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Original Research

Tumour endothelial marker 1/endosialin-mediated targeting of human sarcoma

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Abstract ***Background:*** Tumour endothelial marker 1 (TEM1/endosialin/CD248) is a tumour-restricted cell-surface protein expressed by human sarcomas. We previously developed a high-affinity human single-chain variable fragment (scFv)-Fc fusion protein (78Fc) against TEM1 and demonstrated its specific binding to human and mouse TEM1.
Patient and methods: Clinical sarcoma specimens were collected between 2000 and 2015 at the Hospital of the University of Pennsylvania, as approved by the institutional review board and processed by standard formalin-fixed paraffin embedded techniques. We analysed TEM1 expression in 19 human sarcoma subtypes (n = 203 specimens) and eight human sarcoma–cell lines. Near-infrared (NIR) imaging of tumour-bearing mice was used to validate 78Fc binding to TEM1^{+} sarcoma *in vivo*. Finally, we tested an immunotoxin conjugate of anti-TEM1 78Fc with saporin (78Fc-Sap) for its therapeutic efficacy against human sarcoma *in vitro* and *in vivo*.
Results: TEM1 expression was identified by immunohistochemistry in 96% of human sarcomas, of which 81% expressed TEM1 both on tumour cells and the tumour vasculature.

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NIR imaging revealed specific *in vivo* targeting of labelled 78Fc to TEM1$^+$ sarcoma xenografts. Importantly, 78Fc-Sap was effective in killing *in vitro* TEM1$^+$ sarcoma cells and eliminated human sarcoma xenografts without apparent toxicity *in vivo*.
Conclusion: TEM1 is an important therapeutic target for human sarcoma, and the high-affinity TEM1-specific scFv fusion protein 78Fc is suitable for further clinical development for therapeutic applications in sarcoma.

1. Introduction

Tumour endothelial marker 1 (TEM1/CD248), also known as endosialin, is an 80.9 kDa transmembrane glycoprotein belonging to the C-type lectin receptor superfamily [1]. TEM1 expression was first detected on tumour blood vessels in the early 1990s by Dr. Lloyd Old, with the monoclonal antibody (mAb) FB5 generated from a mouse immunised with human foetal mesenchymal fibroblasts [2], and the protein was later characterised in two independent studies [1,3]. TEM1 expression has since been specifically localised to perivascular cells (pericytes) and fibroblasts of the tumour stroma [4,5], as well as malignant cells [6]. Notably, although TEM1 knockout mice exhibit no obvious phenotype and normal wound healing, transplanted tumours grow more slowly, are less invasive and develop fewer metastases in TEM1 knockout than in wild-type mice [7].

TEM1 is widely expressed during embryonic development and is silenced in normal tissues in the adult but is re-expressed in solid tumours, including sarcomas and a broad range of carcinomas [2,6] and melanomas [8]. TEM1 overexpression has been associated with aggressive tumour behaviour and poor patient prognosis [9–11]. It has been implicated in tumour-cell vascular adhesion and migration, neoangiogenesis, local invasion and metastasis [1,2,4,7,12,13]. Pre-clinical studies indicate that TEM1 is a safe and promising target for cancer therapy [14,15]. Moreover, encouraging phase I safety results have been reported for MORAb-004, a humanised mAb derived from FB5, used to neutralise TEM1 [16].

Sarcomas are a rare but diverse type of cancer of mesodermal origin that can arise from transformed cells of connective tissue (soft-tissue sarcoma) or bone (osteosarcoma) [17]. Although some early sarcomas can be cured by surgery followed by chemotherapy or radiotherapy, the prognosis of advanced sarcomas remains dismal, with various approaches in development (reviewed by Linch *et al.* [18]). To date, there are limited data exploring TEM1 expression across different subtypes of sarcoma [2,6,19,20]. Rouleau *et. al.* [6] analysed 94 clinical sarcoma specimens using an mAb generated against human TEM1 in KM mice and reported 84% expression across all eight subtypes tested. In a retrospective study, they showed that TEM1 is upregulated in high-grade human sarcomas [21].

We have previously isolated single-chain variable fragment 78 (scFv78), a human scFv cross-reactive with both mouse and human TEM1 at high affinity (K_d ~2 nM) [22,23]. We fused it to the Fc region of human IgG1, creating a dimeric protein (78Fc), which we conjugated with a fluorochrome for near-infrared (NIR) optical imaging. Preliminary *in vivo* experiments demonstrated efficient 78Fc homing to TC1 tumours, which strongly express TEM1 in the vasculature and weakly in tumour cells *in vivo*. Moreover, we showed that the 78Fc internalises upon binding to cell-surface TEM1 [24]. Based on these properties, we theorised that 78Fc could be an ideal candidate for drug delivery to TEM1$^+$ sarcomas. Here we developed an immunotoxin that comprise 78Fc conjugated with the type-1 ribosome–inactivating protein saporin (Sap), a powerful toxin that irreversibly blocks protein synthesis and causes cell death. We demonstrate that 78Fc binds specifically to sarcoma xenografts *in vivo* and that scF78-toxin immunoconjugate exhibits strong therapeutic efficacy in pre-clinical sarcoma models. Moreover, we confirmed a high prevalence of TEM1 expression by immunostaining in 203 human sarcoma specimens, covering 19 sarcoma subtypes, further validating the value of scFv78 as a specific theranostic tool worth of further clinical development for human sarcoma.

2. Materials and methods

2.1. Clinical sarcoma specimens

Clinical sarcoma specimens were collected between 2000 and 2015 at the Hospital of the University of Pennsylvania, as approved by the institutional review board, and processed by standard formalin-fixed paraffin-embedded techniques. Please see Supplementary Materials for the rest of materials and methods.

3. Results

3.1. Tumour endothelial marker 1 expression in human sarcoma

We tested the expression of TEM1 on 203 clinical sarcoma specimens using anti-human TEM1 mAb (clone B1/35). Histology and quantification of TEM1