to the lymphatic vessels negatively affects development of antitumour immunity, and that drugs that impair lymphatic vessel regeneration might not be suitable for the use in combination with PDT.

1. Introduction

Lymphatic vessels enable migration of dendritic cells (DCs) and transport of soluble antigens from the tissue interstitium to the lymph nodes, where activation of naïve T cells occurs. Therefore, lymphatics play a critical role in the development of the adaptive immune response [2]. Although for many years solid tumours have been considered to have poorly developed network of lymphatic vessels, increasing number of reports reveal that tumour cells or tumour-infiltrating cells release growth factors such as vascular endothelial growth factor C and D (VEGF-C and D) to induce lymphatic vessel expansion (lymphangiogenesis) that occurs both within the primary tumours and in the draining lymph nodes (LNs) [22]. Since this process promotes LN metastasis, inhibition of lymphangiogenesis has been suggested to be a potential goal for antitumour therapies.

Photodynamic therapy (PDT) is approved for clinical use as the treatment for solid tumours and ocular diseases [1]. Immunogenic cell death and acute inflammation induced by the PDT are typically related to the induction of tumour-specific immune response that develops in tumour-bearing animal as well as in the clinical setting [4–6,26]. Antivascular effects of PDT are relatively well described [3], however very little is known about the recently reported antilymphatic effects of this treatment. It was reported that PDT with verteporfin used as a photosensitiser leads to lymphatic vessel damage that can be used in combination with surgical removal of the primary tumour to eliminate in-transit lymphatic metastases in the B16F10 melanoma model [25]. Also our previous studies revealed that PDT can effectively ablate pre-existing skin lymphatic vessels, causing lymphatic endothelial cells (LECs) and smooth muscle cells death [10,28]. In this study, we sought to investigate the influence of PDT-mediated damage to the lymphatic vessels on the development of antitumour immune response in the orthotopic murine breast cancer models.

2. Results

2.1. PDT leads to destruction of tumour-associated lymphatic vessels

We have previously shown in mice that intradermal administration of liposomal formulation of verteporfin (that accumulates within lymphatic vessels) leads to the functional occlusion of lymphatic vessels in the laser light-exposed areas of normal ears [10]. In this study, we investigated whether antitumour PDT with verteporfin, conventionally injected intravenously (i.v.), may affect the lymph flow in the orthotopic mammary 4T1 tumour model. We observed that PDT completely blocks the functional lymphatic drainage of intratumourally injected fluorescein isothiocyanate (FITC)-labelled dextran from the tumour to the draining, axillary LNs (Fig. 1A). Notably, we observed that the lymphatic drainage started to be recovered from day 5 after PDT and was completely restored in all mice by day 20 after PDT (Fig. 1B).

2.2. Lenalidomide diminishes antitumour efficacy of PDT

Considering recent studies revealing that lenalidomide inhibits lymphangiogenesis in mantle cell lymphoma model in mice [21], we examined whether lenalidomide affects the lymphatic regeneration post PDT. To confirm antilymphangiogenic activity of lenalidomide in the 4T1 tumour model, the percentage of tumour LECs, characterised as cells lacking the expression of CD45 and expressing podoplanin (gp38) and CD31, was evaluated with flow cytometry. Five days after

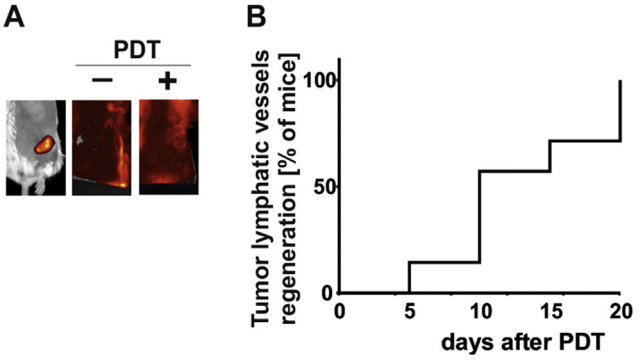

Fig. 1. Antitumour PDT blocks lymphatic drainage to the local axillary lymph nodes. **A.** BALB/c mice were treated with PDT on day 9 after inoculation of tumour cells. To monitor lymphatic drainage mice were intratumourally injected with FITC-labelled dextran (left) and visualised with IVIS Spectrum System before (middle) and 10 min after PDT (right). **B.** FITC-labelled dextran was then injected into PDT-treated mice every 5 d to determine when the lymphatic drainage becomes restored. The graph presents the percentage of mice with restored lymphatic drainage of FITC-labelled dextran after PDT (n = 7).