



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

The Frequency and Impact of  
Fibroblast Growth Factor Receptor 1  
Amplification and p16 Protein  
Expression on Clinical Outcomes  
in Resected Head and Neck Squamous  
Cell Carcinoma



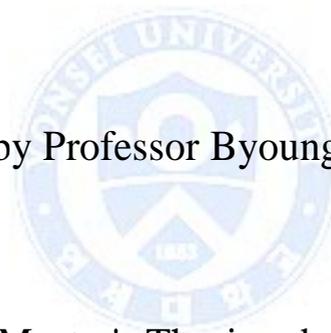
Su Jin Heo

Department of Medicine

The Graduate School, Yonsei University

The Frequency and Impact of  
Fibroblast Growth Factor Receptor 1  
Amplification and p16 Protein  
Expression on Clinical Outcomes  
in Resected Head and Neck Squamous  
Cell Carcinoma

Directed by Professor Byoung Chul Cho



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

Su Jin Heo

December 2015

This certifies that the Master's Thesis of  
Su Jin Heo is approved.



-----  
Thesis Supervisor : Yoon Woo Koh



-----  
Thesis Committee Member#1 : Byoung Chul Cho



-----  
Thesis Committee Member#2 : Sun Och Yoon

The Graduate School  
Yonsei University

December 2015

## ACKNOWLEDGEMENTS

It is a great pleasure to express my deep sense of thanks and gratitude to my mentor and guide Dr. B.C. Cho, Department of Internal Medicine, Division of Medical Oncology, Yonsei Cancer Center, Yonsei University College of Medicine. His prompt inspiration, enthusiasm, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extent to accomplish this task.

I owe a deep sense of gratitude to Dr. Eun Kyung Kim, Department of Pathology, Yonsei University College of Medicine for important charge of this research, having hard time finding lights in darkness.

I express sincere gratitude to Dr. Sun Och Yoon, Department of Pathology, Yonsei University College of Medicine, and Dr. Yoon Woo Koh, Department of Otorhinolaryngology, Yonsei University College of Medicine, whose guidance, suggestion and very constructive criticism have contributed immensely to the evolution of my research.

Finally, I am extremely thankful to my family and friends, for consistent encouragement throughout my research period.

## <TABLE OF CONTENTS>

|  |    |
|--|----|
| ABSTRACT .....   | 1  |
| I. INTRODUCTION .....  | 2  |
| II. MATERIALS AND METHODS .....  | 4  |
| 1. Patients .....  | 4  |
| 2. <i>FGFR1</i> FISH method .....  | 5  |
| 3. p16 Immunohistochemistry .....  | 6  |
| 4. Statistical Analyses .....  | 7  |
| III. RESULTS .....   | 7  |
| 1. Patient Characteristics .....   | 7  |
| 2. <i>FGFR1</i> Amplification and p16 status .....                                   | 11 |
| 3. Survival Outcomes According to <i>FGFR1</i> Amplification and p16<br>status ..... | 14 |
| IV. DISCUSSION .....   | 19 |
| V. CONCLUSION .....  | 20 |
| REFERENCES .....   | 21 |
| ABSTRACT(IN KOREAN) .....  | 25 |

## LIST OF FIGURES

|   |    |
|---|----|
| Figure 1. <i>Fibroblast growth factor receptor1 (FGFR1)</i><br>amplification assessed by fluorescent in situ hybridization .... | 9  |
| Figure 2. Survival analysis on the bases of <i>FGFR1</i> amplification<br>.....   | 14 |
| Figure 3. Survival analysis on the bases of p16 status. ....  | 15 |

## LIST OF TABLES

|   |    |
|---|----|
| Table 1. Definition of <i>FGFR1</i> amplification .....   | 6  |
| Table 2. Baseline characteristics of the patients according to<br><i>FGFR1</i> amplification status ..... | 8  |
| Table 3. Baseline characteristics of the patients according to p16<br>status .....                        | 12 |
| Table 4. Univariate and multivariate analysis of overall survival<br>.....                                | 17 |

## ABSTRACT

### The Frequency and Impact of Fibroblast Growth Factor Receptor 1 Amplification and p16 Protein Expression on Clinical Outcomes in Resected Head and Neck Squamous Cell Carcinoma

Su Jin Heo

*Department of Medicine,  
The Graduate School, Yonsei University*

(Directed by Professor Byoung Chul Cho)

The aim of this study was to investigate the frequency and the impact of *Fibroblast growth factor receptor1 (FGFR1)* amplification and p16 protein expression on clinical outcomes in curatively resected head and neck squamous cell carcinoma (HNSCC). Tumor tissue from 383 patients with HNSCC from November 2005 and December 2012 were collected and analyzed using an *FGFR1* fluorescent in situ hybridization (FISH) assay. High amplification was defined as percentage of tumor cells containing  $\geq 9$  signals in  $\geq 20\%$  cells, and low amplification was defined as percentage of tumor cells containing 2~8 signals in  $\geq 20\%$  cells. High and low amplifications were detected in 1.0% and 41.3%, respectively. In our study, the prognostic impact of *FGFR1* amplification was not observed, probably due to tumor heterogeneity in HNSCC, unstandardized FISH criteria for *FGFR1* amplification, varying adjuvant treatment, and wide variation in *FGFR1* amplification group may contribute to the controversial result. And, as known, p16 protein expression was confirmed as a strong and independent predictor of survival. Further studies are needed to identify the criteria for *FGFR1* amplification and its therapeutic efficacy.

---

Key words : *FGFR1*, p16, squamous cell carcinoma, head and neck

# The Frequency and Impact of Fibroblast Growth Factor Receptor 1 Amplification and p16 Protein Expression on Clinical Outcomes in Resected Head and Neck Squamous Cell Carcinoma

Su Jin Heo

*Department of Medicine  
The Graduate School, Yonsei University*

(Directed by Professor Byoung Chul Cho)

## I. INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) arises from mucosa lining the paranasal sinuses, nasal cavities, oral cavity, oropharynx, hypopharynx, and larynx. It is the sixth leading cancer by incidence worldwide which affect 600,000 patients per year with 40-50% 5-year survival rate<sup>1</sup>. Risk factors included tobacco use, alcohol consumption, human papilloma virus (HPV) infection, and genetic disorders such as Fanconi Anemia<sup>1-3</sup>. Most of patients with HNSCC are treated with largely uniform approach based on stage and anatomic location, typically using surgery, radiation therapy, and chemotherapy alone or in combination. Despite these multidisciplinary treatments, approximately half of all patients will die of the disease because of local aggressiveness and high rate of early relapse<sup>4</sup>. Cetuximab, anti-epidermal growth factor receptor (EGFR) antibody, is the only approved target agent in treatment of HNSCC since 2006 and yielded modest increases in response rate of 10-13% when used in combination with standard chemotherapy and radiation therapy. Unlike other types of cancers, there have been no validated predictive biomarkers for benefit from cetuximab in HNSCC<sup>5</sup>. Recently, the cancer genome atlas (TCGA) profiled 279 HNSCC to provide a comprehensive landscape of somatic genomic alterations according to HPV status, smoking, and primary tumor sites<sup>6</sup>. Nevertheless, there are no effective targeted

therapies available for HNSCC. The effort to identify the novel therapeutic targets and prognostic markers in HNSCC is under way.

The Fibroblast Growth Factor Receptor (FGFR) tyrosine kinase family comprises four kinases: FGFR1, FGFR2, FGFR3, and FGFR4. These kinases play crucial roles in cancer development and targets for dysregulation by amplification, point mutations, and translocation in many cancers<sup>7</sup>. Amplification or activation of *FGFR1* has been reported in breast adenocarcinoma, lung squamous cell cancer (lung SqCC), esophageal squamous cell carcinomas, ovarian cancer, bladder cancer, rhabdomyosarcoma, and oral squamous carcinoma<sup>8-11</sup>. Kim et al<sup>12</sup> recently reported *FGFR1* amplification in 13% of lung SqCC and its negative prognostic impact. Also, their study showed a positive association between *FGFR1* amplification and smoking dosage. In resected esophageal squamous cell carcinoma, high *FGFR1* amplification had a greater risk of recurrence and death. Like in lung SqCC, high amplification was significantly higher in current smokers than former and never-smokers and increased proportional to smoking dosage<sup>13</sup>.

Based on the histomorphological and clinical similarities between HNSCC and other squamous cell carcinoma, many studies have been described a potential role of *FGFR1*. In the first study about *FGFR1* amplification in HNSCC, Freier et al<sup>11</sup> reported *FGFR1* amplifications in 17% oral squamous cell carcinomas in a limited number of patient samples. Recently, Goke et al<sup>14</sup> described *FGFR1* amplification in 15% of patients with HNSCC. It was associated with nicotine and alcohol consumption and parameters of worse outcome, so it represents a potential role of therapeutic biomarker. In vivo studies have demonstrated inhibition of the *FGFR1* pathway with FGFR inhibitors that led to significant tumor shrinkage, suggesting that FGFR inhibitors might be an effective therapeutic option in HNSCC with *FGFR1* amplification<sup>15</sup>.

HPV status in tumors can be determined by several assays, including HPV DNA detection by in situ hybridization (ISH) or polymerase chain reaction (PCR), HPVE6/E7 RNA expression detected by quantitative reverse transcriptase-PCR (qRT-PCR), and/or p16 protein expression by immunohistochemistry (IHC) staining as a surrogate marker of oncogenic HPV infection<sup>16-20</sup>. Among these

assays, detection of HPV E6/E7 RNA expression, which indicates active viral oncogene transcription in tumor cells, is considered to be a gold standard<sup>18,19</sup>. However, because RNA isolation for qRT-PCR requires additional sample preparation steps and a larger amount of tumor cells compared with other assays, the most widely used assays are HPV ISH and p16 IHC. The p16 protein is an important tumor suppressor and cell-cycle regulator<sup>21</sup>. In HPV-positive tumors, the viral protein E7 binds to retinoblastoma susceptibility protein (Rb) through cullin 2 ubiquitin ligase complex and rapidly degrades Rb by ubiquitination<sup>22</sup>. Loss of Rb results in upregulation of p16 protein expression by a feedback interaction<sup>23,24</sup>. It is well established that patients with HPV-positive/p16-positive HNSCC have a more favorable prognosis compared with those with HPV-negative/p16-negative HNSCC<sup>16,17,20,25,26</sup>.

In this study, we sought to determine the frequency and the impact of *FGFR1* amplification and p16 protein expression on clinical outcomes in curatively resected with surgically resected HNSCC.

## II. MATERIALS AND METHODS

### 1. Patients

This study was conducted in a cohort of patients with HNSCC who underwent curative resection at Severance Hospital, Seoul, Korea, between November 2005 and December 2012. The criteria used for patient selection included (1) surgically resected HNSCC for curative aim, (2) availability of tumor tissue and clinical data on smoking status and survival, (3) no preoperative treatment, and (4) no distant metastasis. The primary tumor sites categorized in four groups as following: oral cavity, oropharynx, hypopharynx, and larynx. Oral cavity included hard palate, tongue, and buccal mucosa. Oropharynx included floor of mouth, base of tongue, and tonsils. Hypopharynx included pyriform sinus and larynx included supraglottis and glottis. We excluded 121 cases which had undergone the process of decalcification and 13 cases which were

not profit to produce tissue microarray. Finally, the tumor samples of 384 patients were available for examination of *FGFR1* amplification. Two pathologists (S.O.Y. and E.K.K.) confirmed the diagnosis of HNSCC by hematoxylin and eosin staining. Paraffin-embedded tumor specimens were used to construct a tissue microarray with 2-mm-diameter cores. Each patient was represented by three tissue cores. Patients' information was collected by reviewing the medical records for evaluation of clinicopathologic characteristics and survival outcomes. Staging was determined using the 7<sup>th</sup> edition American Joint Committee on Cancer (AJCC) guideline for tumor, node, and metastasis (TNM) classification. Never-smokers were defined as those with a lifetime smoking dose of fewer than 100 cigarettes, former smokers were those who had stopped smoking for more than 1 year, and current smokers were those who currently smoke or quit smoking for less than 1 year<sup>27</sup>.

## 2. *FGFR1* FISH method

Fluorescent in situ hybridization (FISH) assay was performed on the tissue microarrays by using *FGFR1* probes that hybridized to the 8p12–8p11.23 region using the fluorophore, Spectrum Orange (red) and to the centromere region of chromosome 8 (CEP 8) using the fluorophore, Spectrum Green (Abbott Molecular, Abbott Park, IL) following the manufacturers' instructions. FISH analyses were interpreted by two experienced evaluators (S.O.Y. and E.K.K.) blinded to the clinical data. Cells with sharp borders of nuclei, no signs of overdigestion, non-overlapping nuclei were evaluated. Normal tissue including vessels, fibroblasts, or non-tumor squamous epithelium served as internal positive control. Cases were only further evaluated if control tissue nuclei displayed one or two clearly distinct signals of each color. Tumor tissue was scanned for amplification hot spots by using x 40 or x 63 objectives. If the *FGFR1* signals were homogeneously distributed, then random areas were used for counting the signals. Twenty contiguous tumor cell nuclei

from three hot spots or random areas, resulting in a total of 60 nuclei, were individually evaluated with the x 100 objectives by counting red *FGFR1* and green centromere of chromosome 8 (CEP8) signals. *FGFR1* amplification was defined based on the previous study<sup>14</sup> (Table1).

Table1. Definition of *FGFR1* amplification<sup>14</sup>

|                    |  |
|--------------------|--|
| High amplification | Nine or more red target signals or clusters of target gene signals as compared with the green reference signals displayed in at least 20% nuclei |
| Low amplification  | Lower than nine but more than two red target signals as compared with the green reference signals displayed in at least 20% nuclei               |

### 3. p16 Immunohistochemistry

Tumor p16 expression was evaluated by IHC using a mouse monoclonal antibody (Clone E0037, Ventana, AZ, USA) and was visualized with the Ventana XT autostainer using the the 1-view secondary detection kit (Ventatn, Tuscon, AZ) for details see manufacturer's recommendations. Tumor p16 expression was scored as positive if strong and diffuse nuclear and cytoplasmic staining was present in at least 75% of the tumor cells, and alternatively >50% staining combined with >25% confluent areas<sup>28,29</sup>.

### 4. Statistical Analyses

Our primary objective was to evaluate the frequency of *FGFR1* amplification and p16 protein expression in patients with HNSCC. Our secondary objectives were to identify the clinical features of patients with *FGFR1* amplified and p16 expressed tumors and to analyze its impact of *FGFR1* on disease-free survival (DFS) and overall survival (OS) in patients. DFS was measured from the time of surgery to initial tumor relapse (local recurrence or distant) or death as a result of any cause. OS, calculated from the time of surgery to death or last follow-up date, and 95% confidence intervals (CIs) were evaluated by survival

analysis using the Kaplan-Meier method. We used Chi-square, Fisher's exact, and Mann-Whitney tests to compare the clinical factors among the patients with level of *FGFR1* amplification and p16 status. Statistical significance was set at  $P < .05$  for all analyses. Survival outcomes among the group were compared by using the log-rank test. Multivariate analysis was performed by using Cox regression analysis with the following prespecified variables: sex, smoking, primary tumor site, histologic differentiation, lymphovascular invasion, perineural invasion, resection margin, p16 status, pathologic T stage, pathologic N stage, and *FGFR1* amplification according to both categories. All statistical analyses were performed by using SPSS version 20.0 (SPSS, Chicago, IL).

### III. RESULTS

#### 1. Patient Characteristics

A total of 383 patients with surgically resected HNSCC were analyzed of *FGFR1* amplification. The clinical characteristics of the enrolled patients are shown in Table 2 and 3, divided by categories A and B. There were 287 (74.9%) male and 96 (25.1%) female with a median age of 58 years (range 22-88). The majority of patients were current (40.5%) or former (20.4%) smokers, and median smoking dosage was 17.0 pack-years (range 0-100). Sites of primary tumor were distributed as follows: 51.7% in oral cavity, 31.1% in oropharynx, 7.3% in hypopharynx, and 9.9% in larynx. The histologic differentiations of squamous cell carcinoma were 36.8% in well differentiation, 50.1% in moderated differentiation, and 13.1% in poor differentiation. In pathologic results, lymphovascular invasion was 19.1%, perineural invasion was 13.3%, and the positive of resection margin was 23.2%. About half of tumors had pathologic T1 or N0 stage. The AJCC stages were I in 27.9%, stage II in 11.2%, stage III in 18.0%, stage IVA in

42.3% and stage IVB in 0.5%. Adjuvant treatment was given in 242 (63.2%) patients and of those, 95 patients were received adjuvant concurrent chemoradiation therapy (CCRT) and 147 patients were received adjuvant radiation therapy.

Table 2. Baseline characteristics of the patients according to *FGFR1* amplification status

| Characteristics                   | All patients<br>n (%) | High<br>amplification<br>n (%) | Low<br>amplification<br>n (%) | No<br>amplification<br>n (%) | <i>P</i> -value |
|-----------------------------------|-----------------------|--------------------------------|-------------------------------|------------------------------|-----------------|
| Number of patients                | 383                   | 4 (1.0)                        | 158 (41.3)                    | 221 (57.7)                   |                 |
| <b>Age, year</b>                  |                       |                                |                               |                              | 0.430           |
| Median                            | 58.0                  | 69.5                           | 59.0                          | 58.0                         |                 |
| Range                             | 22-88                 | 42-73                          | 24-87                         | 22-88                        |                 |
| <b>Sex</b>                        |                       |                                |                               |                              | 0.631           |
| Male                              | 287 (74.9)            | 4 (100.0)                      | 120 (75.9)                    | 163 (73.8)                   |                 |
| Female                            | 96 (25.1)             | 0 (0.0)                        | 38 (24.1)                     | 58 (26.2)                    |                 |
| <b>Smoking</b>                    |                       |                                |                               |                              | 0.579           |
| Never smoker                      | 150 (39.2)            | 0 (0.0)                        | 62 (39.2)                     | 88 (39.8)                    |                 |
| Former smoker                     | 78 (20.4)             | 1 (25.0)                       | 32 (20.3)                     | 45 (20.4)                    |                 |
| Current smoker                    | 155 (40.5)            | 3 (75.0)                       | 64 (40.5)                     | 88 (39.8)                    |                 |
| <b>Smoking dosage, pack/years</b> |                       |                                |                               |                              | 0.061           |
| Median                            | 17.0                  | 45.0                           | 15.0                          | 17.0                         |                 |
| Range                             | 0-100                 | 22-60                          | 0-80                          | 0-100                        |                 |
| <b>Primary sites</b>              |                       |                                |                               |                              | 0.206           |
| Oral cavity                       | 198 (51.7)            | 1 (25.0)                       | 78 (49.4)                     | 119 (53.8)                   |                 |
| Oropharynx                        | 119 (31.1)            | 2 (50.0)                       | 45 (28.5)                     | 72 (32.6)                    |                 |
| Hypopharynx                       | 28 (7.3)              | 0 (0.0)                        | 16 (10.1)                     | 12 (5.4)                     |                 |
| Larynx                            | 38 (9.9)              | 1 (25.0)                       | 19 (12.0)                     | 18 (8.1)                     |                 |
| <b>Histologic differentiation</b> |                       |                                |                               |                              | 0.106           |
| Well differentiated               | 141 (36.8)            | 1 (25.0)                       | 67 (42.4)                     | 73 (33.0)                    |                 |
| Moderated differentiated          | 192 (50.1)            | 2 (50.0)                       | 77 (48.7)                     | 113 (51.1)                   |                 |
| Poorly differentiated             | 50 (13.1)             | 1 (25.0)                       | 14 (9.9)                      | 35 (15.8)                    |                 |
| <b>Lymphovascular invasion</b>    |                       |                                |                               |                              | 0.322           |
| Yes                               | 73 (19.1)             | 1 (25.0)                       | 35 (22.2)                     | 37 (16.7)                    |                 |
| No                                | 310 (80.9)            | 3 (75.0)                       | 123 (77.8)                    | 184 (83.3)                   |                 |

|                                 |              |              |             |             |        |
|---------------------------------|--------------|--------------|-------------|-------------|--------|
| <b>Perineural invasion</b>      |              |              |             |             | 0.555  |
| Yes                             | 51 (13.3)    | 1 (25.0)     | 20 (12.7)   | 30 (13.6)   |        |
| No                              | 332 (86.7)   | 3 (75.0)     | 138 (87.3)  | 191 (86.4)  |        |
| <b>Resection margin</b>         |              |              |             |             | 0.310  |
| Positive                        | 89 (23.2)    | 2 (50.0)     | 34 (21.5)   | 53 (24.0)   |        |
| Negative                        | 294 (76.8)   | 2 (50.0)     | 124 (78.5)  | 168 (76.0)  |        |
| <b>T stage</b>                  |              |              |             |             | 0.946  |
| T1                              | 169 (44.1)   | 2 (50.0)     | 69 (43.7)   | 98 (44.3)   |        |
| T2                              | 145 (37.9)   | 2 (50.0)     | 60 (38.0)   | 83 (37.6)   |        |
| T3                              | 29 (7.6)     | 0 (0.0)      | 10 (6.3)    | 19 (8.6)    |        |
| T4                              | 40 (10.4)    | 0 (0.0)      | 19 (12.0)   | 21 (9.5)    |        |
| <b>N stage</b>                  |              |              |             |             | 0.986  |
| N0                              | 167 (43.6)   | 2 (50.0)     | 71 (44.9)   | 94 (42.5)   |        |
| N1                              | 69 (18.0)    | 0 (0.0)      | 28 (17.7)   | 41 (18.6)   |        |
| N2                              | 145 (37.9)   | 2 (50.0)     | 58 (36.7)   | 85 (38.5)   |        |
| N3                              | 2 (0.5)      | 0 (0.0)      | 1 (0.6)     | 1 (0.5)     |        |
| <b>AJCC stage</b>               |              |              |             |             | 0.870  |
| Stage I                         | 107 (27.9)   | 1 (25.0)     | 44 (27.8)   | 62 (28.1)   |        |
| Stage II                        | 43 (11.2)    | 1 (25.0)     | 21 (13.3)   | 21 (9.5)    |        |
| Stage III                       | 69 (18.0)    | 0 (0.0)      | 27 (17.1)   | 42 (19.0)   |        |
| Stage IVA                       | 162 (42.3)   | 2 (50.0)     | 65 (41.1)   | 95 (43.0)   |        |
| Stage IVB                       | 2 (0.5)      | 0 (0.0)      | 1 (0.6)     | 1 (0.5)     |        |
| <b>Adjuvant treatment</b>       |              |              |             |             | 0.079  |
| CCRT                            | 95 (24.8)    | 3 (75.0)     | 43 (27.2)   | 49 (22.2)   |        |
| Radiotherapy                    | 147 (38.4)   | 0 (0.0)      | 47 (29.7)   | 100 (45.2)  |        |
| No                              | 141 (36.8)   | 1 (25.0)     | 68 (43.0)   | 72 (32.6)   |        |
| <b>FGFR1 FISH amplification</b> |              |              |             |             |        |
| Number                          | 2.08         | 7.30         | 2.67        | 2.08        | <0.001 |
| (median, range)                 | (1.55-10.08) | (6.75-10.08) | (1.97-6.08) | (1.55-2.47) |        |
| FGFR1/CEP8                      | 0.99         | 3.40         | 0.97        | 0.99        | <0.001 |
| ratio                           | (0.43-5.13)  | (2.40-5.13)  | (0.43-2.76) | (0.48-1.24) |        |
| (median, range)                 |              |              |             |             |        |
| <b>p16 status</b>               |              |              |             |             | 0.043  |
| Positive                        | 162 (42.3)   | 0 (0.0)      | 59 (37.3)   | 103 (46.6)  |        |
| Negative                        | 221 (57.7)   | 4 (100.0)    | 99 (62.7)   | 118 (53.4)  |        |

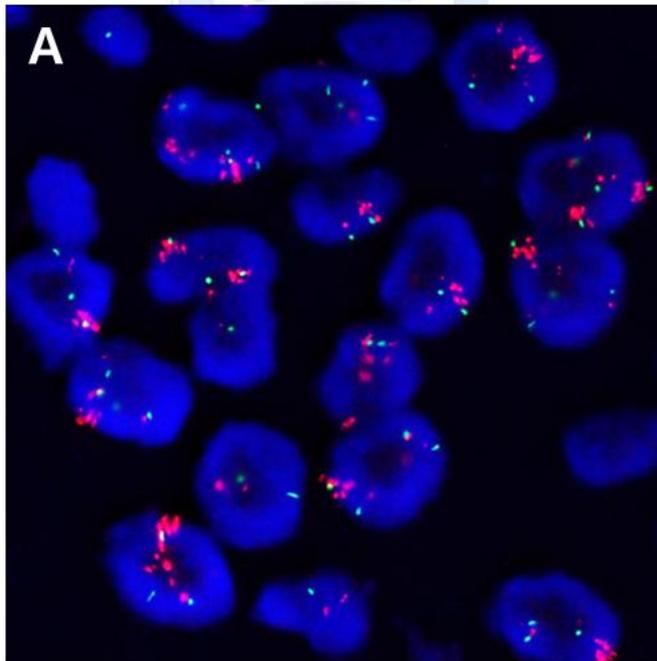
Abbreviations: AJCC, American Joint Committee on Cancer; CCRT, Concurrent chemoradiation therapy ; *FGFR1*, *Fibroblast Growth Factor Receptor1*; FISH, Fluorescent in situ hybridization

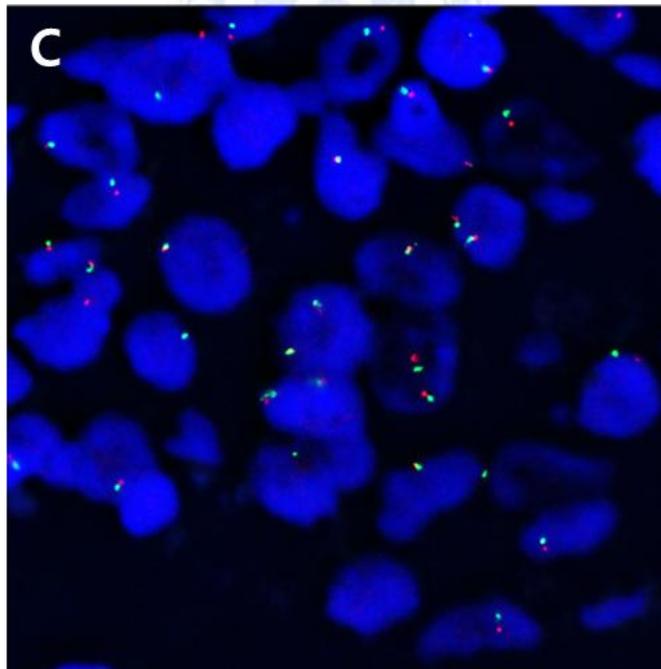
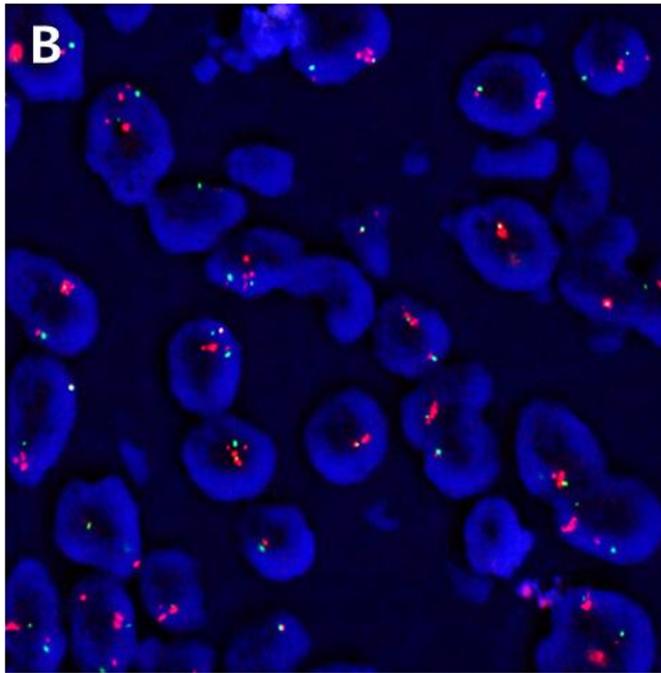
## 2. *FGFR1* Amplification and p16 status

Among a total of 383 patients, 4 (1.0%) were high *FGFR1* amplification, 158 (41.3%) were low *FGFR1* amplification, and 221

(57.7%) were no amplification (Table2; Figure1). The median *FGFR1* gene copy number per nucleus and the mean *FGFR1*/CEN8 ratio in all patients were 2.08 (range, 1.55 to 10.08 copies per nucleus) and 0.99 (range, 0.43 to 5.13). The median *FGFR1* gene copy number was 7.30 (range, 6.75 to 10.08) in high amplification, 2.67 (range, 1.97 to 6.08) in low amplification, and 2.08 (range 1.55 to 2.47) in no amplification group. The median *FGFR1*/CEN8 ratio was 3.40 (range 2.40 to 5.13), 0.97 (range, 0.43 to 2.76), and 0.99 (range, 0.48 to 1.24) in high, low and no amplification group, respectively. The p16 negative status related to unfavorable prognosis was 100% in high amplification, 62.7% in low amplification, and 53.4% in no amplification. There was statistically significant difference among these three groups.

Figure 1. *Fibroblast growth factor receptor1 (FGFR1)* amplification assessed by fluorescent in situ hybridization (FISH). (A) High *FGFR1* amplification; (B) low *FGFR1* amplification; (C) no amplification.





Based on p16 status, 162 (42.4%) patients were p16 positive and 221

(57.6%) patients were p16 negative in total cohort (Table 3). There were significant difference in smoking status, primary sites, histologic differentiation, lymphovascular invasion, resection margin, pathologic stage, and *FGFR1* amplification between two groups. In primary tumor sites, oropharynx was most common in p16 positive group, and oral cavity, hypopharynx, larynx were relatively common in p16 negative group. Favorable histologic differentiation, no lymphovascular invasion, negative resection margin, early pathologic stage which known to related to good prognosis were common in p16 negative group. In contrast, gene copy number of *FGFR1* amplification was higher in p16 negative group than in p16 positive group.

Table 3. Baseline characteristics of the patients according to p16 status

| Characteristics                   | All patients<br>n (%) | p16 positive<br>n (%) | p16 negative<br>n (%) | P-value |
|-----------------------------------|-----------------------|-----------------------|-----------------------|---------|
| Number of patients                | 383                   | 162 (42.4)            | 221 (57.6)            |         |
| <b>Age, year</b>                  |                       |                       |                       | 0.465   |
| Median                            | 58.0                  | 57.0                  | 59.0                  |         |
| Range                             | 22-88                 | 23-87                 | 22-88                 |         |
| <b>Sex</b>                        |                       |                       |                       | 0.925   |
| Male                              | 287 (74.9)            | 121 (74.7)            | 166 (75.1)            |         |
| Female                            | 96 (25.1)             | 41 (25.3)             | 55 (24.9)             |         |
| <b>Smoking</b>                    |                       |                       |                       | 0.037   |
| Never smoker                      | 150 (39.2)            | 69 (42.6)             | 81 (36.7)             |         |
| Former smoker                     | 78 (20.4)             | 23 (14.2)             | 55 (24.9)             |         |
| Current smoker                    | 155 (40.5)            | 70 (43.2)             | 85 (38.5)             |         |
| <b>Smoking dosage, pack/years</b> |                       |                       |                       | 0.979   |
| Median                            | 17.0                  | 15.0                  | 20.0                  |         |
| Range                             | 0-100                 | 0-100                 | 0-100                 |         |
| <b>Primary sites</b>              |                       |                       |                       | <0.001  |
| Oral cavity                       | 198 (51.7)            | 51 (31.5)             | 147 (66.5)            |         |
| Oropharynx                        | 119 (31.1)            | 98 (60.5)             | 21 (9.5)              |         |
| Hypopharynx                       | 28 (7.3)              | 5 (3.1)               | 23 (10.4)             |         |
| Larynx                            | 38 (9.9)              | 8 (4.9)               | 30 (13.6)             |         |
| <b>Histologic differentiation</b> |                       |                       |                       | <0.001  |
| Well differentiated               | 141 (36.8)            | 32 (19.8)             | 109 (49.3)            |         |

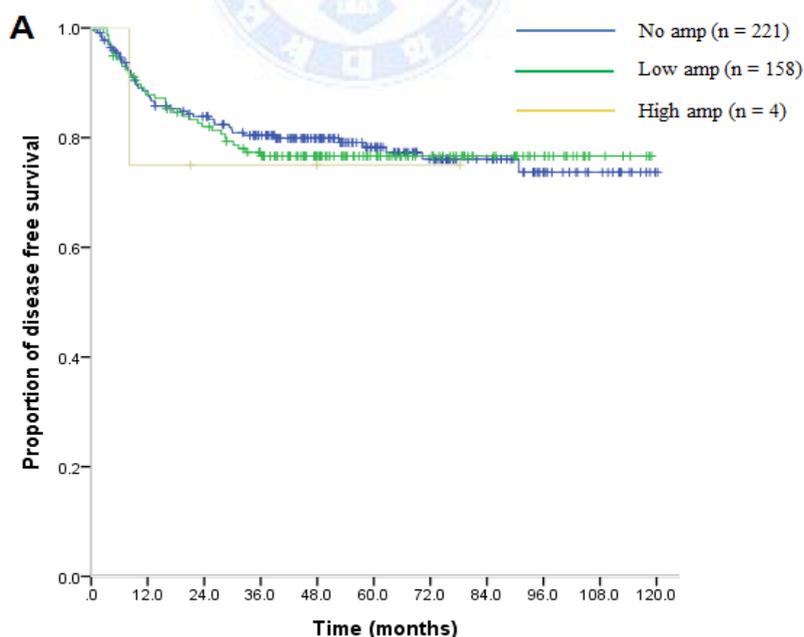
|   |                      |                     |                      |        |
|---|----------------------|---------------------|----------------------|--------|
| Moderated differentiated                    | 192 (50.1)           | 96 (59.3)           | 96 (43.4)            |        |
| Poorly differentiated                       | 50 (13.1)            | 34 (21.0)           | 16 (7.2)             |        |
| <b><i>Lymphovascular invasion</i></b>       |                      |                     |                      | <0.001 |
| Yes   | 73 (19.1)            | 45 (27.8)           | 28 (12.7)            |        |
| No  | 310 (80.9)           | 117 (72.2)          | 193 (87.3)           |        |
| <b><i>Perineural invasion</i></b>           |                      |                     |                      | 0.090  |
| Yes   | 51 (13.3)            | 16 (9.9)            | 35 (15.8)            |        |
| No  | 332 (86.7)           | 146 (90.1)          | 186 (84.2)           |        |
| <b><i>Resection margin</i></b>              |                      |                     |                      | 0.011  |
| Positive                                    | 89 (23.2)            | 48 (29.6)           | 41 (18.6)            |        |
| Negative                                    | 294 (76.8)           | 114 (70.4)          | 180 (81.4)           |        |
| <b><i>T stage</i></b>                       |                      |                     |                      | 0.002  |
| T1  | 169 (44.1)           | 56 (34.6)           | 113 (51.1)           |        |
| T2  | 145 (37.9)           | 78 (48.1)           | 67 (30.3)            |        |
| T3  | 29 (7.6)             | 14 (8.6)            | 15 (6.8)             |        |
| T4  | 40 (10.4)            | 14 (8.6)            | 26 (11.8)            |        |
| <b><i>N stage</i></b>                       |                      |                     |                      | 0.001  |
| N0  | 167 (43.6)           | 56 (34.6)           | 111 (50.2)           |        |
| N1  | 69 (18.0)            | 26 (16.0)           | 43 (19.5)            |        |
| N2  | 145 (37.9)           | 80 (49.4)           | 65 (29.4)            |        |
| N3  | 2 (0.5)              | 0 (0.0)             | 2 (0.9)              |        |
| <b><i>AJCC stage</i></b>                    |                      |                     |                      | 0.001  |
| Stage I                                     | 107 (27.9)           | 30 (18.5)           | 77 (34.8)            |        |
| Stage II                                    | 43 (11.2)            | 21 (13.0)           | 22 (10.0)            |        |
| Stage III                                   | 69 (18.0)            | 26 (16.0)           | 43 (19.5)            |        |
| Stage IVA                                   | 162 (42.3)           | 85 (52.5)           | 77 (34.8)            |        |
| Stage IVB                                   | 2 (0.5)              | 0 (0.0)             | 2 (0.9)              |        |
| <b><i>Adjuvant treatment</i></b>            |                      |                     |                      | <0.001 |
| CCRT  | 95 (24.8)            | 46 (28.4)           | 49 (22.2)            |        |
| Radiotherapy                                | 147 (38.4)           | 81 (50.0)           | 66 (29.9)            |        |
| No  | 141 (36.8)           | 35 (21.6)           | 106 (48.0)           |        |
| <b><i>FGFR1 FISH amplification</i></b>      |                      |                     |                      | 0.001  |
| Number<br>(median, range)                   | 2.08<br>(1.55-10.08) | 2.06<br>(1.62-5.43) | 2.10<br>(1.55-10.08) |        |
| <i>FGFR1</i> /CEP8 ratio<br>(median, range) | 0.99<br>(0.43-5.13)  | 1.00<br>(0.58-2-23) | 0.97<br>(0.43-5.13)  | 0.185  |

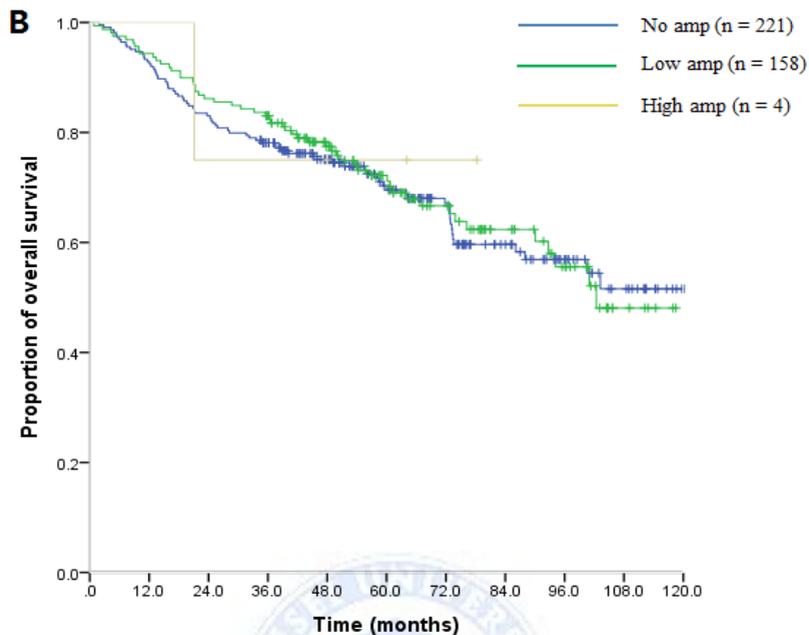
Abbreviations: AJCC, American Joint Committee on Cancer; CCRT, Concurrent chemoradiation therapy ; *FGFR1*, *Fibroblast Growth Factor Receptor1*; FISH, Fluorescent in situ hybridization

3. Survival Outcomes According to *FGFR1* Amplification and p16 status

With a median follow-up time of 53.1 months, the 5-year DFS and OS rates for all patients were 150 (39.2%) and 167 (43.6%), respectively. The median DFS for each of the three *FGFR1* groups were not reached (Figure 2A). In comparison of mean survival, patients with high *FGFR1* amplification showed shorter DFS than those with low and no amplification (60.8 vs 95.2 months in low amplification and 95.9 months in no amplification,  $P=0.955$ ). Figure 2B showed OS in Kaplan-Meier method, the median OS for one of the three *FGFR1* groups were not reached. Regarding the mean survival, patients with high *FGFR1* amplification showed shorter OS than those with low and no amplification (64.0 vs 85.9 months in low amplification and 85.8 months in no amplification,  $P=0.933$ ).

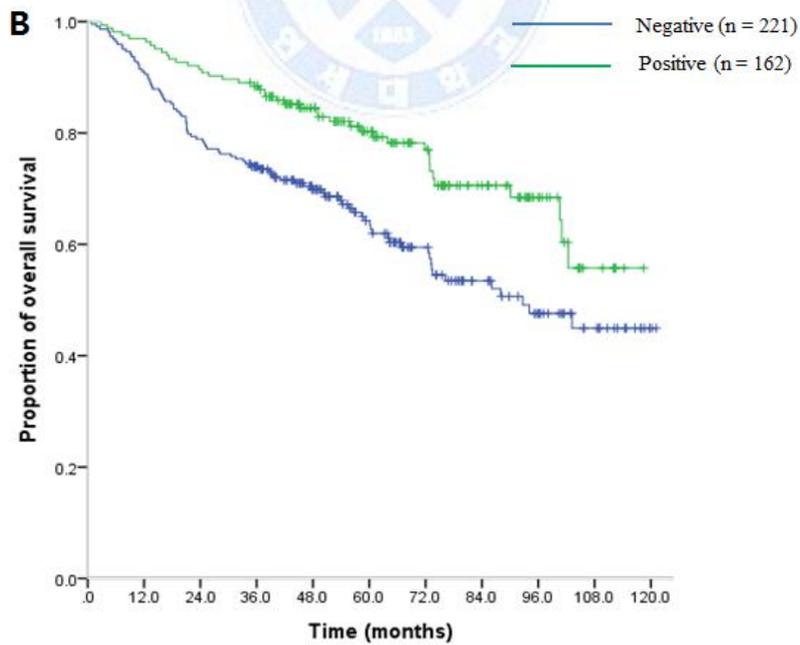
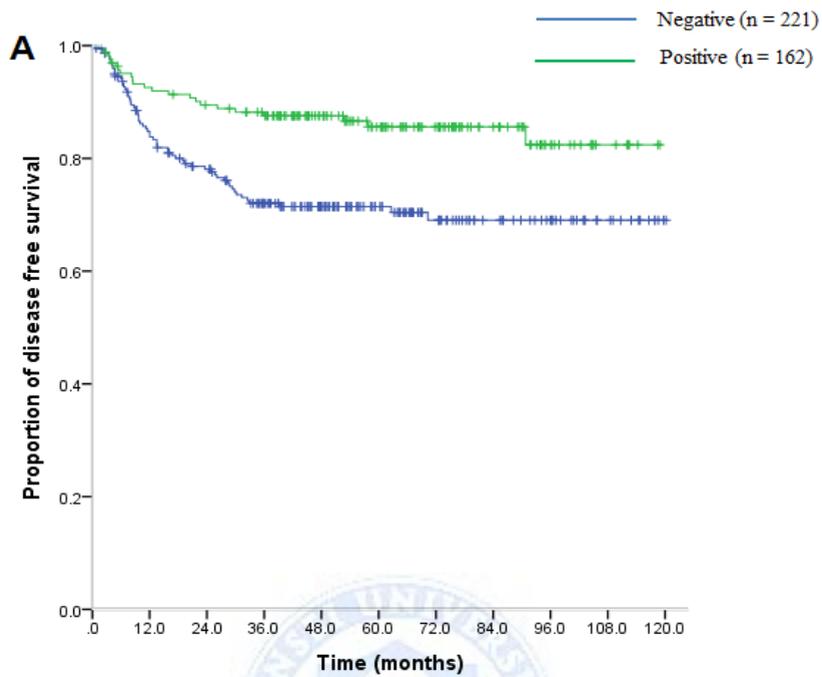
Figure 2. Survival analysis on the bases of *FGFR1* amplification (high, low, and no amplification). (A) Disease free survival, and (B) overall survival were not showed significant difference.





Based on p16 status, patients with p16 positive tumors had significantly better DFS and OS than patients with p16 negative tumors (Figure 3). For DFS, the hazard ratio (HR) was 0.42 (95% CI, 0.26 to 0.70), reflecting a 58% reduction in recurrence for patients with p16 positive tumors ( $P=0.001$ ). For OS, the HR was 0.52 (95% CI, 0.36 to 0.75), reflecting a 48% reduction in death rate for patients with p16 positive tumors ( $P<0.001$ ).

Figure 3. Survival analysis on the bases of p16 status. (A) Disease free survival, and (B) overall survival were significantly better in patients with p16 positive tumors.



In Cox proportional hazard model adjusted for smoking, primary sites (oropharynx, hypopharynx), lymphovascular invasion, perineural invasion, positive resection margin, positive nodal status, p16 positive status and postoperative CCRT, p16 positive status was significantly associated with a longer OS (HR 0.48; 95% CI, 0.30-0.76; P=0.002, Table 4). Lymphovascular invasion, positive resection margin and positive nodal status were significantly related to shorter OS in multivariate analysis. There was no significant difference in OS for sex, smoking status, and *FGFR1* amplification in multivariate analysis.

Table 4. Univariate and multivariate analysis of overall survival

|                            | Univariate analysis |           |       | Multivariate analysis |           |       |
|----------------------------|---------------------|-----------|-------|-----------------------|-----------|-------|
|                            | HR                  | 95%CI     | P     | HR                    | 95%CI     | P     |
| Sex                        | 0.77                | 0.51-1.17 | 0.222 |                       |           |       |
| Smoking                    | 1.36                | 0.94-1.95 | 0.099 |                       |           |       |
| Oral cavity                | 1.10                | 0.78-1.55 | 0.591 |                       |           |       |
| Oropharynx                 | 0.68                | 0.46-1.00 | 0.052 | 0.62                  | 0.37-1.03 | 0.066 |
| Hypopharynx                | 1.70                | 0.96-3.02 | 0.069 |                       |           |       |
| Larynx                     | 1.26                | 0.71-2.24 | 0.438 |                       |           |       |
| Lymphovascular invasion    | 1.89                | 1.28-2.80 | 0.002 | 1.78                  | 1.17-2.73 | 0.008 |
| Perineural invasion        | 1.70                | 1.07-2.68 | 0.024 |                       |           |       |
| Positive resection margin  | 1.57                | 1.08-2.29 | 0.019 | 1.62                  | 1.09-2.41 | 0.018 |
| Positive nodal status      | 2.19                | 1.50-3.19 | 0.001 | 2.34                  | 1.56-3.51 | 0.001 |
| p16 positive               | 0.52                | 0.36-0.75 | 0.001 | 0.48                  | 0.30-0.76 | 0.002 |
| <i>FGFR1</i> amplification | 1.00                | 0.88-1.24 | 0.625 |                       |           |       |
| CCRT                       | 1.59                | 1.08-2.35 | 0.020 |                       |           |       |
| Radiotherapy               | 0.87                | 0.61-1.23 | 0.425 |                       |           |       |

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence intervals; *FGFR1*, *Fibroblast Growth Factor Receptor1*; CCRT, Concurrent chemoradiation therapy ;

#### IV. DISCUSSION

In this study, we investigated the frequency and the impact of *FGFR1*

amplification and p16 protein expression on clinical outcomes in patients with resected HNSCC. To our knowledge, this is the first report on the prognostic impact of *FGFR1* amplification in the largest cohort of resected HNSCC patients from East Asians. Our study demonstrated the frequency of *FGFR1* amplification is common 42.3% (1.0% for high amplification and 41.3% for low amplification) and there was no relation to prognostic impact on clinical outcomes. Regarding p16 status, a surrogate marker of oncogenic HPV infection, there were 42.4% of p16 positive tumors and related to favorable prognosis significantly.

The frequency of *FGFR1* amplification has been reported in squamous cell carcinoma of lung, esophagus, SCLC, and HNSCC, for which smoking is known dominant risk factor<sup>10,12,13,30</sup>. Overall, the frequency of *FGFR1* amplification was reported to be 5.6-24.8% in lung SqCC<sup>12</sup>, and 6-9.4% in esophageal squamous cell carcinoma<sup>13</sup>. As determined by FISH analysis in our study, *FGFR1* amplification was 42.3%, which was higher compared to a value of 15% reported in a recent study in white patients from Western countries<sup>14</sup>. Of them, low *FGFR1* amplification was observed more commonly in our study, 41.3% vs 14.0%, respectively. There could be ethnic differences in the frequency of *FGFR1* amplification and the prevalence of HPV infection. The other types of carcinoma reported variable ethnic difference in prevalence of *FGFR1* amplification. In SCLC, the frequency of *FGFR1* amplification was reported to be 5.6-6% in western population<sup>31</sup> and 1.9% in East Asian population<sup>30</sup>. In comparison, in esophageal squamous cell carcinoma, there are similar frequency between Western Europe and East Asia, 9.4% and 8.6%, respectively<sup>13</sup>.

*FGFR1* amplification has been known to be associated with poor prognosis or unfavorable clinicopathologic parameters in squamous cell carcinoma of lung, esophagus, and head and neck with several controversial results. In resected lung SqCC, Kim et al<sup>12</sup> reported *FGFR1* amplification as negative prognostic factor, whereas Heist et al<sup>32</sup> observed no significant difference in OS. In HNSCC, *FGFR1* amplification was significantly associated with poor prognostic factors such as higher T stage, lymphovascular invasion, and higher numbers of visceral metastases<sup>14</sup>. In our study, the prognostic impact of *FGFR1* amplification was

not founded. Tumor heterogeneity in HNSCC, unstandardized FISH criteria for *FGFR1* amplification, excellent surgical skills, varying adjuvant treatment, wide variation for *FGFR1* amplification, and different frequency of *FGFR1* alteration according to HPV status may contribute to the controversial results. In TCGA data<sup>6</sup>, *FGFR1* alteration was higher (10%) in HPV negative group, and in HPV positive group, *FGFR3* alteration was more common (11%).

Unfortunately, standard definition for *FGFR1* amplification by FISH is not established yet. Indeed, the definition of *FGFR1* amplification by FISH technique has been highly variable in the previous studies<sup>9,10,12,14,32,33</sup>. Unlike breast cancer, lung SqCC exhibits small-clusters and co-amplifications of *FGFR1* and CEN8<sup>33</sup>. Therefore, *FGFR1* FISH assay needs to differentiate between true amplification and polysomy in lung SqCC. In a large cohort study, Schildhause et al<sup>33</sup> proposed a more sophisticated *FGFR1* FISH criteria using average gene copy number per nucleus, *FGFR1*/CEN8 ratio, and percentage of gene clusters at the same time. By the addition of *FGFR1*/CEN8 ratio, 8 out of 47 cases (17.0%) were newly classified as high amplification in that study. In our study, we applied the *FGFR1* FISH criteria in HNSCC previously proposed by Goke et al<sup>14</sup>, which not consider co-amplification of CEP8. Applied in our study, if the criteria for *FGFR1*/CEN8 ratio included, 14 patients (3.5%) might have been classified from low amplification group to high amplification group additionally.

In the previous studies<sup>10,12,13</sup>, *FGFR1* amplification may be an oncogenic driver mutation in tobacco-associated cancers of the aerodigestive tract. In our study, there was no relation between smoking status and survival outcomes. It's caused by small sample size of high amplification and depending on medical record, underestimated amount of smoking by patients' statement when they had visited to clinic in first time of diagnosis.

In clinicopathologic characteristics of p16 negative group, several factors were known to be related good prognosis: favorable histologic differentiation, no lymphovascular invasion, negative resection margin, early pathologic stage. Nevertheless, p16 negative group had poor disease free and overall survival outcomes. As known in previous studies<sup>26,34,35</sup>, p16 protein expression was

confirmed as a strong and independent predictor of survival in oropharyngeal and nonoropharyngeal HNSCC. In p16 negative group, percentage of high/low FGFR1 amplification, and gene copy number of FGFR1 amplification were higher than in p16 positive group, suspiciously in which FGFR1 amplification had tendency for poor prognosis.

Our study had several limitations. The main limitation includes its retrospective nature and selection bias for patient's cohort. This is likely related to selection of surgically resected, earlier stage patients who relatively have favorable prognosis. And during process of manufacturing TMA, we excluded about 170 tissues for hypopharynx and larynx which had been decalcified. Because the sample included only a few high *FGFR1* amplified tumors, we did not have enough statistical power to identify significant differences between the clinical characteristics of patients with *FGFR1* amplification and those without *FGFR1* amplification. To identify such characteristics, a dedicate criteria with HNSCC will be needed.

Several potent selective FGFR tyrosine kinase inhibitors are already in early clinical development. Tyrosine kinase inhibitors targeting multiple receptors including *FGFR1* have been tried in lung SqCC. There are recent reports of promising results with the non-ATP competitive pan-FGFR selective inhibitor LY2874455<sup>36</sup>, the FGFR1-3 selective inhibitor AZD4547<sup>37</sup>, and the FGFR1-3 selective inhibitor BGJ398<sup>15</sup>. Those agents are reported to have manageable toxicities including hyperphosphatemia, hypercalcemia, and ectopic tissue calcification; correlated with abnormal phosphate and vitamin D homeostasis caused by the blockage of *FGF23* signaling<sup>38</sup>. Further clinical trials could show that *FGFR1* inhibition has a therapeutic effect in *FGFR1* amplified HNSCC. These emerging treatment strategies may shed light on treatment for patients with HNSCC who lack a specific therapeutic target. Further investigation for finding profit biomarkers and promising candidate drugs in clinical trials are currently ongoing and needed to proceed.

## V. CONCLUSION

In conclusion, we did not demonstrate the prognostic impact of *FGFR1* amplification in resected HNSCC. As known, p16 protein expression was confirmed as a strong and independent predictor of survival. Further research for finding a dedicate criteria for *FGFR1* amplification and promising candidate drugs in clinical trials will be needed.

## REFERENCES

1. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. *J Clin Invest* 2012;122:1951-7.
2. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, et al. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. *Arch Otolaryngol Head Neck Surg* 2003;129:106-12.
3. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer* 2011;11:9-22.
4. Conley BA. Treatment of advanced head and neck cancer: what lessons have we learned? *J Clin Oncol* 2006;24:1023-5.
5. Specenier P, Vermorken JB. Cetuximab in the treatment of squamous cell carcinoma of the head and neck. *Expert Rev Anticancer Ther* 2011;11:511-24.
6. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 2015;517:576-82.
7. Brooks AN, Kilgour E, Smith PD. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clin Cancer Res* 2012;18:1855-62.
8. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. *Nat Rev Cancer* 2010;10:116-29.
9. Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res* 2010;70:2085-94.
10. Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med* 2010;2:62ra93.
11. Freier K, Schwaenen C, Sticht C, Flechtenmacher C, Muhling J, Hofele C, et al. Recurrent FGFR1 amplification and high FGFR1 protein expression in oral squamous cell carcinoma (OSCC). *Oral Oncol* 2007;43:60-6.

12. Kim HR, Kim DJ, Kang DR, Lee JG, Lim SM, Lee CY, et al. Fibroblast growth factor receptor 1 gene amplification is associated with poor survival and cigarette smoking dosage in patients with resected squamous cell lung cancer. *J Clin Oncol* 2013;31:731-7.
13. Kim HS, Lee SE, Bae YS, Kim DJ, Lee CG, Hur J, et al. Fibroblast growth factor receptor 1 gene amplification is associated with poor survival in patients with resected esophageal squamous cell carcinoma. *Oncotarget* 2015;6:2562-72.
14. Goke F, Bode M, Franzen A, Kirsten R, Goltz D, Goke A, et al. Fibroblast growth factor receptor 1 amplification is a common event in squamous cell carcinoma of the head and neck. *Mod Pathol* 2013;26:1298-306.
15. Goke F, Franzen A, Hinz TK, Marek LA, Yoon P, Sharma R, et al. FGFR1 Expression Levels Predict BGJ398 Sensitivity of FGFR1-Dependent Head and Neck Squamous Cell Cancers. *Clin Cancer Res* 2015;21:4356-64.
16. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;363:24-35.
17. Rischin D, Young RJ, Fisher R, Fox SB, Le QT, Peters LJ, et al. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol* 2010;28:4142-8.
18. Shi W, Kato H, Perez-Ordóñez B, Pintilie M, Huang S, Hui A, et al. Comparative prognostic value of HPV16 E6 mRNA compared with in situ hybridization for human oropharyngeal squamous carcinoma. *J Clin Oncol* 2009;27:6213-21.
19. Jordan RC, Lingen MW, Perez-Ordóñez B, He X, Pickard R, Koluder M, et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. *Am J Surg Pathol* 2012;36:945-54.
20. Rietbergen MM, Brakenhoff RH, Bloemena E, Witte BI, Snijders PJ, Heideman DA, et al. Human papillomavirus detection and comorbidity: critical issues in selection of patients with oropharyngeal cancer for treatment De-escalation trials. *Ann Oncol* 2013;24:2740-5.
21. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1993;366:704-7.
22. Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989;243:934-7.
23. Parry D, Bates S, Mann DJ, Peters G. Lack of cyclin D-Cdk com

- plexes in Rb-negative cells correlates with high levels of p16INK4/MTS1 tumour suppressor gene product. *Embo j* 1995;14:503-11.
24. Andl T, Kahn T, Pfuhl A, Nicola T, Erber R, Conradt C, et al. Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. *Cancer Res* 1998;58:5-13.
  25. Posner MR, Lorch JH, Goloubeva O, Tan M, Schumaker LM, Sarlis NJ, et al. Survival and human papillomavirus in oropharynx cancer in TAX 324: a subset analysis from an international phase II I trial. *Ann Oncol* 2011;22:1071-7.
  26. Chung CH, Zhang Q, Kong CS, Harris J, Fertig EJ, Harari PM, et al. p16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. *J Clin Oncol* 2014;32:3930-8.
  27. Couraud S, Zalzman G, Milleron B, Morin F, Souquet PJ. Lung cancer in never smokers--a review. *Eur J Cancer* 2012;48:1299-311.
  28. Chen ZW, Weinreb I, Kamel-Reid S, Perez-Ordóñez B. Equivocal p16 immunostaining in squamous cell carcinoma of the head and neck: staining patterns are suggestive of HPV status. *Head Neck Pathol* 2012;6:422-9.
  29. Lewis JS, Jr., Chernock RD, Ma XJ, Flanagan JJ, Luo Y, Gao G, et al. Partial p16 staining in oropharyngeal squamous cell carcinoma: extent and pattern correlate with human papillomavirus RNA status. *Mod Pathol* 2012;25:1212-20.
  30. Park JS, Lee JS, Kim EY, Jung JY, Kim SK, Chang J, et al. The frequency and impact of FGFR1 amplification on clinical outcomes in Korean patients with small cell lung cancer. *Lung Cancer* 2015;88:325-31.
  31. Schultheis AM, Bos M, Schmitz K, Wilsberg L, Binot E, Wolf J, et al. Fibroblast growth factor receptor 1 (FGFR1) amplification is a potential therapeutic target in small-cell lung cancer. *Mod Pathol* 2014;27:214-21.
  32. Heist RS, Mino-Kenudson M, Sequist LV, Tammireddy S, Morrissey L, Christiani DC, et al. FGFR1 amplification in squamous cell carcinoma of the lung. *J Thorac Oncol* 2012;7:1775-80.
  33. Schildhaus HU, Heukamp LC, Merkelbach-Bruse S, Riesner K, Schmitz K, Binot E, et al. Definition of a fluorescence in-situ hybridization score identifies high- and low-level FGFR1 amplification types in squamous cell lung cancer. *Mod Pathol* 2012;25:1473-80.
  34. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsague X, Laporte L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. *Lancet Oncol* 2014;15:1319-31.

35. Fakhry C, Zhang Q, Nguyen-Tan PF, Rosenthal D, El-Naggar A, Garden AS, et al. Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2014;32:3365-73.
36. Zhao G, Li WY, Chen D, Henry JR, Li HY, Chen Z, et al. A novel, selective inhibitor of fibroblast growth factor receptors that shows a potent broad spectrum of antitumor activity in several tumor xenograft models. *Mol Cancer Ther* 2011;10:2200-10.
37. Gavine PR, Mooney L, Kilgour E, Thomas AP, Al-Kadhimi K, Beck S, et al. AZD4547: an orally bioavailable, potent, and selective inhibitor of the fibroblast growth factor receptor tyrosine kinase family. *Cancer Res* 2012;72:2045-56.
38. Lim SM, Kim HR, Shim HS, Soo RA, Cho BC. Role of FGF receptors as an emerging therapeutic target in lung squamous cell carcinoma. *Future Oncol* 2013;9:377-86.



ABSTRACT (IN KOREAN)

수술로 절제된 두경부 편평세포암에서  
FGFR1 유전자 증폭과 p16 단백질 발현의  
빈도 및 임상적 영향에 관한 연구

<지도교수 조 병 철>

연세대학교 대학원 의학과

허 수 진

본 연구의 목적은 수술로 절제된 두경부 편평세포암에서 Fibroblast growth factor receptor 1 (*FGFR1*) 유전자 증폭과 p16 단백질 발현의 빈도 및 임상적 영향에 대해 알아보고자 함이다. 2005년 11월부터 2012년 12월까지 383명의 환자로부터 얻은 조직으로 형광동소보합법의 기법을 사용하여 *FGFR1* 유전자 증폭을 분석하였다. 이전 연구를 참고하여, 고증폭의 기준은 9개 이상의 신호를 가진 종양세포가 20% 이상일 경우, 저증폭의 기준은 2개 이상, 9개 미만의 신호를 가진 종양세포가 20% 이상일 경우로 정하였다. 그 결과 *FGFR1* 고증폭은 1.0%, *FGFR1* 저증폭은 41.3% 였고 *FGFR1* 증폭의 정도는 생존율과 관련성을 보이지 않았고 p16 유전자 발현 양성은 42.4% 의 빈도로 생존율 향상과 관련을 보였다. 두경부 종양의 부위별 이질성, 형광동소보합법 기준의 비표준화, 수술 후 치료의 다양성, 넓은 범위의 저증폭 환자비율 등이 이전 연구들의 결과와 차이점을 보이는 것으로 생각된다. 추후 *FGFR1* 증폭 분석 기준의 표준화에 대한 연구와 *FGFR1* 의 치료적 효용성에 대한 추가적인 연구가 필요하겠다.

-----  
핵심되는 말 : *FGFR1*, p16, 두경부 편평세포암