The Effects of EEF1A on Cell Proliferation by Intracellular Alkalinization

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The Effects of EEF1A on Cell Proliferation by Intracellular Alkalinization

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The Master's Thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University In partial fulfillment of the requirements for the degree of Master of Medical Science

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This certifies that the Master's Thesis of

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ABSTRACT

Formation of pH gradient in tumor cells, which is characterized by intracellular alkalinization and extracellular acidification, plays a key role in the growth and metastasis of tumor cells. Maintaining intracellular alkalinization in tumor cells is produced by the constitutive activation of Na^+/H^+ exchanger 1 (NHE1), and inhibition of NHE1 activity has been shown to suppress tumor cell proliferation. However, the underlying molecular mechanisms of alkalinization-induced cell growth are not known. One of the key proteins in cell proliferation and growth is the eukaryotic elongation factor 1a (EEF1A), which plays a critical role in translating mRNA into proteins by recruiting amino-acyl tRNA. Interestingly, it has been suggested that cellular distribution of EEF1A and its actin-binding properties are affected by intracellular pH in *Dictyostelium*. In the present study, the effects of intracellular alkalinization on cell growth and EEF1A were investigated using molecular functional approaches in mammalian cells containing different isoforms of EEF1A. In all cell lines tested, NIH3T3, HEK 293 and HeLa cells, a weak intracellular alkalinization pH around ~7.6 achieved by reducing

carbon dioxide concentrations in a CO_2-incubation chamber resulted in increased cell growth. Over-expression of EEF1A by transfection with mammalian expressible EEF1A1 and EEF1A2 plasmids increased the alkalinization-induced cell growth in HeLa and NIH3T3 cells. Conversely, attenuation of EEF1A levels by treatment with EEF1A1 and EEF1A2 siRNAs reduced the effects of alkalinization-induced cell growth in HeLa cells which expressed both isoforms of EEF1A. Finally, the amount of actin-bound EEF1A was greatly reduced under alkaline conditions without the changes in the total amount of EEF1A, suggesting an increase in the functionally active, free form of EEF1A in NIH3T3 cells. The above findings provide sufficient and necessary evidence that EEF1A is a critical factor in alkalinization-induced tumor growth.

Key Words: Cell growth, Intracellular alkalinization, Na^+/H^+ exchanger, Elongation factor

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Ⅰ. INTRODUCTION

Tumor cells which harbor a significant growth advantage have many different hallmarks including a high degree of intracellular alkalinization[1] and abnormal glycolytic metabolism[2]. Cellular alkalinization is one of the most common phenotypes of tumor cells and is due to their ability to secrete protons and acidify their extracellular environment[1]. The alkaline intracellular pH (pH_i) and acidic extracellular pH (pH_e) create a reversed pH gradient across the cell membrane that is the earliest step of neoplastic progression[1, 3].

Tumor cells activate membrane-based ion exchangers such as the Na^+/H^+ exchanger 1 (NHE1)[4], the HCO_3^-/Cl^- exchanger[5] and the H^+/lactate cotransporter (the monocarboxylate, MCT)[6] in order to maintain the reverse pH gradient. Transformation of human keratinocytes (HPKIA) by the E7 oncogene of human papillomavirus type 16 (HPV16) showed activation of NHE1 as a key mechanism in malignant transformation[7]. NHE1, the housekeeping isoform of the Na^+/H^+ exchanger, is ubiquitously distributed in most tissues[8]. NHE1 plays a pivotal role in

the regulation of pH_i by exchanging intracellular protons $[H^+]_i$ for extracellular sodium $[Na^+]_e$ in a 1:1 stoichiometric ratio, thereby regulating the cell volume by osmotic homeostasis[9-11]. The transformed cells and tumor cells differ from normal tissues in having constitutive NHE1 activity at the resting pH_i, resulting in an increase in pH_i. The intracellular alkalinization induced by activation of NHE1 in tumor cells has been shown to play a pivotal role in the maintenance and progression of the neoplastic state[1, 16]. This activation of NHE1, with consequent cytoplasmic alkalinization, appears to be the universal progression when quiescent cells commit to proliferate[7, 12-15].

The redistribution of eukaryotic elongation factor 1a (EEF1A) is associated with increased pH_i[17, 20]. EEF1A is the most abundant protein in the cell, comprising 1 - 2% of total protein[18, 19], and is highly conserved[20]. During protein translation, EEF1A recruits amino-acylated tRNAs to the ribosome and translocates the growing polypeptide from the ribosomal A site to the P site[21]. In addition to its role in protein synthesis, EEF1A binds actin in many species[22]. The binding affinity of EEF1A for F-actin diminishes with increasing pH_i, and EEF1A dissociates from the F-actin. The total amount of the free form of EEF1A in *Dictyostelium* increased by 60% upon intracellular alkalinization from pH 6.0 to 8.0[17]. EEF1A has two isoforms, EEF1A1 and EEF1A2 which share greater than 90% homology in amino acid sequence. EEF1A1 is expressed ubiquitously whereas expression of EEF1A2 is restricted to the heart, brain and skeletal muscle in humans, rats and rodents[23-24]. In mice and rats, EEF1A2 substitutes for the EEF1A1 protein synthesis function in specific tissues[25, 26]. Recently, it has been reported that EEF1A1 and EEF1A2 are oncogenes, and are over-expressed in some tumors in breasts, prostates, and pancreases[20, 27-33]. The oncogenicity of EEF1A1 and EEF1A2 may be related to their role in protein synthesis and F-actin binding.

In this study, we evaluated the role of EEF1A in intracellular alkalinization-dependent

tumor cell growth using an experimental model with varying carbon dioxide concentration. We found that low carbon dioxide concentration induced cellular alkainization, and stimulated tumor cell growth through the increased activity of EEF1A. These results imply that reducing the increased EEF1A activity due to intracellular alkalinization might be a therapeutic strategy to suppress the earliest step in the tumorigenesis.

Ⅱ. MATERIALS AND METHODS

1. Material, Solutions and Cells

2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) was purchased from Molecular Probes (Eugene, OR, USA). EEF1A and c-Myc monoclonal antibodies were obtained from Upstate (Lake Placid, NY, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. The siRNAs targeted to EEF1A1 and EEF1A2 were purchased from Dharmacon (Lafayette, CO, USA). Actin-biotin and Immobilized Streptavidin were purchased from Cytoskeleton (Denver, CO, USA) and PIERCE (Rockford, IL, USA), respectively. All other chemicals including MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and sodium dodecyl sulfate (SDS) were obtained from Sigma (St. Louis, MO, USA). The HCO_3^--buffered solution B contained (mM/L) 120 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, 5 HEPES, and 25 $NaHCO_3$ (pH 7.4). pCMV-EEF1A1 (PCMV-SPORT6) and pCMV-EEF1A2 (pCNS) clones were purchased from the Korea Research Institutive of Bioscience and Biotechnology (KRIBB, Daejon, KOREA). pCMV-EEF1A1 and pCMV-EEF1A2 were subcloned into the pCDNA 3.1/Zeo(+) vector with an Myc tag at the carboxy-terminus, respectively. NIH3T3, HEK293 and HeLa cells were maintained in Dulbecco's modified Eagle's medium-high glucose medium (DMEM-HG; Invitrogen, Carlsbad, CA, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA), penicillin (50 IU/㎖), and streptomycin (50 ㎍/㎖) at 37°C in 5% carbon dioxide incubator.

2. Cell Growth Assay

Cell growth conferred by EEF1A was determined by MTT viability assay. The pCMV-EEF1A constructs were transiently transfected into HeLa and NIH3T3 cell lines in 60-mm dishes using Lipofectamin Plus Reagent (Invitrogen, Carlsbad, CA, USA). After 12 h of incubation at 37°C in 5% carbon dioxide, cells were trypsinized and seeded into a 12-well plate. The media were replaced after a 12 h incubation with normal DMEM-HG and pH-modified DMEM-HG containing Hepes (pH 6.7) which were pre-incubated at 37°C in 2% and 10% carbon dioxide, respectively, and the cells were incubated in each concentration for 3 days. At 1-day intervals, MTT stock solution (5 ㎎/㎖ in PBS) at 0.2 volume equivalents was added, giving a final MTT concentration of 1 ㎎/㎖. After the three day incubation, the cells were treated overnight with one volume of lysis buffer (20% SDS in 50% N, N-dimethyl formamide with water; pH 4.7). Absorbance was read at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader.

With the same protocols, NIH3T3, HEK293 and HeLa cells were seeded into 12-well plates and incubated for 24 h in each carbon dioxide concentration to measure the effect of alkalinization on cell growth by MTT viability assay.

3. Actin Binding Assay and Immunoblotting

The pCMV-EEF1A1 and pCMV-EEF1A2 with Myc epitope constructs were transiently transfected into NIH3T3 cells in a 100-mm dish using the Lipofectamin Plus Reagent (Invitrogen, Carlsbad, CA, USA). After a 48 h of incubation at 37°C in 5% carbon dioxide, cells were lysed with one of three kinds of actin binding buffers (20 mM Pipes pH 6.0, 20 mM Tris-HCl pH 7.0 and pH 8.0 were added to 50 mM KCl, 2 mM $MgCl_2$, 1 mM ATP, 1% Triton X-100, 0.2 mM DTT, 2 mM EGTA, proteinase inhibitor cocktail tablet

respectively). 1.5 ㎎ of protein samples from EEF1A1 or EEF1A2-expressing cells were resuspended in each of the actin binding buffers and incubated with 10 ㎍ of Actin-biotin at 4°C for >18 h. After incubation, Immobilized Streptavidin was added to the sample and incubated at 4°C for 2 h, followed by washing 5 times with actin binding buffer, and electrophoresis. The biotin-binding proteins or lysates (30 ㎍ of protein) were analyzed by immunoblotting as previously detailed[34]. Briefly, the samples were suspended in SDS sample buffer and separated by SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred onto a nitrocellulose membrane and probed with the c-Myc monoclonal antibody. After being treated with appropriate secondary antibody, protein bands were visualized using an enhanced chemiluminescence kit (Amersham Pharmacia).

4. siRNA Transfections

siRNA-mediated knockdowns of EEF1A1 and EEF1A2 were performed according to manufacturer's instructions. Briefly, EEF1A1 siRNA and EEF1A2 siRNA were transiently transfected into HeLa cells in 6-well and 12-well plates using Lipofectamin 2000 (Invitrogen, Carlsbad, CA, USA). After a 6-hour incubation at 37°C in 5% carbon dioxide, the media were replaced with normal DMEM-HG that was pre-incubated at 37°C in 2%, 10% and 20% carbon dioxide. The cells were then incubated at each respective carbon dioxide concentration for 48 h. After incubation, cells in 6-well plates were lysed with a standard lysis buffer containing proteinase inhibitor cocktails, and 50 ㎍ of the protein sample suspended in SDS sample buffer, and immunoblotted as described above. HeLa cells in 12-well plates were analyzed with the MTT viability assay described above.

5. Intracellular pH Measurements

Intracellular pH (pH_i) was measured as previously described[35]. Briefly, for measurement of pH_i in NIH3T3 HEK293 and HeLa cells, cells on coverslips were incubated with normal DMEM-HG at 37°C in 2%, 10% and 20% carbon dioxide, and pH-modified DMEM-HG with Hepes (pH 6.7) at 37°C in 10% carbon dioxide for 1 day. The cells were then washed with pre-incubated HCO_3^--buffered solution B at each carbon dioxide concentration and assembled to form the bottom of a perfusion chamber. The cells were loaded with BCECF by a 10-min incubation in solution B containing 2.5 mM BCECF-AM at room temperature. After dye loading, the cells were perfused with the appropriate solutions, and pH_i was measured by photon counting using a fluorescence measuring system (Delta Ram; PTI Inc., South; Brunswick, NJ, USA). The fluorescence ratios of 490/440 nm were calibrated intracellularly by perfusing the cells with solutions containing 145 mM KCl, 10 mM HEPES, and 5 μM nigericin with pH adjusted to 6.2–7.8.

6. Statistical Analysis

Results are presented as the means ± S.E. of the indicated number of experiments. The results of multiple experiments were analyzed using the non-paired Student's t test or analysis of variance, as appropriate. Cell growth analysis was performed by the one-way ANOVA method.

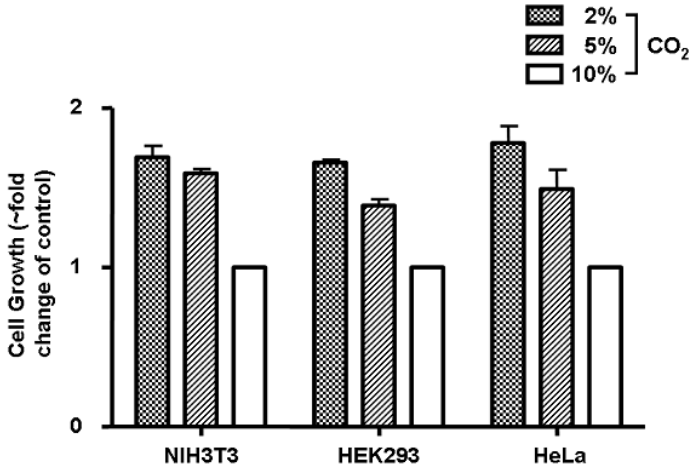
Ⅲ. RESULTS

1. Effects of Alkaline Treatments on Cell Growth

We initially evaluated the effects of alkaline treatment on NIH3T3, HEK293 and HeLa cells. These cells were incubated in different carbon dioxide concentrations (2%, 5% and 10% carbon dioxide in the incubation chamber) for 24 h, and the altered growth rates due to cytoplasmic pH change were determined by MTT viability assay (Fig. 1-A). Under alkaline conditions, the cells grew faster than those under control conditions, and HeLa cells showed the largest growth difference, compared to the other cells. Since HeLa is the human cervical adenocarcinoma cell line that has both EEF1A1 and EEF1A2[32], it was chosen for further study.

To further examine the growth of HeLa cells in alkaline and acidic conditions, HeLa cells were incubated at normal DMEM-HG in 2%, 10% and 20% carbon dioxide, and pH-modified DMEM-HG with Hepes (pH 6.7) in 10% carbon dioxide for 3 days (Fig. 1-B). At 1-day intervals, cell growth was measured with the MTT viability assay. The cells in 2% carbon dioxide grew faster than cells grown at the other concentrations, and tumor cell proliferation was inhibited in 20% carbon dioxide and pH-modified DMEM-HG. The medium and intracellular pH values of each chamber condition (2%, 10%, 20% and pH-modified media) are shown in Table 1.

A.



B.

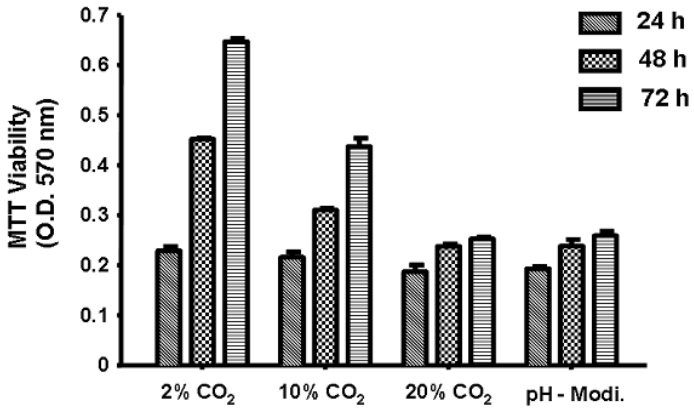


Figure 1. Cell growth by alkaline treatment. (A). NIH3T3, HEK293 and HeLa were incubated under alkaline conditions for 24 hours. (B) HeLa cells were incubated in normal DMEM-HG in 2%, 10% and 20% CO_2, and pH-modified DMEM-HG in 10% CO_2 for 3 days. At 1-day intervals, cell growth was measured by MTT viability assay.

Cell Type	CO_2	Medium pH	Intracellular pH
NIH3T3	2%	7.9 ± 0.02	7.50 ± 0.06
	10%	7.54 ± 0.03	6.87 ± 0.01
	20%	7.06 ± 0.03	6.44 ± 0.06
	pH-Modified	6.7 ± 0.04	6.52 ± 0.06
HEK293	2%	7.9 ± 0.02	7.57 ± 0.02
	10%	7.54 ± 0.03	6.99 ± 0.06
	20%	7.06 ± 0.03	6.52 ± 0.06
	pH-Modified	6.7 ± 0.04	6.40 ± 0.03
HeLa	2%	7.9 ± 0.02	7.46 ± 0.03
	10%	7.54 ± 0.03	7.00 ± 0.04
	20%	7.06 ± 0.03	6.42 ± 0.01
	pH-Modified	6.7 ± 0.04	6.46 ± 0.01

Table 1. pH measurements with prolonged carbon dioxide incubation. Values are expressed as means ± S.E.M..

The medium pH value was estimated from the Henderson–Hasselbach equation. Measurements of pH_i in HCO_3^--containing (25 mM/L) media using BCECF showed that low carbon dioxide concentrations induced cytoplasmic alkalinization. Despite differences in carbon dioxide concentration, cells in normal media in 20% carbon dioxide and pH-modified media yielded similar media pH and pH_i values. These results indicate that intracellular alkalinization stimulates tumor cell growth.

2. Effect of EEF1A over-expression on the growth of HeLa cells

As oncogenes, EEF1A1 and EEF1A2 must have two basic properties: they must be hyperactivated in human cancer cells and be able to activate tumor cell proliferation. Over-expression of EEF1A1 and EEF1A2 occurs in some human tumors, which is important to tumor cells which have abnormal growth, different from proliferation induced by cellular alkalinization.

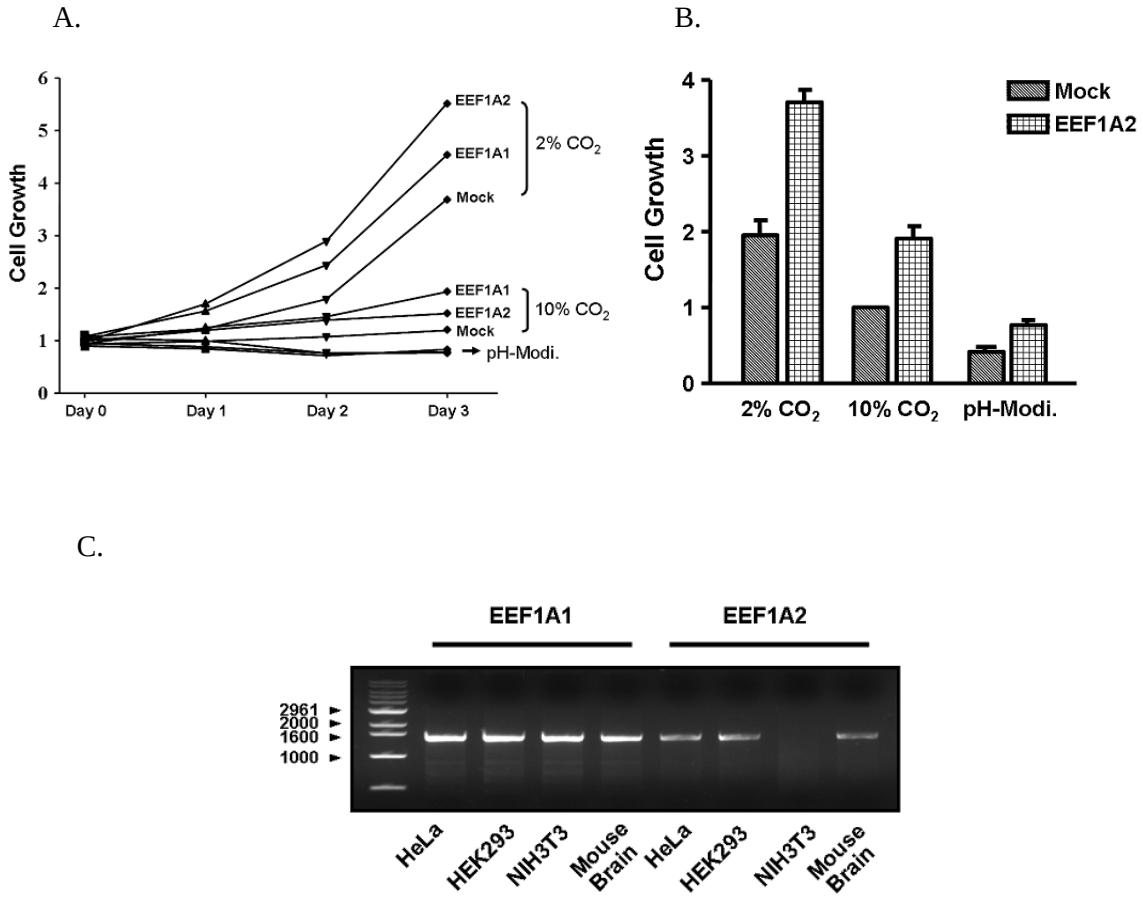


Figure 2. Effect of EEF1A over-expression on tumor cell growth. After transfection,

EEF1A-over-expressing cells were incubated for 3 days. (A) Over-expression of EEF1A enhances the proliferation of tumor cells. EEF1A2 had more influence than EEF1A1 on growth with akalinization. (B) Effect of EEF1A on cell growth was reproduced in NIH3T3 fibroblasts. (C) RT-PCR analyses of EEF1A1 and EEF1A2.

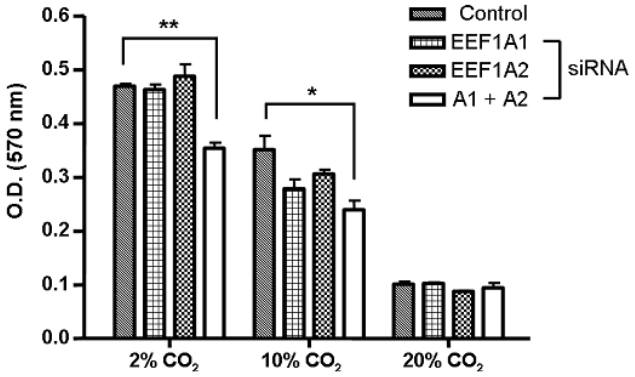
To over-express EEF1A, each of EEF1A1 and EEF1A2 was transfected into HeLa cells. After transfection, cells were incubated with normal DMEM-HG in 2%, 10% carbon dioxide and at pH-modified DMEM-HG in 10% carbon dioxide for 3 days, and samples were taken at 1-day intervals to measure the capacity of EEF1A to trigger tumor cell growth by MTT viability assay. As shown in Fig. 2-A, both EEF1A1 and EEF1A2 enhance tumor cell growth. EEF1A1 and EEF1A2-over-expressing cells showed an exponential increase in cell growth compared to HeLa control cells transfected with GFP. The factors that increased the growth rate of EEF1A1 and EEF1A2 transfectants in 2% carbon dioxide were due not only to the over-expression of these proteins, but also to intracellular alkalinization. Since NIH3T3 cells do not contain EEF1A2 isoforms (Fig. 2-C), the effect of 2% carbon dioxide incubation and EEF1A over-expression was analyzed in NIH3T3 cells transfected with pCMV-EEF1A2 to further investigate the effect of EEF1A on cell growth (Fig. 2-B). The EEF1A2 transfected NIH3T3 cells divided more quickly than controls transfected with GFP.

3. Inhibition of tumor cell growth by knockdown of EEF1A isoforms

To investigate the function of EEF1A in human cancer cells, we used the RNA interference technique to specifically deplete the EEF1A protein in the HeLa tumor cell line. After EEF1A1 siRNA and EEF1A2 siRNA were transfected into HeLa cells, they were

incubated in 2%, 10% and 20% carbon dioxide incubation chambers for 48 h. In the 10% carbon dioxide-grown cells, MTT viability three days after the cell growth of each EEF1A1 and EEF1A2 siRNA transfectants was significantly lower than that of the HeLa controls transfected with the scrambled siRNA. Moreover, in a siRNA-cotransfection assay, the tumor cell proliferation was 33% lower than either of the EEF1A1 and EEF1A2 siRNA-transfected cells, suggesting that siRNA-mediated EEF1A knockdown prevented the tumor cell growth in normal media pH. In 2% carbon dioxide-treated cells, each of the EEF1A1 and EEF1A2 siRNAs did not inhibit tumor cell growth. However, the double knockdown of EEF1A1 and EEF1A2 significantly reduced the growth of cervical cancer cells, strongly suggesting that intracellular alkalinization in 2% carbon dioxide-grown cells at least partially rescued growth inhibition mediated by depletion of EEF1A isoforms (Fig. 3-A).

A.



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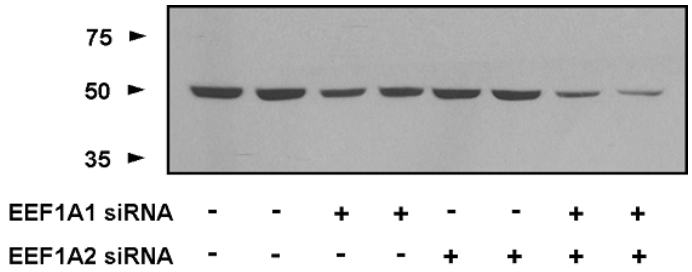

Figure 3. Inhibition of tumor cell growth by EEF1A depletion. After EEF1A1 and EEF1A2 siRNA transfection, HeLa cells were divided into 3 groups and incubated at different CO_2 concentrations. After 48 h, HeLa cell growth was measured by MTT viability assay (A), and expression of EEF1A isoforms was detected by immunoblotting. *P< .05 and **P< .01.

We used an immunoblot assay to confirm the siRNA-mediated EEF1A knockdown. HeLa cells were harvested two days after transfection with EEF1A1 and EEF1A2 siRNA, and the cells separated by SDS-PAGE on 10% gels. As shown in Fig. 3-B, EEF1A1 and EEF1A2 protein expression levels were similar to each other and were the same relative to HeLa controls. However, the total EEF1A in siRNA-cotransfected cells decreased by 70 ~ 80%. Taking advantage of the amino acid similarity between EEF1A1 and EEF1A2, the EEF1A monoclonal antibody was used to assess EEF1A2 expression levels.

4. Effect of intracellular pH on the binding of EEF1A to F-actin

Fig. 1 shows that intracellular alkalinization enhanced the proliferation of tumor cells. This raised the possibility that EEF1A expression may be influenced by alkaline treatments. However, immunoblot analysis showed that this was not the case. Neither EEF1A1 nor EEF1A2 expression was altered by 2% carbon dioxide treatment for 12 or 24 h (data not shown). It has been shown that EEF1A protein not only shuttled the aminoacyl -tRNA in protein translation, but bound F-actin. This binding was regulated by $pH_{i,}$ both *in vitro* and *in vivo*. When the pH_i is increased, the EEF1A-mediated cross-links between actin filaments dissociate, and EEF1A is liberated from actin and binds to aminoacyl-tRNA for protein synthesis[17-19]. Due to the DNA sequence homology between EEF1A1 and EEF1A2, we sought to determine whether EEF1A1

and EEF1A2 would bind F-actin, and whether its distribution would be controlled by cytoplasmic pH. To address this question, we measured the affinity of EEF1A1 and EEF1A2 for F-actin using the actin binding assay described in Material and Methods.

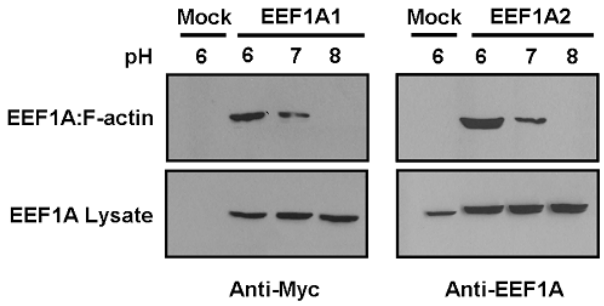

Figure 4. Effect of intracellular pH on the distribution of EEF1A. The intracellular pH of cells was clamped at the pH of actin binding buffer. Constructs expressing pCMV-EEF1A1 with Myc epitope and pCMV-EEF1A2 were transiently transfected into NIH3T3 cells. After 48 h, the NIH3T3 cells were lysed in actin binding buffer, and the lysates were incubated with actin-biotin in actin binding buffer with pH values ranging from 6 to 8. After incubation, Immobilized Streptavidin was added to the sample and biotin-binding proteins or lysates were analyzed by immunoblotting. The Mock transfection was made with GFP.

In the pH 6 actin binding buffer, the majority of EEF1A1 and EEF1A2 proteins were bound to F-actin. EEF1A1 and EEF1A2 protein levels bound to F-actin at pH 7 were lower than those at pH 6, and F-actin binding was almost non-existent at pH 8. These results showed that EEF1A1 and EEF1A2 are actin-binding proteins and that their binding affinity to F-actin is reduced as the pH is increased without the change in the total amount EEF1A. The Mock

transfected with GFP yielded undetectable results. The 30 μg of protein lysate showed that EEF1A1 and EEF1A2 proteins were over-expressed, and we confirmed that the expression levels of EEF1A1 and EEF1A2 were not noticeably altered by alkaline treatment.

Ⅳ. DISCUSSION

In this study, we provide several lines of evidence that intracellular alkalinization stimulates the proliferation of tumor cells, and both EEF1A1 and EEF1A2 have a direct and pivotal role in this proliferation. Low carbon dioxide treatments alkalinized HeLa human cervical adenocarcinoma cells to proliferate faster, and this was reproduced in NIH3T3 and HEK293 cells. Carbon dioxide treatments changed the intracellular pH from 6.4 to 7.6. Tumor cells had physiological pH_i values of 7.12-7.65 and pH_e values of 6.2-6.9[1], indicating that the carbon dioxide experiment model is suitable for this study. Moreover, growth pattern of cells at pH-modified media in 10% carbon dioxide incubation chamber and that of at normal media in 20% carbon dioxide incubation chamber were very similar. Accordingly, the change in cell proliferation cannot be ascribed to CO_2 toxicity, O_2 deficiency or bicarbonate ion concentration[36], but cell growth can be ascribed to cytoplasmic alkalinization and acidosis.

Intracellular signals including alkalinization weaken EEF1A:F-actin bundles to release EEF1A and favor its binding to t-RNA and promotion of polypeptide elongation. Actin and t-RNA are mutually exclusive competing ligands for binding EEF1A, such that when EEF1A are complexed with t-RNA, the binding of actin is excluded[19]. In NIH3T3 cells, artificially raising pH_i by incubating them in 2% carbon dioxide reduced the actin-binding affinity of EEF1A and allowed EEF1A to be free form which not bound to F-actin without the change of total amountof EEF1A, suggesting an increase of its functionally active form of in cytoplasm. This indicated that increasing the concentration of active EEF1A forms by alkalinization enhances cell proliferation. In the same manner, tumor cells have alkaline pH_i, and exhibit an abnormal growth rate.

The primary function of EEF1A is to shuttle aminoacyl -tRNAs in protein translation.

Recently, studies have shown that these two isoforms of EEF1A serve as growth-enhancing genes, where an increase in the copy number of *EEF1A1* and *EEF1A2* plays a pivotal role in the malignant tumor progression[31]. The amplification of the *EEF1A2* gene, which maps to 20q13, occurs in 20–30% of ovarian and breast tumors[28-33]. Moreover, EEF1A2 is a potential oncoprotein that is over-expressed in 67% of breast tumors[32]. As it bears a strong sequence similarity to EEF1A2, EEF1A1 is also involved in cancer. In prostate carcinoma, a dominant Prostate tumor inducing gene 1 (PTI-1), with a 97.7% DNA sequence homology to EEF1A1, was identified by PCR[27]. Its expression occurs in breast, colon and lung cancer cells, but not in normal cells, and it can transform rodent fibroblasts. Antisense-mediated blocking of PTI-1 inhibits tumorigenesis and results in reversion of tumor cells to a normal cellular phenotype[37]. *EEF1A1*, which maps to 6q14, is amplified in some childhood brain tumors, and EEF1A1 is over-expressed in pancreas, breast, lung and colon tumors[27, 38-40].

What relationship will there be between protein synthesis and tumorigenesis? It has been previously suggested that an increase of EEF1A expression is associated with an increase of cell proliferation, oncogenic transformation and delaying of cellular senescence[17, 41]. Moreover, EIF4E, an mRNA cap-binding factor, is highly expressed in breast, colorectal, and squamous larynx tumors[42-44]. Furthermore, *EIF4E* is amplified in lung and breast cancers[45, 46], and is thought to regulate tumorigenesis by enhancing the translation of genes promoting cellular growth[31, 47]. Both EEF1A1 and EEF1A2 may function in the same manner through the protein translation machinery. In metastatic cells, EEF1A have a reduced affinity for F-actin[41]. As illustrated in Fig. 2, HeLa cervical cancer cells incubated in 2% carbon dioxide increased the functional expression of EEF1A and enhanced cell growth, and this was reproduced in NIH3T3 cells which do not contain EEF1A2 isoforms. Of note, the pH-dependent distribution of EEF1A protein induced cell growth; that is, 2% carbon dioxide–grown cells exhibited an accelerated

growth rate relative to 10% carbon dioxide–grown cells. Furthermore, over-expression of EEF1A stimulated tumor cell growth. Therefore, disassociation of EEF1A from F-actin by intracellular alkalinization occurs in tumor cells and over-expression of EEF1A has a direct role in tumor proliferation and transformation.

Ⅴ. CONCLUSIONS

This study determined that intracellular alkalinization increased tumor cell growth, and it also characterized the role of EEF1A in tumor cell growth using molecular and electrophysiological approaches. Our results are summarized as follows:

1. Intracellular alkalinization enhances tumor cell growth.

2. The binding affinity of EEF1A to F-actin is reduced with increasing intracellular pH.

3. EEF1A, which disassociates from F-actin, has a direct role in tumor cell growth.

4. Over-expression of EEF1A increases tumor cell growth.

5. Depletion of EEF1A expression remarkably decreases tumor cell growth.

Taken together, we demonstrated that EEF1A is an important modular protein involved in tumor cell proliferation, and that its activity is regulated by intracellular pH.

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세포 내 염기성화에 의한 암세포의 성장 기전에서 EEF1A의 역할

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Abstract (IN KOREAN)

종양 세포의 세포 내 pH 는 7.12~7.65 으로 알칼리화 되어 있으며, 외부의 pH 는 6.2~6.9 로 산성화 되어 있다. 이러한 pH 차이는 종양세포의 성장, 조직 침해, 전이 과정에 중요한 역할을 하며, 이 알칼리화의 유지는 주로 활성이 크게 증가한 Na^+/H^+ exchanger 1 (NHE1)을 통한 세포 내 H^+의 방출에 의해 이루어진다. 그러나 알칼리화에 따른 세포 성장 의 분자생물학적 메커니즘은 아직 알려지지 않은 상태이며 이에 관한 많은 연구들이 진행되고 있다. 세포의 성장과 분열에서 중요한 역할을 담당하고 있는 단백질 중의 하나인 eukaryotic elongation factor 1a (EEF1A)는 단백질 합성 과정에서 amin-acyl tRNA 를 ribosome 으로 운반하여 mRNA 의 번역을 조절하는 단백질로서, 아메바의 한 종류인 *Dictyostelium* 에서 세포내의 pH 변화에 따라 actin 과의 결합력이 변하는 것은 잘 알려진 사실이다. 본 연구에서는 세포의 알칼리화가 세포성장과 EEF1A 에 미치는 영향을 여러 분자 생물학적 방법을 통해 연구하였다. 여러 세포 주를 낮은 CO_2 농도에서 키우는 방법으로 세포 내 알칼리화 (pH ~7.6)를 유도한 결과 세포의 성장 속도가 증가하는 것을 확인하였다. 또한 EEF1A 의 과발현이 pH 변화와는 별도로 세포의 성장을 활성화시키는 것을

밝혀내었으며, RNA interference 에 의한 EEF1A 의 발현 감소는 세포성장을 억제시켰다. 마지막으로 전체 EEF1A 양의 변함 없이 actin 과 결합한 EEF1A 의 양이 세포 내의 알칼리화에 따라서 감소하는 것을 확인하였다. 즉, 알칼리화가 진행됨에 따라 EEF1A 가 기능적으로 활성화된다고 여겨진다. 이상의 결과를 통해 본 연구에서는 EEF1A 가 알칼리화에 따른 암세포의 성장에 관여하는 중요한 단백이라는 것을 밝혀내었다. 이는 암세포의 초기 진행과정을 억제하는 데에 많은 도움을 줄 것으로 생각된다.

핵심되는 말: 세포 성장, 알칼리화, NHE1, Elongation factor