# CREB mediates ICAM-3 – inducing radio-resistance, cell growth and migration/invasion of the human non-small cell lung cancer cell

Jong Kuk Park, Kwang –sup So, In Hwa Bae and Hong-Duck Um
Laboratory of Radiation Cancer Biology, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-Dong, Nowon-Gu, Seoul, 139-706, Korea

*Corresponding author: Jong Kuk Park, *jkpark@kcch.re.kr*

## 1. Introduction

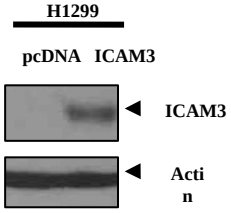
The ICAM family proteins comprises cell surface molecules that are homologous to NCAM and are members of the single passed type 1 immunoglobulin superfamily (IgSF) that are anchored at the cellular membrane [1]. The ICAM family consists of five subfamilies (ICAM-1 to ICAM-5) of heavily glycosylated cell surface receptors with common functional or structural homology [2]. The extracellular domains of ICAM protein have roles in immune response and inflammation through various cell-cell interactions [3]. The cytoplasmic tail residues of ICAM-3 participate in intracellular signaling such as calcium mobilization and tyrosine phosphorylation. Interestingly, the ICAM proteins appear to have a dual role in cancer. ICAM molecules may target and block tumor progression by stimulation of an immune response such as leukocyte activation. Conversely, other investigations have shown that ICAM molecules are involved in cancer malignancy because their increased expressions are associated with a poor diagnosis, lower survival rates and invasion in several cancers including melanoma, breast cancer and leukemia. We have also reported that an increase of ICAM-3 expression in several cancer cells and specimens of cervical cancer patient induce enhanced radio-resistance by the activation of focal adhesion kinase (FAK) [4] and promote cancer cell proliferation by the activation of Akt and p44/42 MAPK [5]. Therefore, these previous reports imply that ICAM-3 has various undefined roles in cancer.

In this study, we investigated whether ICAM-3 increase cell migration and invasion through CREB activation and CREB has a role of increase of radio-resistance and cell growth.

## 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




### *2.1. ICAM-3 induces activation of CREB*

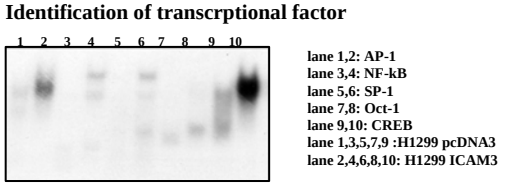



Fig.2. ICAM-3 activated CREB. CERB is one of downstream transcriptional factors of Akt and activated in H1299 ICAM-3 stable transfectant. This activation is detected with Electromotility Shift Assay (EMSA).

### *2.3. CREB is downstream molecule of PI3 kinase/Akt pathway.*

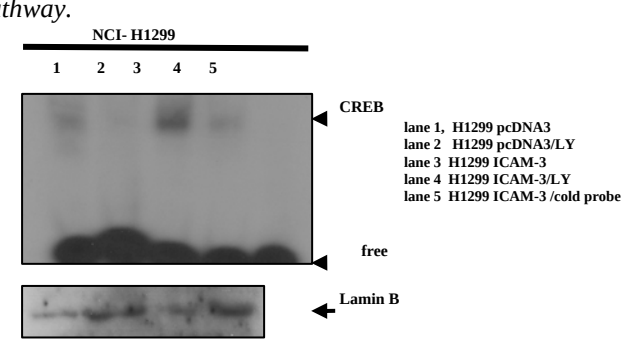



Fig. 3. Activation of CREBinduced by ICAM-3 is blocked by treatment of specific inhibitor(LY294002) of PI3 kinase/Akt. EMSA (upper panel) and Immunoblotting assay (lower panel).

### *2.4. Elimination of CREB protein by siRNA treatment.*

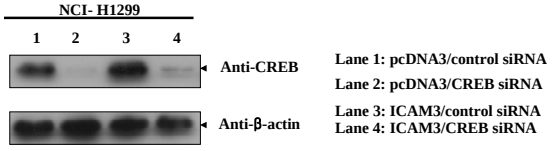



Fig. 4. Elimination of CREB protein by siRNA treatment is detected with Immunoblotting assay.

### *2.5. Elimination of CREB protein by siRNA treatment decreased activity of MMP proteins.*

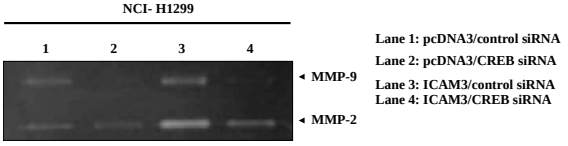


Fig. 5. Gelatin Zymography. CREB specific siRNA treatment induced decrease of activity of MMP-2 and -9.

*2.6. CREB induced migration.*

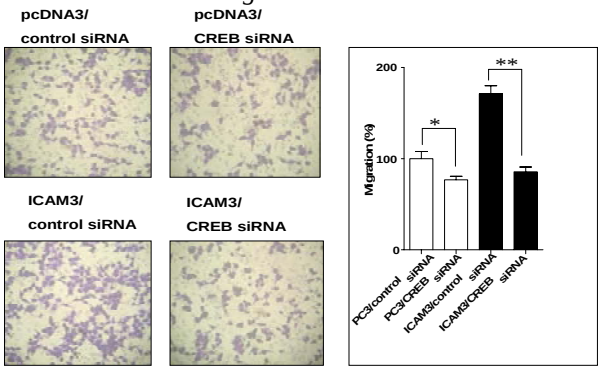



Fig. 6. Cell migration assay. Elimination of CREB protein by siRNA treatment decreased cell migration.

*2.7. CREB induced invasion.*

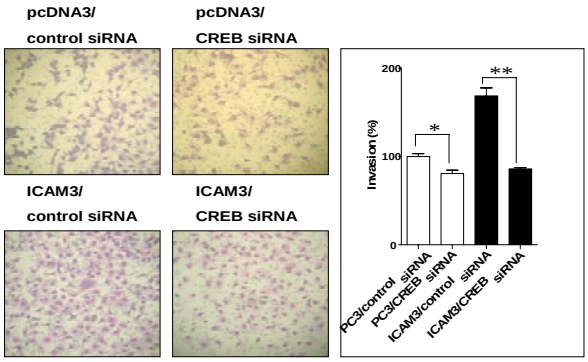



Fig.7. Invasion assay. Elimination of CREB protein by siRNA treatment decreased cell invasion.

*2.8. CREB induced radio-resistance.*

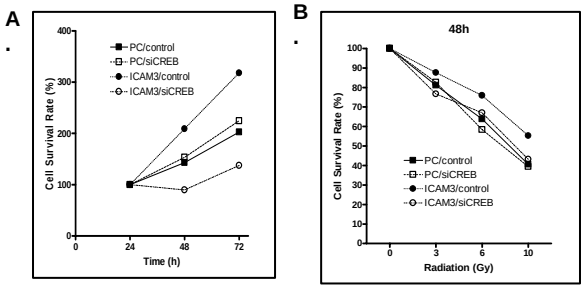



Fig. 8. Cell counting. Elimination of CREB protein by siRNA treatment decreased radio-resistance induced by ICAM-3. A. Radiation-inducing cell death in time-dependent manner (6 Gy). B. Radiation-inducing cell death in dose-dependent manner (48h).

*2.9. Elimination of CREB protein by siRNA treatment decreased cell growth.*

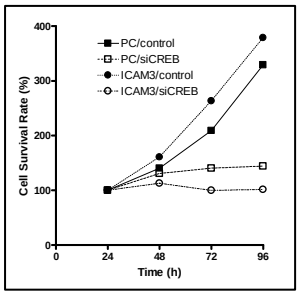



Fig. 9. Cell counting. Elimination of CREB protein by siRNA treatment decreased cell growth induced by ICAM-3.

*2.10.ICAM-AKT-CREB-MMPs pathway.*

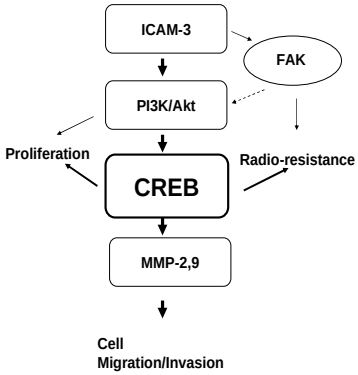



Fig.10. Scheme of ICAM-3 - Akt - CREB - MMPs pathway. CREB has a role of intermediator in the pathway.

## 3. Conclusions

In our previous study, we made the ICAM-3 stable expressing H1299 (human non-small cell lung cancer cell) cell line, and this cell line showed the increase of cell motility and invasiveness through ICAM-3 - PI3K/Akt – MMPs pathway. In this study, we also confirmed that CREB, which is one of transcription factor and located downstream of PI3K/Akt pathway, is activated and mediate increase of migration and invasion induced by ICAM-3. 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

[1] Simmons D, Makgoba MW, Seed B. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. *Nature* 1998;331: 624 – 627.

[2] Springer, TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 1994;76:301-314..

[3] Figdor CG, van Kooyk Y, Adema GJ. C-type lectin receptors on dendritic cells and Langerhans cells. *Nat. Rev. Immunol.* 2002; 2, 77-84.

[4] Chung YM, Kim BG, Park CS *et al.* Increased expression of ICAM-3 is associated with radiation resistance in cervical cancer. *Int J Cancer.* 2005;117(2):194-201.

[5] Kim YG, Kim MJ, Lim JS, Lee MS, Kim JS, Yoo YD. ICAM-3-induced cancer cell proliferation through the PI3K/Akt pathway. *Cancer Lett.* 2006;239(1):103-10.