ICAM-3, radiation resistance gene, activates PI3K/Akt – CREB – MMPs pathway and promotes migration/invasion of the human non-small cell lung cancer cell NCI-H1299

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1. Introduction

Cancer cell is characterized by various distinctive functions difference from normal cell. The one of specific properties of cancer is invasion and metastasis. Invasion and metastasis is a multi-step process involving over-expression of proteolytic enzymes such as matrix metalloproteinases (MMPs) and critically dependent on the ability of cells to move away from the primary tumor to gain access to the vascular or lymphatic systems which disperses cells to distant sites, where they can grow in a permissive microenvironment at a secondary location. All of these processes are critically dependent upon the ability of cancer cells to breach the basement membrane and to migrate through neighboring tissues. Cancer cell invasion is an important, tightly regulated process that is related with development, immune response and wound healing. This invasive response is dependent on activation of signaling pathways that result in both short-term and long-term cellular responses. The gene expressions of the cancer cell invasion related-proteolytic enzymes are regulated at the transcriptional level (through AP-1 and NF-kB via mitogen activated protein kinases (MAPKs) and PI3K-Akt pathways) and post-transcriptional levels, and the protein level via their activators or inhibitors, and their cell surface localization. Therefore, the related proteins such as MMPs, MAPK, PI3K, Akt and their regulatory pathway have been considered as promising targets for anti-cancer drugs [1, 2]. In previous reports, Intercellular adherin molecule-3 (ICAM-3) showed increase of radio-resistance and proliferation [3, 4]. We have made ICAM-3 overexpressed cancer cells which shows elevated level of invasion compared with normal cancer cells and its invasion capacity was down-regulated with treatment of specific inhibitor for PI3K. These results suggest that ICAM-3 – related invasion is associated with PI3K signaling pathway.

2. Methods and Results

2.1 Contruction of ICAM-3 overexpression model

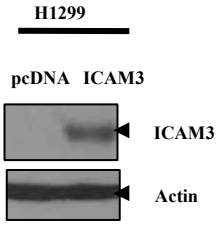


Fig.1. Construction of ICAM-3 stable transfectant of NCI-H1299 non-small cell lung cancer (NSCLC) cell line. This transfectant was confirmed with Immunoblotting assay.

2.2 ICAM-3 induces up-regulation of MMP-2 and -9

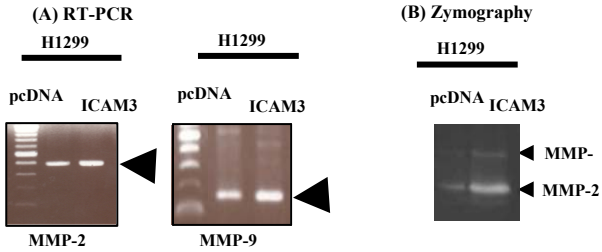


Fig.2. ICAM-3 stable transfectant of NCI-H1299 showed overexpression and increase of of MMP-2 and -9 activation. (A) RT-PCR assay. (B) Zymography assay.

2.3 ICAM-3 promotes migration and invasion.

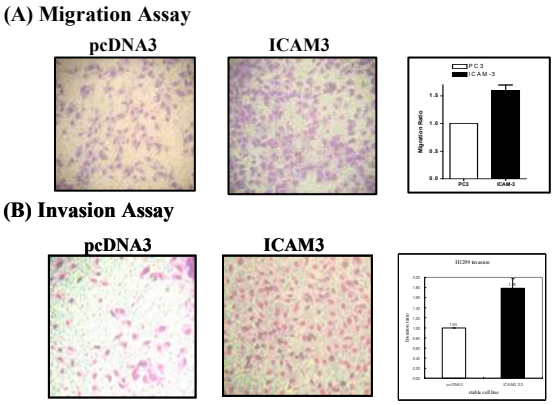
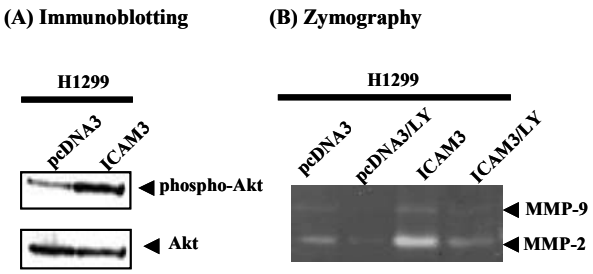


Fig.3. ICAM-3 stable transfectant of NCI-H1299 showed increase of migration and inavasion.. (A) Migration assay. (B) Invasion assay.

2.4 PI3K/Akt pathway involved ICAM3-induced cell migration, invasion



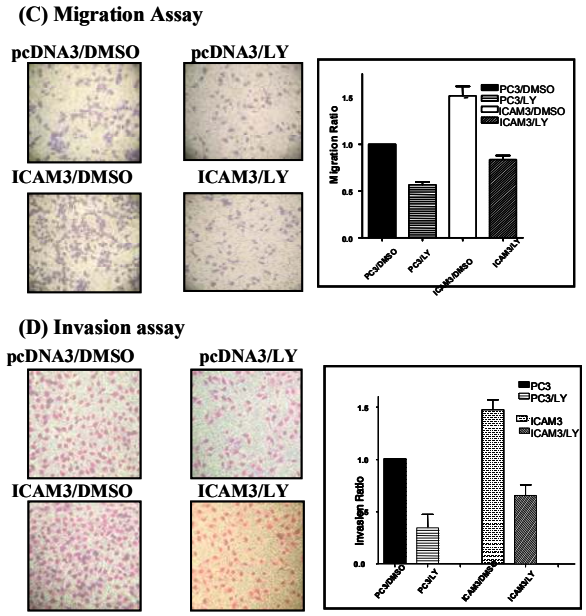


Fig.4. ICAM-3 induced phosphorylation of Akt and inhibition of PI3K/Akt pathway showed decrease of MMPs activity and migration and Invasion. (A) Immunoblotting assay. (B) Zymography assay. (C) Migarion assay. (D) Invasion assay.

2.5 ICAM-3 induces activation of CREB

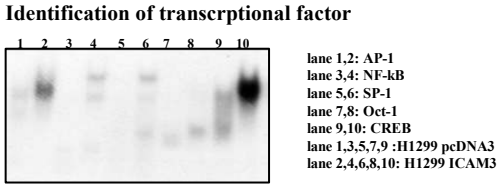


Fig.5. ICAM-3 activated CREB. CERB is one of downstream transcriptional factors of Akt and activated in H1299 ICAM-3 stable transfectant. This activation is detected with Electromotility Shift Assay (EMSA).

2.6. ICAM-AKT-CREB-MMPs pathway

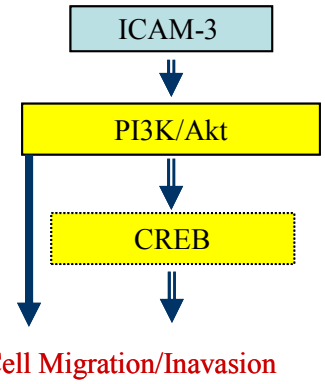


Fig.6. Scheme of ICAM-3 - Akt - CREB - MMPs pathway. ICAM-3 might induce activation of Akt, CREB and MMPs in a row.

3. Conclusions

In this study, we made the ICAM-3 stable expressing H1299 (human non-small cell lung cancer cell). ICAM-3 over-expression in ICAM-3 stable expression cell lines is confirmed with immunoblotting and PCR. Motility or invasiveness of the ICAM-3 stable expression cell lines are tested by migration/invasion and wound healing assay. The ICAM-3 over-expression cancer cells showed the increase of cell motility and invasiveness. We also observed the up-regulation of MMP (Matrix Metalloproteinases) -2 and -9 in ICAM3 stable expression cell lines with RT-PCR and zymography. ICAM-3 over-expression cancer cells showed activation of Akt and the lower migration/invasiveness in condition of LY294002 treatment. And we also observed that CREB, which is one of transcription factor and located downstream of PI3K/Akt pathway, is activated in ICAM3 stable cell line. Taken together, these results showed that the activation of ICAM-3 - PI3K/Akt – CREB – MMPs pathway induces up-regulation of cancer cell migration/invasion.

REFERENCES

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