PD-L1 Upregulation by UVB in Melanoma

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Abstract

Increasing evidence shows that UV radiation-induced DNA damage causes most skin cancer, among which melanoma is the most deadly form. Besides inducing melanomagenesis, UV suppresses the immune system in several ways, including inhibition on antigen presentation, release of immunosuppressive cytokines, and apoptosis of immune cells, which impair the self-removal of tumor cells. In recent years, a great breakthrough in melanoma treatment in immunotherapies has been performed for patients with advanced melanoma. An important immunotherapeutic target for melanoma is the programmed death ligand 1 (PD-L1/PD-1) pathway. PD-L1 binds to its receptor of PD-1 to suppress T-cell function and to inhibit the autoimmune disease. However, cancer cells could upregulate PD-L1 to inhibit anti-tumor immunity in microenvironment, which contributes to evading the immune system. Increased PD-L1 expression is observed in many tumors, including melanoma. Here, we use biochemical experiment and cellular biology study to analyze if the PD-L1 expression is related with UVB exposure. The western blot showed that UVB exposure phosphorylated and activated ERK and AKT, which are important in cellular proliferation and survival, but always inappropriately activated in human cancers. Moreover, flow cytometry assay indicated that UVB exposure has resulted into the upregulation of PD-L1 in melanomas. Our works suggests a novel molecular mechanism of UVB-induced immune suppression through induction of PD-L1 upregulation in melanoma, which provided an important insight into the melanoma research and immunotherapy.

Introduction

Role of PD-L1

Figure 1: The role and function of PD-L1 in tumor cells. PD-L1 is needed in the body to regulate the immune system, with the function of preventing autoimmune disease caused by the immune system overworking. PD-L1 works by binding to the PD-1 protein in T-cells to inhibit T-cells function. Tumor cells upregulate PD-L1 to suppress the function of T-cell, leading the tumor cell to escape from immune system. The medications have been developed to block the PD-L1/PD-1 signaling pathway to increase the tumor-rejection immunity (Casey, S. C., et al, 2007).

Role of Phosphorylated AKT

Figure 2: The role of phosphorylated AKT and ERK. When AKT or ERK are phosphorylated, they become active. In tumor cells these pathways show abnormal activated. The activated pathways are contributing to cell growth, proliferation, motility invasion and survival.

Results

Figure 3: UVB induced activation of AKT pathway. A375 treated with UVB were used for the whole cell protein extract, which were applied for the western blot to test activated phosphorylated AKT, with β-actin as a loading control.

Figure 4: PD-L1 is upregulated by UVB. A375 cells treated with UVB were assayed with flow cytometry for PD-L1 expression. Compared to the cells where UVB was not present, when UVB was present, 97.8% of the cells showed PD-L1 positive.

Methods

A375 cells are common human malignant melanoma cells found in skin. Western blot was used to assay the expression of phosphorylated and total AKT following UVB exposure, a common trigger of melanoma. Flow cytometry was used to test the expression of UVB-induced PD-L1 in A375.

A375 cells were treated with UVB exposure. After 0.5 hour the whole cell extract were prepared for the western blot assay. The proteins went through the gel electrophoresis, before the protein were transferred to the membrane. Specific antibodies of anti-p-AKT and anti-AKT were used to detect the proteins amount, with anti-β-actin using as the loading control.

Flow cytometry is a system for sensing individual cells in a physiological saline solution as they move in a focused liquid stream through a laser beam and emit fluorescence that is measured and converted to digital data. Flow cytometry takes advantage of cells fluorescing after excitation from a laser light. Data produced by flow cytometry is clear, cohesive, efficient, and statistically powerful. To detect a particular protein using flow cytometry, a fluorochrome can be used to stain a protein of interest. Then the fluorochromes can detect light that target proteins emit from being excited by the laser. Sensors called photomultiplying tubes (PMTs) are able to detect the fluorescence only at a specified wavelength. A375 cells treated with UVB exposure for 4 hours, and in the presence of external PD-L1/FITC were examined.

Conclusion

• UVB exposure activates AKT pathway with upregulation of p-AKT
• PD-L1 production is upregulated by UVB in melanomas

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