Squamous cell carcinoma of the larynx and hypopharynx is one of the most common head and neck squamous cell carcinoma (HNSCC). HNSCC represents a major worldwide health problem with patients exhibiting a 5-year survival rate lower than 50%. G9a is a histone methyltransferase (HMTase) like DOT1L (DOT1-like HMTase) and it was reported that G9a-mediated H3K9me2 aberrantly promotes transactivation of endogenous cancer genes. These findings support the idea that G9a might be correlated with cell growth, so we could prove the importance of G9a regulatory mechanism. It may be useful to develop an anti-G9a strategy to improve the poor survival rate of HNSCC.

**METHODS AND MATERIALS**

- **Cell viability assay**
  - Cells (2x10⁶) were seeded in 96-well plate per well. The cell growth rate were determined by using MIT (3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay. Each experiment was repeated at least three times.

- **Mouse model for tumorigenesis**
  - Age-matched random-diet-related obese (ND-RD) female mice (6–8 weeks old) were used. The mice were anesthetized by exposure to 1–3% isoflurane and subsequently marked with cells (5x10⁶ FaDu cells) injected subcutaneously in each side (the upper side of the left mouth). During the period of breeding, tumor size was measured weekly using Delta Light Imaging software. As the effects of implanted tumor cells on body weight, mice weights were recorded at each visit. Six weeks after implantation, the mice were sacrificed. Prostate-specific antigen (PSA) was measured from blood samples collected from the retro-orbital sinus of sacrificed mice. In-vivo fluorescence activity is expressed as a percentage of mean of saline-injected

**RESULTS**

- **G9a strongly expressed in human HNSCC**
  - To determine the potential relevance of G9a to human head and neck cancers, we analyzed G9a mRNA expression through the Oncomine database. According to the database, we found that G9a mRNA expression is higher in all HNSCC tumor samples in non-tumor part samples from patients (Fig 1A). The sample sizes in each category were determined by the number of tumors that was able to be matched to the above criteria. We also analyzed G9a mRNA expression in cancer tissues by real-time PCR. We found that the G9a mRNA was overexpressed in 9/9 HNSCC specimens compared with matching healthy mucosa control (Fig 1B). These results indicated that G9a expression was significantly associated with tumor progression.

- **G9a downregulation of G9a related to cell growth and clonogenicity in vitro**
  - To determine the potential relevance of G9a to human head and neck cancers, we analyzed G9a mRNA expression through the Oncomine database. According to the database, we found that G9a mRNA expression is higher in all HNSCC tumor samples in non-tumor part samples from patients (Fig 1A). The sample sizes in each category were determined by the number of tumors that was able to be matched to the above criteria. We also analyzed G9a mRNA expression in cancer tissues by real-time PCR. We found that the G9a mRNA was overexpressed in 9/9 HNSCC specimens compared with matching healthy mucosa control (Fig 1B). These results indicated that G9a expression was significantly associated with tumor progression.

**DISCUSSION AND CONCLUSIONS**

Our findings suggested that G9a possessed strong oncogenic properties and correlated with sustained malignant phenotype. Inhibition of G9a expression could suppress HNSCC cells growth and in vivo tumorigenicity. These results provide a new information that G9a plays an important role in the HNSCC tumor progression and G9a knockdown will be a new anti-cancer strategy in HNSCC.

**REFERENCES**