Bcl-w, a Radio-resistant Protein, Promotes the Gastric Cancer Cell Migration by inducing the phosphorylation of Focal Adhesion Kinase

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1. Introduction

Gastric cancer is one of the leading malignancies in many countries and lethal for the high incidence of recurrence even after drastic surgical resection. Because local invasion and subsequent metastasis contributes to the failure of anticancer treatments of gastric cancer, a better understanding of the mechanisms involved in tumor invasiveness within the stomach seems to be essential for the control of this disease.

Bcl-w is a prosurvival member of the Bcl-2 protein family [1, 2], and thus protects cells from γ-irradiation [3]. Recent reports suggest that Bcl-w can be up-regulated in gastric cancer cells in a manner associated with the infiltrative (diffuse) types of the tumor [4]. An analysis of Bcl-w function consistently revealed that Bcl-w can also promote the migratory and invasive potentials of gastric cancer cells [5]. While it was shown that Bcl-w increases the invasiveness of cancer cells by sequentially inducing PI3K, Akt, SP1, and MMP-2 [5], cellular components involved in Bcl-w-induced cell migration remain to be determined. This was the reason why we undertook the present study, which shows that FAK is a critical mediator of the cell migration induced by Bcl-w.

2. Methods

2.1 Cell culture and transfection

Human SNU-484 gastric adenocarcinoma cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and gentamicin (50 ug/ml). Expression constructs were prepared using pcDNA vectors and transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Transfected cells were selected by using 2 mg/ml G418 sulfate.

2.2 Wound healing assays

Cell were harvested with buffered EDTA and plated into 12-well plates (6×10^4 cells/well). The confluent monolayer were scratched after 24h and then allowed to migrated for 24hours at 37 °C. Cell in five fields in the scratched area (200×500 um^2 area) were counted under a light microscope [6]. Results were analyzed for statistical significance using Student's *t* test. Differences were considered significant at $P < 0.05$.

2.3 Western blot analysis

Proteins either in conditioned media or in cell lysates, prepared using a previously described method [7], were separated by SDS-PAGE, and electrotransferred to Immobilon membranes (Millipore, Bedford, MA), which were subsequently blotted using the indicated antibodies and visualized by the enhanced chemiluminescence detection system (Amersham, Uppsala, Sweden).

3. Results

3.1 Bcl-w enhances SNU-484 cell migration.

We previously reported that Bcl-w increased gastric cancer cells, SNU-484 invasion and migration [5]. However, to confirm previous data, the control and Bcl-w-induced cells were compared by wound healing assay (Fig 1). The data reconfirmed that Bcl-w can promote the migratory potentials of SNU-484.

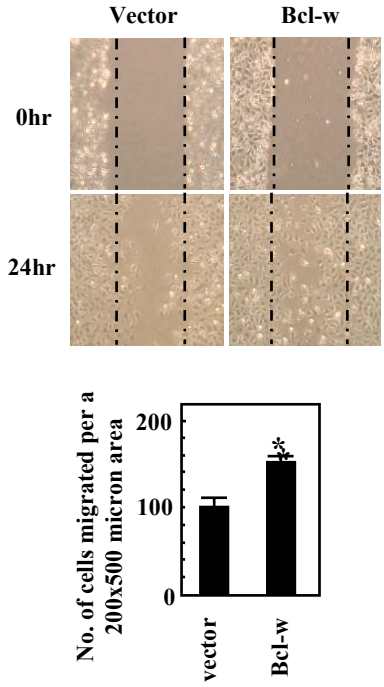

Fig. 1. Bcl-w promotes the migration of SNU-484 gastric cancer cells. *Top*, monolayers of SNU-484 cells

were scratched, photographed, washed, and rephotographed after 24h. Cell in five fields in the scratched area (200 x 500 um^2 area) were counted under a light microscope. *Bottom*, mean of triplicate experiments; *bars*, SD. *, $P < 0.05$, statistically different from controls.

3.2 Bcl-w induces the phosphorylation of FAK

To investigate whether FAK acts in Bcl-w-induced signaling pathways, levels of FAK phosphorylation in the control and Bcl-w-overexpressing cells were compared by Western blot analysis. The data shown in Fig 2A show that Bcl-w overexpression significantly enhanced the phosphorylation of FAK. This suggests that Bcl-w promotes the signaling actions of FAK.

3.3 FAK is required for Bcl-w-induced cell migration

To determine the role of FAK in Bcl-w-induced cell migration, the dominant negative mutant of FAK (FAKY397F) was introduced into the cells. This treatment significantly attenuated the ability of Bcl-w to promote the cell migration (Fig. 2B). Overall, Bcl-w seems to promote SNU-484 cell migration by increasing FAK activation.

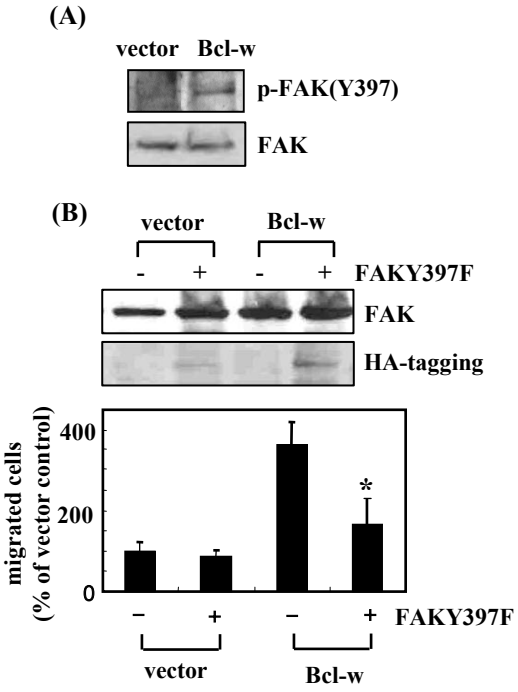

Fig. 2. Bcl-w enhances SNU-484 cell migration by inducing the phosphorylation of FAK. FAK mutant was introduced into the indicated SNU-484 transfectants, and after 24 hours of incubation, cellular levels of FAK and HA were compared by Western blotting. Migration assay was done using the FAK mutant-treated and untreated cells. *, $P < 0.05$ versus untreated Bcl-w control ($n = 5$).

4. Conclusions

The present study shows that FAK is a critical mediator of Bcl-w-induced cell migration. Further analysis of Bcl-w action may provide new insight into how the migratory and invasive potentials of cancer cells are linked to their radio-resistance.

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