Introduction

The protein Cluster of Differentiation 74 (CD74) is a type II transmembrane molecule that acts as a receptor for macrophage migration inhibitory factor (MIF) when it displays on the cell surface. The interaction between MIF and CD74 initiates oncogenic signaling in cancer to control the growth of a tumor. Our previous study showed this interaction was relevant in advanced melanoma1 (Fig. 1). CD74 alone does not function to signal these pathways but does so with help of others including CD44, a non-kinase transmembrane glycoprotein, acting as a transducing co-receptor. Although it has been demonstrated that CD74 has four different isoforms (p31, p33, p41, and p43)2, it is unclear which isoform makes this complex with CD44 to initiate the oncogenic signaling pathways in melanoma.

Objectives

Our group has previously shown that autocrine MIF-CD74 interactions in response to interferon-γ (IFN-γ) induced oncogenic pAKT pathway and tumor progression in cutaneous melanoma3. However, we have not shown whether the interaction between MIF and CD74/CD44 binding induces the oncogenic signaling.

In this proposal, we aim to identify
(1) if the binding of MIF/CD74/CD44 signaling is involved in oncogenic pAKT pathways
(2) which CD74 isoform makes the complex with CD44 in melanoma.

Methods

Cell lines:
A375 (BRAF-mutant melanoma cell line),
SB2 (NRAS-mutant melanoma cell line)

Experimental overview
(1) Induction of the cell surface CD74 by recombinant human (rh) IFN-γ stimulation.
[Methods] We stimulated A375 and SB2 with different concentration of rhIFN-γ (0, 20, 100, and 500 IU/mL) and evaluated the CD74 expression by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), western blotting (WB), and flow cytometry.
(2) Activation of the AKT pathway by the binding of MIF to cell-surface CD74, induced by IFN-γ.

Methods (continued)

[Methods] We pre-treated A375 and SB2 with 100 IU/mL rhIFN-γ (no IFN-γ stimulation for control) for 24 h to induce the cell-surface CD74 expression and sequentially stimulated by 100 ng/mL rhMIF. After 0, 10, 30, and 120 min exposures to rhMIF, we prepared the cell lysates to evaluate p-AKT expression by WB.
(3) Interaction between MIF and CD74/CD44 binding is involved in the AKT pathway.

[Methods] The change in p-AKT expression 0, 10, and 30 min after 100 ng/mL rhMIF exposure was evaluated by WB with knocking down of CD74 and CD44 (as single and double) by short interfering (si)RNA in A375 and SB2. Scramble (SC) siRNA was used as a control.

Results

(1) Induction of the cell surface CD74 by IFN-γ stimulation.
CD74 mRNA expression was dramatically upregulated in response to IFN-γ in A375 and SB2 by q-RT PCR (Fig. 2A). The upregulation of 3 isoforms of CD74 (p33, p35, and p41) under IFN-γ stimulatory conditions were found in A375 and SB2 by WB (Fig. 2B). On the contrary, the CD44 expression was not affected by IFN-γ (Fig. 2B). In addition, flow cytometry analysis revealed that the cell-surface CD74 was induced by IFN-γ, particularly in SB2 (Fig. 2C).
(2) Activation of the AKT pathway by the binding of MIF to cell-surface CD74, induced by IFN-γ.
In order to explore if binding of MIF to cell-surface CD74 initiates AKT signaling, we compared the p-AKT expression 0, 10, 30, and 120 min after rhMIF exposure under basal and IFN-γ stimulatory conditions. As shown in Fig. 3, the upregulation of p-AKT expression after rhMIF stimulation was more marked in A375 and SB2 pre-treated with rhIFN-γ than in those cultured in basal conditions, particularly clear in SB2. These results indicate the interaction between MIF and the cell-surface CD74, induced by IFN-γ, activates oncogenic signaling pathway in melanoma.
(3) Interaction between MIF and CD74/CD44 binding is involved in the AKT pathway.

Conclusions

The present study showed that the three isoforms of CD74 (p33, p35, and p41) was upregulated by IFN-γ, and the interaction between MIF and CD74/CD44 was associated with the activation of the AKT signaling pathway in melanoma.

We are currently studying to identify the isoforms of CD74 (p33, p35, and p41) that complexes with CD44. Given that CD74 also works as a chaperon for MHC-class II, it may be unfavorable to inhibit the functions of all isoforms of CD74.

In this regard, our result may not only provide a better understanding of the signaling pathways, but also help develop the therapeutic drug against the specific isoform of CD74 that is associated with the promotion of oncogenic signaling in melanoma.

References