Mutation in Ras-signaling occurs most commonly in follicular thyroid cancer, and is associated with tumor initiation. Most of the mutations were observed in H-RasG12V, N-RasQ61L, and K-RasG13D in follicular thyroid carcinoma, respectively. However, a genetic modification by Ras in the thyrocytes results in oncogene induced senescence (OIS). Morphology of the cells was found to change to large and elongated shape, and cytoplasmic vacuoles were observed. Furthermore, cell growth, senescence associated β-galactosidase (SA-β-gal) staining and p16INK4A data clearly showed cellular senescence in H-RasG12V, N-RasQ61L, and K-RasG13D expressing thyrocytes. Ras mutation markedly induced Erk1/2 phosphorylation. These data clearly indicated that Ras mutation caused oncogene induced senescence (OIS) in primary normal thyrocytes. We will further evaluate the factors involved in the senescence overcome program in thyroid carcinoma.

**RESULTS**

**Fig. 1.** The overexpression of H-RasG12V in Human diploid fibroblast (HDF). (A) Morphology of transduced H-RasG12V infected HDF. (B) Western blotting of H-RasG12V infected HDF cell line. (C) Growth rate of H-RasG12V infected HDF cells were shown lower than control cells.

**Fig. 2.** Cellular senescence was detected in mutated Ras-overexpressed HDF cells. (A) Senescence morphology was observed in Ras-overexpressed cells at 10 days after lentivirus infection. (B) The activation of Erk1/2 and Akt by mutant Ras were clearly observed in Western blotting. (C) The cell growth of K-RasG13D, H-RasG12V, N-RasQ61L-overexpressed HDF cells were shown by proliferation assay. (D) OIS was induced by H-RasG12V and N-RasQ61L, not by K-RasG13D. Mutated Ras-lentiviral infected HDF cells were stained by SA-β-Galactosidase staining for 14 hours with citric acid and pH 6.0.

**Fig. 3.** The overexpression of H-RasG12V in thyrocytes. (A) Morphology of thyrocytes induced by lentivirus H-RasG12V. (B) The immune blotting data show the high expression of Ras and Ras-Raf in H-RasG12V overexpressed thyrocytes. (C) The increasing of cell proliferation in lentivirus H-RasG12V infected thyrocytes.

**Fig. 4.** Ras-induced senescence in human thyrocytes. (A) Induced human thyrocytes were induced by H-RasG12V, N-RasQ61L, B-RasV12-GRants. and maintained for 15days. This morphology show that human normal thyrocytes were changed by Ras and Raf-Raf overexpression. (B) Ras-overexpressed thyrocytes decreased cell growth than control after initiation factor. (C) Cell lysates were prepared from Ras-overexpressed thyrocytes, H-Ras and N-Ras overexpression in thyrocyte induced phosphorylation of Erk1/2, was detected by Western blot. (D) Ras-overexpressed thyrocytes were fixed, stained by SA-β-gal for overnight and showed the positive cells increased in Ras-infected cells.