MIIP downregulates PD-L1 expression through HDAC6 in malignant melanoma

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Abstract

Background Immune checkpoint inhibitors have improved the objective response rate and survival of melanoma patients. However, there are still many melanoma patients suffering from disease progression due to primary or secondary immune checkpoint inhibitor resistance, as is observed in the failure of anti-PD1/PD-L1 therapy. It is urgent to determine the function of PD-1/PD-L1 expression in melanoma and its associated pathways to enhance the efficacy of anti-PD-1 therapies.

Methods A cohort of 133 patients with histologically confirmed melanoma from Tianjin Medical University Cancer Institute & Hospital were included in this study. We performed immunohistochemical staining to detect the expression of MIIP, HDAC6 and PD-L1. Kaplan-Meier and log-rank test were used for survival analyze. As for vitro, Western blot was used to verify the signaling pathway that MIIP regulates PD-L1 expression.

Results Our present data demonstrate MIIP expression was decreased in melanoma and that the negative expression of MIIP was correlated with worse overall survival. We also found the positive expression of HDAC6, a molecule that is downstream of MIIP, had a positive trend with decreased overall survival, because the p value was not statistically significant. At the same time, the positive expression of PD-L1, a crucial costimulatory molecule, was associated with decreased overall survival. Furthermore, there was a positive association between HDAC6 and PD-L1 protein expression ($p < 0.01$). In vitro experiment, we found that increasing MIIP led to decreased HDAC6, pSTAT3, and PD-L1 expression. Knocking down MIIP led to increased HDAC6, pSTAT3, and PD-L1 expression. Combining the published results, showing that HDAC6 can regulate PD-L1 expression through STAT3, our present data suggest that MIIP inhibits the expression of PD-L1 by downregulating HDAC6 in melanoma. Most importantly, methods for targeting MIIP-HDAC6-PD-L1 pathways, such as treatment with HDAC6 inhibitors, might indicate a new therapeutic approach for enhancing immune checkpoint inhibitor therapies in melanoma.

Conclusions Our findings highlight the immunomodulatory effects of MIIP in the inhibition of PD-L1 expression by downregulating HDAC6 in melanoma. Our data provide a sensible framework to consider the targeting of the MIIP-HDAC6-PD-L1 pathway.

Background

Malignant melanoma is a relatively common, aggressive tumor with an increasing incidence. It has a high mortality and a poor prognosis among patients with refractory and metastatic disease [1, 2]. In 2019, approximately 96,480 people were diagnosed with melanoma in the US, and estimated 7,230 patients died of this disease [3]. For patients with advanced and metastatic melanoma, the prognosis is still poor, The overall survival (OS) of 5 years is less than 10% with a median survival varying from 8 to 16 months [4]. Along with the rapid development of tumor immunology, treatment of melanoma by immunotherapy using the checkpoint inhibitors ipilimumab or the inhibitors of PD-1/PD-L1, e.g., nivolumab and pembrolizumab, shows great success in improving the prognosis for melanoma patients [5].

Immune evasion includes immune suppressive mechanism network in tumor microenvironment, which enables tumor cells to evade the recognition and attack of the immune system, thus allowing cell survival and proliferation [6]. CD8⁺ tumor infiltrating T lymphocytes (TILs) frequently express activated and produced inhibitory receptors, including PD-1 and CTLA-4 [7]. The immunosuppressive ligand PD-L1 (also known as B7-H1 or CD274) has been identified as a prognostic biomarker associated with poor survival and upregulated in many solid tumors [8]. The combination of PD-1 and PD-L1 deliver inhibitory signals that contribute to immune evasion, making this combination a potential immune checkpoint target [9]. In particular, several studies have indicated the effectiveness of anti-PD-1/PD-L1 treatment in patients with advanced malignant melanoma, which led PD-1/PD-L1 inhibitors to rapidly emerge as a common therapeutic option for advanced melanoma patients [10–12]. Treatment with immune checkpoint inhibitors of malignant melanoma patients exhibited a 5-year OS in 34% of the patients with a median OS of 23.8 months (95% CI, 20.2–30.4) and a 5-year PFS of in 21% of the patients with a median PFS of 8.3 months (95% CI, 5.8–11.1) [13]. However, immunotherapy is still largely ineffective in patients with tumors that have not been infiltrated by immune cells [13]. In addition, treatment-related AEs (TRAEs) occurred in more than 80% of melanoma patients [5]. Therefore, the discovery of new therapeutic methods and/or adjuvants that target multiple cellular processes is of special significance.

Migration and invasion inhibitory protein (MIIP), also known as invasion inhibitory protein 45 (IIP45) [14], was originally named for its ability to inhibit migration and invasion of tumor cells. It has recently been demonstrated to target downstream proteins to participate in tumor development and immune suppression [15–18]. In the yeast two-hybrid screen experiment to identify cellular regulators of insulin like growth factor binding protein 2 (IGFBP2), the promoter of human tumor cell migration, Song et al. first discovered MIIP gene and initially named IIP45 [14]. Furthermore, previous studies have demonstrated that MIIP can restrain the enzymatic activity of histone deacetylase 6 (HDAC6). Decreased acetylation activity of HDAC6 will result to α -tubulin acetylation and reduce cell migration [19]. In addition, MIIP was able to promote EGFR protein degradation and exert a negative effect on lung cancer cells' proliferation [20].

As a main downstream target of MIIP and critical factor of histone acetylation and deacetylation, increased expression or the destroyed functional integrity of HDAC6 is inseparable with tumor [21–23]. It has been reported that HDAC6 is overexpressed in malignant melanoma, bladder cancer and lung cancer [2, 24]. In addition to being able to play the role of deacetylated histones, HDAC6 can also participate in the occurrence and development of tumors by targeting nonhistone substrates, such as heat shock protein 90 (HSP90), α -tubulin, and cortactin [24]. Interestingly, HDAC6 is also involved in the production of MHC class I proteins, specific tumor-associated antigens, cytokines, and the expression of costimulatory molecules [25]. Furthermore, melanoma antigens mart1, TYRP2, gp100, and TYRP1 were increased in mRNA expression levels after using HDAC6 inhibitors (Nexturastat A or Tubastatin A) [25]. Additionally, the major targets of cancer immunotherapy, programmed death receptor-1 (PD-1) and programmed death receptor ligand-1 (PD-L1) are also regulated by HDAC6 [4].

Although HDAC6 has been confirmed to be involved in the regulation of PD-L1 expression, the relationship between MIIP and the immune system is still unclear. Our present data suggest that MIIP inhibits the expression of PD-L1 by downregulating the expression of HDAC6 in melanoma. Methods of targeting MIIP-HDAC6-PD-L1 pathways, such as treatment with HDAC6 inhibitors, might be a new therapeutic approach for enhancing immune checkpoint inhibitor

therapies in malignant melanoma. Thus, the already described participation of MIIP as an invasion-inhibitory protein and its new role as an upstream regulator of PD-L1 expression lays the foundation for its potential as a new treatment targeting MIIP-HDAC6-PD-L1 pathways.

Methods

Melanoma samples and Immunohistochemical Staining

All melanoma tissues and clinical information were collected from Tianjin Medical University Cancer Institute & Hospital. The present study was approved by the Institutional Ethics Committee of Tianjin Cancer Hospital. All patients signed written informed consent forms. All samples were evaluated by two pathologists to confirm the diagnosis and ensure that each specimen contained at least 90% of the tumor. The primary antibodies used were as follows: rabbit antibodies against human MIIP (HPA044948, Sigma, St. Louis, USA, 1:200 dilution), HDAC6 (# 7558S, CST, USA, 1:200 dilution) and PD-L1 [28–8] (ab205921, Abcam, USA, 1:200). An SP staining kit and a DAB reagent kit were purchased from Beijing Zhong Shan Golden Bridge Biotechnology.

All the immunohistochemical staining was observed by two senior pathologists who had no knowledge of the clinicopathological data. MIIP expression was assessed by staining intensity and the distribution of positively stained tumor cells as in previous studies [15, 26]. It was scored by semiquantitative system. The staining intensity (0 = no intensity, 1 = weak staining, 2 = medium staining, and 3 = strong staining) and proportion of positive melanoma cells on each view has at least 200 melanoma cells. The staining score was obtained by multiplying the percentage of positive cells by the staining intensity score, which ranged from 0 to 300. For HDAC6 staining, the staining intensity in tumor cells ranged from 0 to 3 (0 = no staining, 1 = weak staining, 2 = medium staining, and 3 = strong staining), and the percentage of positive cells was calculated as 0: no staining, 1: <25%, 2: ≥25 but < 50%, 3: ≥50 but < 75%, 4: ≥75%; Then, the positive cell proportion score was added to the cell staining intensity score and was classified based on the final summed score as follows: score of 0–3 = negative, score of 4–7 = positive. The expression of PD-L1 was considered positive when ≥ 5% of tumor cells were positive, according to previous studies [27–29].

Cell Lines, Cell Culture, Reagents, and siRNA Transfection

A875 and A375 melanoma cells (Beijing Cellular Research Institute, China) were cultured in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and the cells were incubated under 5% CO₂ at 37 °C. Overexpression of MIIP was achieved via transfection of Lipofectamine 3000 (Invitrogen) by following the manufacturer's instructions with MIIP vector (GenePharma, Shanghai, China) for 48 hours. Depletion of MIIP in A375 and A875 cells was achieved by transfection with two different pools of siRNAs targeting MIIP (Thermo Fisher Scientific, ID: #1-123298 and #2-127111) using Lipofectamine RNAiMAX (Life Technologies, Grand Island, NY, USA); the transfection occurred over 24 h according to the manufacturer's protocol.

Western Blot Analysis

After complete cell lysis, 30 µg of whole cell lysate was added to 10% polyacrylamide gels for electrophoresis. The cells were not treated with any cytokine. Primary antibodies for MIIP (#14472; Cell Signaling Technology, USA, 1:1000), HDAC6 (#13116; Cell Signaling Technology, USA, 1:1000), PD-L1 (#13684; Cell Signaling Technology, USA, 1:1000), and pSTAT3 (#8875; Cell Signaling Technology, USA, 1:1000) were used to analyze the expression of the proteins.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 software for Windows (SPSS, Inc., Chicago, IL). GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA) is used for graphing. The data are presented as the mean ± standard deviation of at least three independent experiments. The correlation of protein expression with clinicopathological characteristics was determined by Pearson's chi-square test or Fisher's exact tests. Correlation analysis between proteins was evaluated by Spearman's rank correlation. Survival curves were generated by the Kaplan-Meier method and a log-rank test. Cox proportional hazard method was used to determine independent predictors of survival in univariate and multivariate analyses. Confidence intervals (95%) were calculated. All the tests were bilateral tests. The indicated annotations correspond to the following P-values: *P < 0.05, **P < 0.01, and ***P < 0.001.

Results

Protein expression levels of MIIP, HDAC6 and PD-L1 and their correlations with clinicopathological parameters in malignant melanoma

The positive staining of MIIP and HDAC6 was mainly located in the cytoplasmic of cancer cells in melanoma tissues (Fig. 1a, b). PD-L1 was mainly expressed in the cytoplasmic and membrane of melanoma cells (Fig. 1c). Among the 133 malignant melanoma tissues, positive expression of MIIP could be detected in only 27.07% (36/133) patients. Positive expression of HDAC6 and PD-L1 was present in 61 (61/133, 45.86%) and 78 (78/133, 58.65%) cases, respectively.

We next investigated the associations of MIIP, HDAC6 and PD-L1 expression with clinicopathological parameters in malignant melanoma (Table 1). According to the results, the proportion of positive expression of PD-L1 was significantly higher in patients with at a higher Clark level [82.8% (53/64)] than it was in patients at a lower Clark level [56.5% (39/69)] (chi-square test, $p < 0.01$, Table 1). However, there were no statistically significant associations between MIIP, HDAC6 and PD-L1 expression level and patients' genders, ages, serum LDH level, tumor size, or pathological TNM stages (chi-square test, $p > 0.05$, Table 1).

Table 1
Correlation of MIIP, HDAC6 and PD-L1 expression levels with clinicopathological variables in melanoma.

Clinical parameters	MIIP				HDAC6				PD-L1			
	Positive expression	Negative expression	χ^2	P	Positive expression	Negative expression	χ^2	P	Positive expression	Negative expression	χ^2	P
Sex			0.369	0.657			0.012	1.000			0.406	0.568
male (80)	17	63			37	43			57	23		
female (53)	9	44			24	29			35	18		
Age			0.076	0.811			0.303	0.701			0.088	0.837
≥ 55 years (95)	18	77			45	50			65	30		
< 55 years (38)	8	30			16	22			27	11		
Clark stage I-II (69) III-IV (64)	12 14	57 50	0.424	0.642	30 31	39 33	0.329	0.604	39 53	30 11	10.763	0.001
Tumor size			0.121	0.769			0.411	0.542			0.491	0.511
≥ 2 cm (44)	9	35			21	30			29	15		
< 2 cm (51)	9	42			21	23			37	14		
AJCC stage			0.000	1.000			0.005	1.000			3.559	0.069
I-II (92) III-IV (41)	18 8	74 33			42 19	50 22			59 33	33 8		
Serum LDH			0.615	0.453			0.003	1.000			0.260	0.669
high (33)	8	25			15	18			24	9		
normal (100)	18	82			46	54			68	32		
Chemotherapy			0.000	1.000			0.685	0.454			0.306	0.685
yes (92)	18	74			40	52			65	27		
no (41)	8	33		0.181	21	20		0.377	27	14		0.568
Recurrence or Metastasis			2.253				0.915				0.406	
yes (53)	19	61			34	46			57	23		
no (80)	7	46			27	26			35	18		

Melanoma patients with negative MIIP and positive PD-L1 expression have worse overall survival

Among the 133 malignant melanoma patients, the median follow-up time was 55.7 months (1.7–123.6 months), and there were 69 cancer-related deaths during the follow-up time. Kaplan-Meier analysis showed that OS in the positive MIIP expression group was longer than that in the negative MIIP expression group ($p = 0.042$) (Fig. 1d). In addition, patients with positive PD-L1 expression exhibited a shorter OS time than those with negative PD-L1 expression ($p = 0.01$) (Fig. 1f). A similar tendency was obtained for HDAC6, although statistical significance was not observed ($p = 0.718$) (Fig. 1e).

Correlation among MIIP, HDAC6 and PD-L1 expression

By analyzing the correlation between MIIP, HDAC6 and PD-L1 protein expression in Tissue Microarray (TMAs) constructed from 133 melanoma patient tissues, we demonstrated that HDAC6, a downstream molecule of MIIP, was positively associated with PD-L1 expression ($p = 0.028$, Table 2). Furthermore, we also found that when MIIP expression was positive, HDAC6 and PD-L1 were negative (Fig. 2a). Moreover, when MIIP expression was negative, HDAC6 and PD-L1 protein levels were positive (Fig. 2b). Thus, in malignant melanoma, there were positive trends in MIIP and HDAC6 protein expression as well as MIIP and PD-L1, although the statistical significance was not observed ($p = 0.640$, Table 3, $p = 0.322$, Table 4). Therefore, we speculated that in melanoma, MIIP might be involved in regulating PD-L1 expression through the MIIP-HDAC6-PD-L1 pathway.

Table 2

The positive correlation of HDAC6 and PD-L1 protein expression in melanoma.

	PD-L1	r	p
	(-) (+)		
HDAC6		0.191	0.028
(-)	36 36		
(+)	19 42		

Table 3

The correlation of MIIP and HDAC6 protein expression in melanoma.

	HDAC6	r	p
	(-) (+)		
MIIP		-0.041	0.640
(-)	59 48		
(+)	13 13		

Table 4

The correlation of MIIP and PD-L1 protein expression in melanoma.

	PD-L1	r	p
	(-) (+)		
MIIP		-0.087	0.322
(-)	42 65		
(+)	13 13		

MIIP regulates PD-L1 expression by downregulating HDAC6

To verify our speculation that MIIP regulates the expression of PD-L1 through HDAC6, the human malignant melanoma cell lines A875 and A375 were used as an in vitro model. For in vitro experiments, human melanoma cell lines were transfected with an MIIP overexpression vector. In melanoma cells that overexpressed MIIP, we observed a lower expression of HDAC6 and a decrease in phosphorylated STAT3 (Y705); we also observed a decrease in expression of PD-L1 (Fig. 3a). To ensure that off-target effect was not affecting our analysis, we used two different siRNAs to target MIIP for side-by-side experiments. The results showed that decreases in MIIP by siRNA treatment led to increased HDAC6 and pSTAT3 (Y705) activation, along with increased PD-L1 expression (Fig. 3b). These in vitro data provide further evidence that MIIP downregulates PD-L1 expression through HDAC6 in malignant melanoma. Thus, based on in vivo and in vitro data, we suggest that in malignant melanoma, MIIP downregulates PD-L1 expression through HDAC6 (Fig. 4).

Discussion

Anti-PD-1 treatment have achieved advanced success in malignant melanoma. While the expression of valuable markers, such as TMB, MSI, and PD-L1, could serve as effective predictors of anti-checkpoint inhibitor therapies, not all melanoma patients are effective to this treatment. In a recent study, the objective response rate (ORR) is 41% in all melanoma patients and 52% for treatment-naïve melanoma patients [5]. Additionally, treatment-related AEs (TRAEs) occurred in 86% of patients and resulted in study discontinuation in 7.8% of patients; 17% experienced grade 3/4 TRAE [5]. Although immunotherapy has greatly improved the prognosis of patients, immunotherapy remains largely ineffective in patients with tumors not infiltrated by immune cells. In order to incrementally advance the immunotherapeutic options in melanoma treatment, the discovery of new therapeutic methods and/or adjuvants that target multiple cellular processes shows special significance.

Previous studies have reported that MIIP plays a role as a tumor suppressor gene. In gliomas, through binding to HDAC6, highly expressed MIIP causes decreased HDAC6 expression and inhibition of HDAC6 deacetylase activity, thereby inhibiting HDAC6-mediated cell migration [19]. The tumor suppressor function of MIIP has been demonstrated in tissues from breast cancer [30] and colorectal cancer [31]. In the current study, we verified that melanoma patients exhibited a negative MIIP expression and predicted worse overall survival when compared with patients with a positive MIIP expression. We also found the positive expression of HDAC6, a molecule that is downstream of MIIP, had a positive trend with decreased overall survival, because the p value was not statistically significant. The reason for this inconsistency may be due to our insufficient sample size and rough melanoma subtype classification. We will collect more samples and perform more precise subtype classification to explore the role of HDAC6 in melanoma. At the same time, the positive expression of

PD-L1, an important costimulatory molecule expressed in cancer cells, was associated with worse overall survival. Furthermore, there was a positive association between HDAC6 and PD-L1.

When we studied the relationship between the expression levels of MIIP, HDAC6 and PD-L1 and the clinicopathological factors in melanoma patients, a correlation between PD-L1 and melanoma cell Clark stage was identified. The expression rate of PD-L1 was significantly greater in higher Clark levels [82.8% (53/64)] than it was in lower Clark levels [56.5% (39/69)] (chi-square test, $p < 0.01$). The Clark stage classification is defined by measuring the depth of skin invasion of melanoma cell to the anatomical level. And it provides a correlation between the degree of skin invasion by melanoma and the 5-year survival rate after surgery. In malignant melanoma, the relationship between PD-L1 expression and Clark stage may exhibit a greater likelihood of malignant behaviors. However, this needs further study for validation.

Some HDACs have received particular attention for their recently endowed roles in regulating tumorigenesis and immune response [32, 33]. However, HDAC's ability to regulate cellular immune microenvironment and their therapeutic potential as targeted agents combined with immunotherapy are not clear. Histone deacetylases (HDACs) and selective HDAC inhibitors (HDACi), alone or in combination with other anti-cancer agents, are promising therapeutic methods in many cancers [34–37]. As a major transcription factor regulating PD-L1, Lienlaf et al demonstrated that HDAC6 was crucial adjective for the recruitment and activation of STAT3 and the upregulation of PD-L1. Additionally, a study has demonstrated that in the mouse model of B16F10 immunotherapy, HDACi combined with PD-1/PD-L1 checkpoint inhibitors can significantly improve the therapeutic effect of immunotherapy alone [38] In multiple myeloma, the expression of PD-L1 is immediately correlated with disease progression. By contrast, the highest PD-L1 expression was observed in patients with recurrent / refractory multiple myeloma.[39] ACY-241, the HDAC6 selective inhibitor, combined treatment with anti-PD-L1 treatment can enhance anti-multiple myeloma immunity in the bone marrow microenvironment through down-regulating the interaction between pDC-T cell and pDC-NK cell.[36] A recent study also showed that a HDAC6i, ricolinostat, promoted phenotypic changes that supported the activation of T cells and improved the function of antigen presenting cells.[40] Furthermore, The use of histone deacetylase 6 (HDAC6) inhibitors limited the growth of ovarian cancer with mutations in ARID1A, which is the most common mutations in human cancer epigenetic regulation factor; Mutations in ARID1A occur in more than 50% of ovarian clear cell carcinomas and it modulates the tumor immune microenvironment.[41] Regarding our present study which suggests that MIIP inhibits the expression of PD-L1 by downregulating the expression of HDAC6 in melanoma, methods that target MIIP-HDAC6-PD-L1 pathways, such as treatment with HDAC6, might provide a new therapeutic approach to enhance immune checkpoint inhibitor therapies in malignant melanoma.

In addition to HDAC6, MIIP can also regulate the expression of insulin-like growth factor-binding protein 2 (IGFBP2), and it can accelerate epidermal growth factor receptor (EGFR) protein turnover and attenuate proliferation [14, 20]. Accumulating evidence has suggested that IGFBP2 modulates the immune response in cancer patients and can be a potential target for cancer immunotherapy [42]. Although an IGFBP2 vaccine was shown to be immunosuppressive, removing the IL-10-inducing T helper epitopes from the vaccine was suggested to ensure potent IGFBP2 anti-tumor activity [43]. Furthermore, many clinical trials have investigated EGFR-mediated tumor immune escape as a target for immunotherapy through the use of immune checkpoint inhibitors. Concha-Benavente et al. (2013) found that overexpression of EGFR in response to IFN- γ through the JAK2/STAT1 pathway upregulated PD-L1 expression and that specific inhibition of JAK2 abolished PD-L1 upregulation in head and neck cancer. In another study, the mutated and constitutively active EGFR/KRAS-MAPK pathway was suggested to cause upregulation of PD-L1 in non-small-cell lung cancer [44]. However, as an upstream gene of IGFBP2, HDAC6 and EGFR, the mechanism of MIIP involvement in immune regulation requires further investigation.

Conclusion

Our findings highlight the immunomodulatory effects of MIIP, which include the inhibition of the expression of PD-L1 by downregulating the expression of HDAC6 in melanoma. Our data provide a sensible framework to consider the targeting of the MIIP-HDAC6-PD-L1 pathway. For example, HDAC6 might indicate a new therapeutic approach to enhance immune checkpoint inhibitor therapies in malignant melanoma.

Abbreviations

PD-1: programmed cell death 1; PD-L1; programmed cell death-ligand 1; STAT3: signal transducer and activator of transcription 3; MIIP: migration and invasion inhibitory protein; HDAC6: Histone deacetylase 6; DMEM: Dulbecco-modified essential medium; FBS: fetal bovine serum; OS: overall survival.

Declarations

Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee of Tianjin Cancer Hospital. All patients signed written informed consent forms.

Consent to publish

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to data privacy according to the license for the current study but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

JLY designed the study and provided funding for the study. RWX and TL participated in the entire experiment and writing and modifying the manuscript. HTL, JQW, JL participated in the editing and revision of the manuscript. LJX participated in the statistical analysis of the data. All authors read and approved the manuscript.

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Figures

Figure 1

The expression of MIIP, HDAC6, and PD-L1 and their relationship with overall survival. (a) Positive MIIP staining in melanoma patient tissue samples was detected by immunohistochemistry (36/133); (b) Positive HDAC6 staining in melanoma patient tissue samples was detected by immunohistochemistry (61/133); (c) Positive PD-L1 staining in melanoma patient tissue samples was detected by immunohistochemistry (78/133); (d) TMA data from 133 melanoma patient tissues showed that a negative MIIP expression was associated with worse OS ($p < 0.05$); (e) TMA data from 133 melanoma patient tissues showed that a positive trend between positive HDAC6 expression and worse OS, statistical significance was not observed ($p=0.718$); F, TMA data from 133 melanoma patient tissues showed that a positive level of PD-L1 expression was associated with worse OS ($p < 0.05$).

Figure 2

The expression of MIIP, HDAC6, and PD-L1 and their relationship. (a) When MIIP expression was positive (left), HDAC6 protein levels showed negative expression (middle), PD-L1 was negative as well (right). (b) When MIIP expression was negative (left), HDAC6 (middle) and PD-L1 (right) showed positive expression levels, although statistical significance was not observed ($p=0.064$ and $p=0.322$, respectively).

Figure 3

MIIP regulates PD-L1 expression through HDAC6/STAT3. (a) In both the A875 and A375 cell lines, overexpressing MIIP by transfecting a MIIP plasmid induced significant decreases in HDAC6, pSTAT3, and PD-L1 compared with that of the control cells. (b) Decreased MIIP expression following MIIP siRNA treatment induced increased HDAC6, pSTAT3, and PD-L1. Western blot analysis was performed to analyze the effects in cells after MIIP was knocked down by two different siRNAs (si-MIIP #1 and si-MIIP #2) for 24 h, and the results are compared with controls (si-control). The original, full-length images were added as supplementary files.

Figure 4

Summary of the role of MIIP in regulating PD-L1 expression through HDAC6/STAT3 in malignant melanoma. The expression of HDAC6 is downregulated by MIIP, resulting in the inactivation of STAT3 on the PD-L1 promoter, which further inhibits the transcriptional activation of PD-L1. As a result, MIIP downregulates the expression of PD-L1 in malignant melanoma.

Supplementary Files

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