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Targeting CD26 suppresses proliferation of malignant mesothelioma cell via downmodulation of ubiquitin-specific protease 22

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ABSTRACT

Malignant pleural mesothelioma (MPM) is an aggressive malignancy arising from mesothelial lining of pleura. It is associated with a poor prognosis, partly due to the lack of a precise understanding of the molecular mechanisms associated with its malignant behavior. In the present study, we expanded on our previous studies on cell cycle control of MPM cells by targeting CD26 molecule with humanized anti-CD26 monoclonal antibody (HuCD26mAb), focusing particularly on ubiquitin-specific protease 22 (USP22). We showed that USP22 protein expression is detected in clinical specimens of MPM and that USP22 knockdown, as well as CD26 knockdown, significantly inhibits the growth and proliferation of MPM cells *in vitro* and *in vivo*. Moreover, depletion of both USP22 and CD26 suppresses MPM cell proliferation even more profoundly. Furthermore, expression levels of USP22 correlate with those of CD26. HuCD26mAb treatment induces a decrease in USP22 level through its interaction with the CD26 molecule, leading to increased levels of ubiquitinated histone H2A and p21. By demonstrating a CD26-related linkage with USP22 in MPM cell inhibition induced by HuCD26mAb, our present study hence characterizes USP22 as a novel target molecule while concurrently suggesting a new therapeutic strategy for MPM.

1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive malignancy arising from mesothelial lining of pleura [1]. It is generally associated with a history of asbestos exposure and has a very poor prognosis. Once rare, the incidence of MPM has increased in industrialized nations as a result of past wide spread exposure to asbestos [1]. Its incidence is predicted to increase further in the next decades, especially in developing countries where asbestos has not yet been prohibited [1]. Due to the lack of efficacy of conventional treatments, novel therapeutic strategies are urgently needed to improve outcomes [2].

We recently showed that mesothelioma cells expressing high level of CD26 displayed high proliferative activity and invasiveness, and microarray analysis of CD26 knockdown and CD26-transfected mesothelioma cells showed that CD26 expression was closely linked to the expression of genes contributing to cell proliferation and cell cycle regulation [3–5]. We have reported that treatment with anti-CD26 antibody induced G1 cell cycle arrest and enhanced cyclin-dependent kinase inhibitor (CDKI) p21 (CIP1/WAF1) expression [6–8]. More recently, we demonstrated that humanized anti-CD26 monoclonal antibody (HuCD26mAb) exhibited a favorable safety profile and substantial clinical activity in heavily pretreated CD26-positive MPM patients who had previously progressed on conventional standard chemotherapies [9]. However, the precise cellular mechanisms involved in the regulation of

Abbreviations: CD26si, siRNA against CD26; CSC, cancer stem cell; Csh, control shRNA; Csi, control siRNA; CDKI, cyclin-dependent kinase inhibitor; HuCD26mAb, humanized anti-CD26 monoclonal antibody; MPM, malignant pleural mesothelioma; s.c., subcutaneous; USP22, Ubiquitin-specific protease 22; USP22-shRNA, shRNA against USP22.

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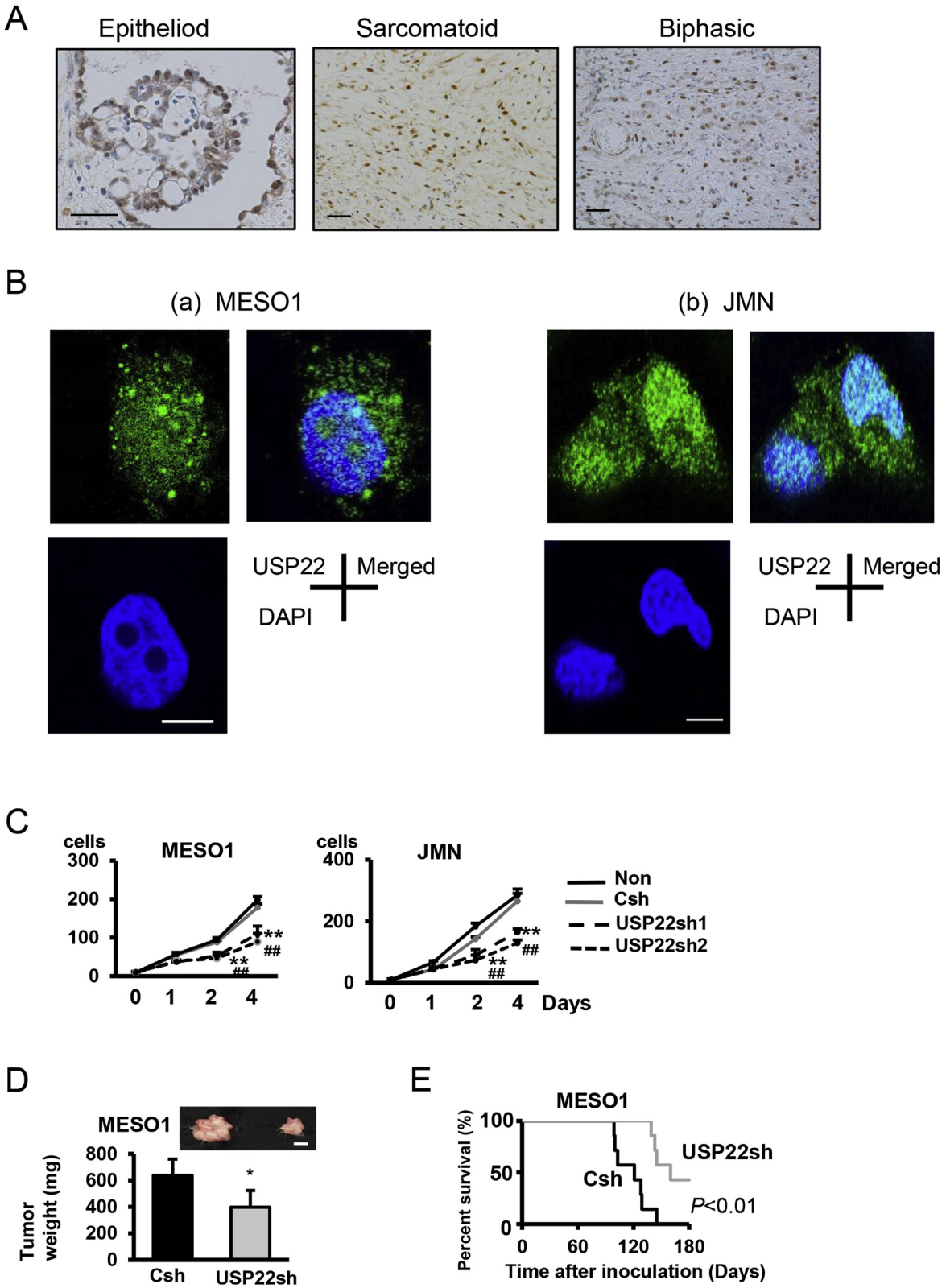

Fig. 1. Suppression of USP22 decreases tumor growth and proliferation in MPM cells.
(A) Representative immunohistochemistry images of USP22 in MPM clinical specimens, including epithelioid, sarcomatoid, and biphasic type. USP22 (brown staining in nuceli) was highly expressed in each MPM type. Scale bars, 50 μm
(B) Confocal microscopy images of USP22 (green) in MPM cell lines, (a) MESO1 and (b) JMN. Nuclei (blue) were stained with DAPI. USP22 was expressed in both the cytosol and the nuclei, and was barely detectable on the cell surface. Scale bars, 10 μm
(C) MESO1 or JMN cells were stably transfected with USP22-shRNA-1, USP22-shRNA-2 or control shRNA (Csh). Cell proliferation was directly examined at the indicated days. Proliferation was significantly decreased following transfection of USP22-shRNA-1 or -shRNA-2. $^{**}p < 0.01$, USP22-shRNA-1 *vs* Csh; $^{\#\#}p < 0.01$, USP22-shRNA-2 vs Csh.
(D) MESO1 cells were stably transfected with USP22-shRNA-1 or control shRNA (Csh), and were inoculated s.c. into the dorsal region of SCID mice (3×10^5 cells/mouse, n = 8). Tumors were resected at day 10 to be weighed. Tumor weight was significantly decreased in the group transplanted with USP22-shRNA-1-transfected cells ($^{*}p < 0.01$).

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