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The overriding of TRAIL resistance by the histone deacetylase inhibitor MS-275 involves c-myc up-regulation in cutaneous, uveal, and mucosal melanoma

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ABSTRACT

Malignant melanoma is a highly aggressive tumor which may occur in the skin, eye, and mucous membranes. The prognosis of melanoma remains poor in spite of therapeutic advances, emphasizing the importance of innovative treatment modalities. Currently, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is showing promising clinical responses, however its use is hampered by intrinsic or acquired melanoma resistance to apoptosis. Recently, we showed that the combination of TRAIL with the class I-specific histone deacetylase inhibitor (HDACi) MS-275 was a privileged way to override TRAIL resistance through down-regulation of cellular Fas-associated death domain (FADD)-like interleukin-1beta-converting enzyme-inhibitory protein (c-FLIP). Here, we elucidated the underlying mechanism and provided evidence that a crucial step in the *c-FLIP* downregulation triggered by MS-275 implies the up-regulation of *c-myc*, a transcriptional repressor of *c-FLIP*. Notably, MS-275 caused H3 histone acetylation at the promoter of *c-myc* and increased its binding to the *c-FLIP* promoter, that in turn led to reduced *c-FLIP* gene transcription. Knockdown of *c-myc* prevented the MS-275-mediated downregulation of *c-FLIP* and hindered TRAIL-plus MS-275-induced apoptosis. Findings reported here provide additional knowledge tools for a more aware and effective molecular therapy of melanoma.

1. Introduction

Malignant melanoma is an aggressive form of cancer predominantly located in the basal layer of the epidermis. However, less common types may be found in other body districts, such as uveal tract and mucous surfaces. Melanoma risk has been positively linked to constitutional as well as lifestyle factors, such as ultraviolet and heavy metal exposure history [1–3], which contribute to both genetic and epigenetic modifications [4–7]. Melanoma is considered one of the most difficult cancers, as either conventional treatments, like chemotherapy and radiotherapy, or biological therapies, both alone and in combination, have not led to a median survival of patients exceeding 5-years from the time of initial diagnosis [8].

Among the encouraging antitumor activities with mild side effects, treatment with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has aroused particular interest because of its ability to induce apoptosis only in malignant cells [9]. However, most melanomas exhibit either the intrinsic or acquired resistance to apoptosis, and such a property often thwarts the TRAIL-based therapy [10]. Dysregulation of death receptors [11,12] or increased expression of inhibitors of apoptosis proteins (IAPs) [13], anti-apoptotic Bcl-2 family members [14,15] or cellular FADD-like interleukin-1beta-converting enzyme-inhibitory protein (c-FLIP) [16] are the major causal events.

Recently, increasing evidences showed that melanoma therapy with TRAIL could benefit from the introduction of small molecules that are able to revert aberrant epigenetic states, such as histone deacetylase inhibitors (HDACis) [17–19], but the way by which the susceptibility to apoptosis is reinstated remains still far from clear. Understanding the molecular mechanism that mediates the synergistic interaction between TRAIL and HDACis could be crucial to successfully translate such approach into a clinical setting of melanoma in a more rational and systematic manner. Previous studies by us [20], showing that the HDACi MS-275 sensitized TRAIL-resistant melanoma cells to TRAIL-induced apoptosis through the down-regulation of *c-FLIP*, have raised many questions about the descending pathways.

Since the most plausible hypothesis appeared to be the activation by MS-275 of a repressor of *c-FLIP*, we investigated the impact of MS-275 on the expression of the canonical transcriptional repressors of *c-FLIP*, namely *c-myc* and *c-fos*, and the role they exert in the MS-275-triggered phenotype switching from a resistant to a sensitive type in mucosal, ocular and cutaneous melanoma cells.

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2. Materials and methods

2.1. Cell cultures and treatments

The uveal melanoma cell lines OCM-1 (provided by J. Mellon, Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX), OCM-3 and 92.1 (provided by Martine J. Jager, Leiden University Medical Center, Leiden, The Netherlands), and the cutaneous melanoma cell line GR-M (ECACC, European Collection of Cell Cultures, Salisbury, UK) were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 1% penicillin/streptomycin, and 10% FBS. WM266-4 cells, derived from a metastatic site of a malignant cutaneous melanoma, (obtained from the

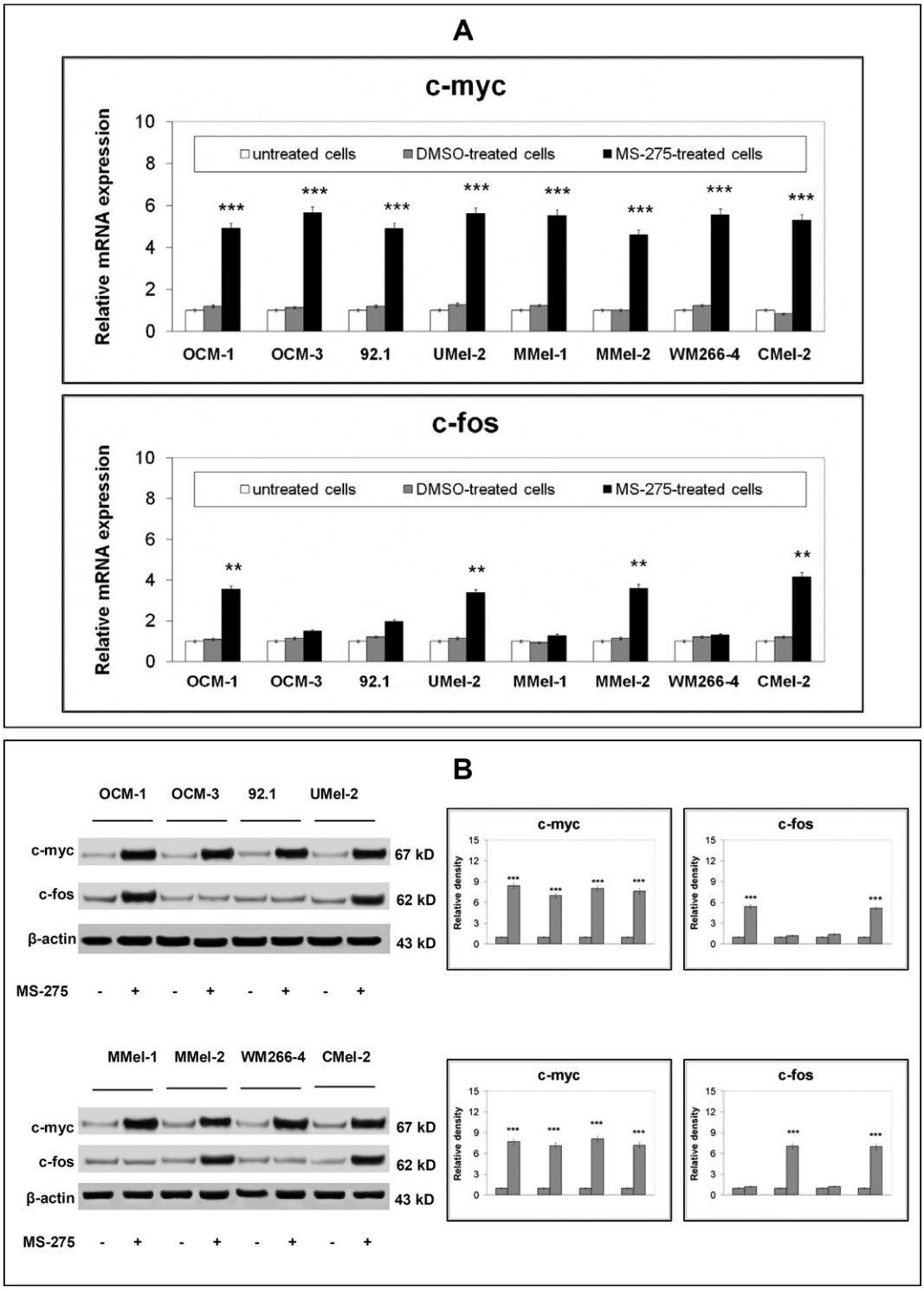

Fig. 1. Expression of c-myc and c-fos at both mRNA and protein levels in untreated and MS-275-treated melanoma cells. (A) Cells were treated with either DMSO vehicle control or 1 μM MS-275 for 24 h. Untreated cells were used as an additional control against DMSO. The mRNA expression levels of *c-myc* and *c-fos* were analyzed by real-time PCR and normalized by using the housekeeping gene β-actin as the internal control. Data are depicted as the mean ± SD of three independent experiments. Significant $^{**}p < 0.01$, and $^{***}p < 0.001$, as compared to untreated cells. (B) Cells were treated for 24 h with 1 μM MS-275 and protein expression levels of c-myc and c-fos were assessed by western blotting. β-Actin was used as an internal control for normalization. The relative densities were calculated by dividing the density of c-myc and c-fos bands by the density of β-actin band at the same point. Data are depicted as means ± S.E. of three independent experiments. Significant $^{***}p < 0.001$, as compared to untreated cells.