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Regulatory and Functional Connection of Microphthalmia-Associated Transcription Factor and Anti-Metastatic Pigment Epithelium Derived Factor in Melanoma[1]

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Abstract

Pigment epithelium-derived factor (PEDF), a member of the serine protease inhibitor superfamily, has potent anti-metastatic effects in cutaneous melanoma through its direct actions on endothelial and melanoma cells. Here we show that PEDF expression positively correlates with microphthalmia-associated transcription factor (MITF) in melanoma cell lines and human samples. High PEDF and MITF expression is characteristic of low aggressive melanomas classified according to molecular and pathological criteria, whereas both factors are decreased in senescent melanocytes and *naevi*. Importantly, MITF silencing down-regulates PEDF expression in melanoma cell lines and primary melanocytes, suggesting that the correlation in the expression reflects a causal relationship. In

Abbreviations: PEDF, pigment epithelium-derived factor; MITF, microphthalmia-associated transcription factor; RGP, radial growth phase of melanoma; VGP, vertical growth phase of melanoma; CM, cutaneous metastasis of melanoma; VM, visceral metastasis of melanoma; BRAF, v-raf murine sarcoma viral oncogene homolog B; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; OIS, oncogene induced senescence; hnRNA, heterogeneous nuclear RNA

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agreement, analysis of Chromatin immunoprecipitation coupled to high throughput sequencing (ChIP-seq) data sets revealed three MITF binding regions within the first intron of *SERPINF1*, and reporter assays demonstrated that the binding of MITF to these regions is sufficient to drive transcription. Finally, we demonstrate that exogenous PEDF expression efficiently halts *in vitro* migration and invasion, as well as *in vivo* dissemination of melanoma cells induced by MITF silencing. In summary, these results identify PEDF as a novel transcriptional target of MITF and support a relevant functional role for the MITF-PEDF axis in the biology of melanoma.

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Introduction

Pigment epithelium-derived factor (PEDF), a member of the serine protease inhibitor (SERPIN) superfamily, is a 50 kDa secreted glycoprotein that displays multiple biological activities relevant for cancer biology [1,2]. PEDF was first described as a pro-differentiation and survival factor in neuronal cells [3] and later was identified as the most potent endogenous inhibitor of angiogenesis in the eye [4]. Evidence accumulated over the last decade consolidated its role as a general anti-angiogenic factor in solid tumors [1,2]. Additionally, PEDF impinges on proliferation, survival, migration and differentiation of a broad spectrum of cancer cell types [1,2,5].

We have previously reported that PEDF has potent anti-tumor and anti-metastatic activities in mouse models of melanoma as a consequence of its combined actions: 1) in the tumor microvasculature; hindering melanoma neovascularization through direct actions on endothelial cells and indirect actions modulating the angiogenic profile of melanoma cells; 2) in the melanoma cells; restricting their survival, migratory and invasive capabilities [5,6]. Using a combination of gene expression profiling analysis and functional assays we have also demonstrated that loss of PEDF expression enables melanoma cells to acquire a migratory and invasive phenotype and consequently it contributes to the metastatic spread of melanoma [7].

Despite the demonstrated relevance of PEDF in melanoma biology, the mechanisms underlying the variations of PEDF levels during malignant progression of melanoma are still largely unknown. In this regard we have recently shown that hypoxia, a condition frequently found in solid tumors and associated to malignant progression, negatively regulates PEDF expression in melanocytes and melanoma cells [8].

Here, we aimed to investigate the regulatory mechanisms controlling PEDF expression in the context of melanoma malignization and melanocyte senescence.

Microphthalmia-associated transcription factor (MITF) has been established as a master regulator of melanocyte and melanoma biology [9–11]. MITF functions as a rheostat to determine different biological responses in melanocytic cells depending on the level of MITF expression and its post-translational regulation [12,13]. Very low MITF levels lead to G1 arrested cells with invasive and stem-like properties [12,14]. Moderate levels of MITF are required for melanocytic cell proliferation [12]. By contrast, elevated MITF levels drive the expression of lineage specific differentiation genes like *TYR* [9]. In the context of melanoma progression, decreased MITF levels lead to acquisition of invasive properties; whereas high MITF levels favor less aggressive melanoma cells likely more sensitive to therapeutic strategies [15].

The striking similarities in the multiple functional effects described for PEDF and MITF in melanocytic cells prompted us to investigate whether a regulatory and functional link between MITF and PEDF is operative in melanocytic cells.

Herein, we describe that the expression of PEDF and MITF positively correlates and varies with the pathological and molecular staging of melanoma and in the context of melanocyte senescence. Using a combination of approaches we demonstrate that MITF is a direct positive regulator of PEDF expression in melanocytic cells. And finally, we identify PEDF as a novel functional mediator of MITF in the control of melanoma cell migration, invasion and metastatic dissemination.

Materials and Methods

Cell Lines and Cell Culture

Human melanoma cell line 501mel was provided by C. Bertolotto (Institute de Génétique et de Biologie Moléculaire et Cellulair, Illkirch, France) and cultured as described [16]. WM278, WM164, WM88 and 1205Lu melanoma cell lines were provided by M. Herlyn (The Wistar Institute, Philadelphia, PA, USA) and cultured as described previously [17]. M000921, M080306 and M010308 melanoma cell lines were provided by K. Hoek (University Hospital of Zürich, Zürich, Switzerland) and cultured as described [18]. MaMel69, MaMel82 and MaMel26a1 melanoma cell lines provided by D. Schadendorf (Skin Cancer Unit of the Dermatology Department, University Hospital, University Duisburg-Essen, Germany) were cultured in RPMI (Gibco, Carlsbad, CA, USA) medium supplemented with 10% FBS. HEK293T cell line was cultured in DMEM (Gibco) medium supplemented with 10% FBS. Primary human melanocytes (NHEM) were obtained from Lonza (Basel, Switzweland) and cultured in MBM-4 medium with MGM-4 supplements (Lonza).

Melanoma cell lines were classified as weakly or highly aggressive according to the molecular profiling analysis by K. Hoek and collaborators [19]. Molecular profiling classification was validated by functional assays in representative cell lines from the weakly aggressive and highly aggressive cohorts [7,18].

Western Blot

Whole-cell extracts were prepared by lysing the cells in 125 mM Tris-HCl pH 7.5 and 2% SDS buffer containing protease and phosphatase inhibitors (10 μg/ml leupeptin; 10 μg/ml aprotinin; 1mM sodium orthovanadate; 1 mM PMSF (all from Sigma, St Louis, MO, USA)). Conditioned medium was concentrated as described [20]. Concentrated or direct conditioned medium and whole-cell extracts were separated by SDS-PAGE, transferred to PVDF membranes and