Expression of Pdcd4 Tumor Suppressor in Human Melanoma Cells

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Abstract. Background: Programmed cell death 4 (Pdcd4) is a tumor suppressor frequently lost in tumors of various origins thus contributing to tumor progression. Expression of Pdcd4 in melanoma, however, has not been extensively studied to date. Materials and Methods: Pdcd4 protein levels were assessed in 23 human melanoma cell lines and in normal melanocytes by western blot analysis. Also, effects of LY294002, rapamycin and PD098059 on Pdcd4 protein levels were analyzed. Results: Pdcd4 is suppressed in ~25% of human cell lines established from advanced melanoma lesions. Pdcd4 protein levels in melanoma cells were up-regulated by treatment with inhibitors of Akt signaling, one of the key pathways leading to Pdcd4 suppression, and to a lesser extent by inhibiting MEK/ERK pathway. Conclusion: Pdcd4 loss is not a common event in melanoma progression yet suppression of Pdcd4 defines a subset of melanoma cells and can be used for molecular typing of melanoma. Our results help determine the significance of Pdcd4 loss in melanoma as well as its up-regulation by Akt pathway inhibitors, which are promising tools in melanoma treatment.

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In the present study firstly we questioned whether Pdcd4 is expressed in human normal epidermal melanocytes. Anti-Pdcd4 antibodies readily detected a single ~60 kDa protein band in human epidermal melanocyte lysate (Figure 1a) which coincides with the expected mobility of Pdcd4 protein (2). Thus Pdcd4 is expressed in normal melanocytes of the

Results and Discussion

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Western blot analysis. For Western blotting, cell lysates were resolved in 10% SDS-PAGE and transferred to Hybond P membrane (GE Healthcare, Little Chalfont, UK). Western blot analysis was performed with primary rabbit polyclonal anti-Pdcd4 (2) and mouse monoclonal anti-α-tubulin, clone DM1a (Sigma, St. Louis, MO, USA) antibodies. Primary rabbit anti-Pdcd4 and mouse anti-α-tubulin antibodies were detected with secondary anti-rabbit and anti-mouse horseradish peroxidase-conjugated antibodies (GE Healthcare, Little Chalfont, UK), respectively, and Immobilon™ Western Chemiluminescent HRP Substrate (EMD Millipore Corporation, Billerica, MA, USA). ChemiDoc XRS gel documentation system (BioRad, Hercules, CA, USA) was used for image acquisition.
human skin. Next, we evaluated Pdcd4 levels in 23 cell lines established from human melanoma lesions and compared them to Pdcd4 levels in normal epidermal melanocytes. In 6 cell lines, Pdcd4 protein levels were substantially lower than those in normal melanocytes, with Pdcd4 found moderately decreased in 3 additional cell lines (Figure 1b). In the remaining 14 cell lines Pdcd4 protein levels were similar to or occasionally higher than those in normal melanocytes. Thus, substantial loss of Pdcd4 is observed in approximately a quarter of melanoma cell lines. As all cell lines were established from lesions of advanced-stage melanoma, this likely indicates that the loss of Pdcd4 expression is not essential for melanoma progression.

MicroRNA-dependent suppression is one of the main mechanisms leading to decreased Pdcd4 protein levels in tumor cells (20). However, neither miR-21 nor miR-183 capable of targeting Pdcd4 transcripts were identified as up-regulated in melanoma genesis and progression (21), suggesting that other mechanisms are responsible for Pdcd4 down-regulation in melanoma cells. Akt signaling which is another key pathway responsible for Pdcd4 suppression, is frequently activated in melanomas and contributes significantly to tumor progression (22). Thus, we assayed if blocking upstream Akt activator, PI3 kinase, with LY294002 or its downstream target mTORC1 essential for Pdcd4 deregulation, with rapamycin, results in up-regulation of Pdcd4 in melanoma cells. Treatment of Mel_253, Mel_Ch and Mel_R cells which have low Pdcd4 level (Figure 1b) with LY294002 or rapamycin resulted in up-regulation of Pdcd4 (Figure 2a). A similar effect was observed in Mel_Kor and Mel_Cher cells with moderately decreased Pdcd4 levels, and in Mel_Me and Mel_Si cells in which Pdcd4 levels remained unchanged compared to normal melanocytes (Figure 2b and 2c). At the same time, neither inhibitor affected Pdcd4 level in Mel_335.2 and Mel_P cells (Figure 2d) which are characterized by elevated Pdcd4 expression (see Figure 1b). Thus, Akt signaling contributes to Pdcd4 suppression in melanoma cells, and its inhibition results in a pronounced

Figure 1. Pdcd4 expression in normal human melanocytes and in human melanoma cell lines. Results of western blot analysis of normal human epidermal melanocyte (HEM) lysates (a) and indicated melanoma cell lines (b) with anti-Pdcd4 antibodies are shown. Protein molecular weight markers (MW) are shown on the left. Membranes were also stained with anti-α-tubulin antibodies used to monitor for total protein loading.
up-regulation of Pdcd4 not only in cells characterized by low levels of Pdcd4 but also in those with apparently unaltered levels. As Pdcd4 expression is known to suppress cell proliferation and facilitate apoptosis (5, 11, 12, 13), up-regulation of Pdcd4 might be one of the mechanisms of anti-tumor action for Akt pathway inhibitors, which constitute promising tools for treatment of melanoma (21).

Akt and mTORC1 are believed to suppress Pdcd4 post-translationally, resulting in Pdcd4 phosphorylation which primes it for ubiquitination (23) as well as directly suppresses Pdcd4 gene transcription (24). Besides Akt signaling, MEK/ERK cascade was reported to contribute to Akt-dependent Pdcd4 degradation by facilitating proteasomal degradation of ubiquitinated Pdcd4 (15). Inhibiting MEK1/2 with PD098059 indeed resulted in elevated levels of Pdcd4 in melanoma cells with intrinsically suppressed Pdcd4 protein although the effect was not so pronounced as for rapamycin (Figure 2a). No effect of PD098059 was observed in Mel_Kor, Mel_Cher, Mel_Me and Mel_Si cells in which blocking Akt/mTOR signaling resulted in Pdcd4 up-regulation (Figure 2b and 2c). These results might indicate that pronounced loss of Pdcd4 in melanoma cells is in part due to MEK/ERK-dependent facilitated proteasomal degradation.

In summary, we found that Pdcd4 protein level is significantly suppressed in approximately a quarter of the cell lines established from advanced-stage melanoma lesions. As melanoma is a diverse group of tumors (25), Pdcd4 expression due to its variability in melanoma cells can be potentially used as a molecular parameter for molecular melanoma typing. The loss of Pdcd4 in melanoma, at least in part, depends on activated Akt signaling, and up-regulation of Pdcd4 upon Akt signaling inhibition might contribute to anti-tumor effects of Akt pathway inhibitors currently recognized as a promising tool for melanoma treatment. Our findings warrant further studies aiming to establish role of Pdcd4 loss for melanoma progression and to investigate specific features of melanoma cells with different Pdcd4 expression status.

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References


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