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# Comprehensive Bioinformatical Analysis of Association of IGFL2 Expression and Methylation Profiles with Prognosis of Head and Neck Cancer

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**ABSTRACT**

Insulin-like growth factor like (IGFL) family is a gene family that contains 11 cysteine residues, including two CC motifs, and has structural homology with Insulin like Growth Factor (IGF) family. Expression of IGFL genes has been detected in various tissues, including fetal skin, spinal cord, cerebellum, placenta, spleen, stomach, testis, and fetal heart. The biological functions and gene interactions of IGFL molecules have not yet been fully elucidated, but they are thought to perform similar functions to IGFs due to their structural similarities with the IGF family. Therefore, it is aimed to determined the relationship between IGFL2 expression and Head and Neck Cancer (HNSC) through bioinformatical approaches. GEPIA2, UALCAN, OncoDB, Mexpress, Cancersea and Kaplan-Meier Plotter databases were used for bioinformatical analyses. Our results showed that expression of IGFL2 increased in tumor tissues especially early stages of HNSC. In addition, we detected that promotor methylation status of IGFL2 decreased in tumor tissues. Gene correlation analyses also showed that IGFL2 expression is positively correlated with gene products that are responsible for the development of HNSC such as IRS1, EGFR, CDK6. As a result, IGFL2 induces tumorigenesis in early stages of HNSC and may be used as a prognostic factor in the development of HNSC.

**Keywords:** IGFL2, HNSC, Pan-cancer.

## Introduction

Head and Neck Cancer (HNSC) is the seventh most common type of cancer worldwide, with more than 660,000 new cases annually. It has a poor prognosis and associated with 325,000 deaths (1-3). The majority of head and neck cancers are squamous cell carcinomas that occur in the oral cavity, pharynx and larynx. Alcohol and tobacco products consumptions and Human Papilomma Virus (HPV) infections are the primary risk factors for HNSC. It is assumed that the incidence of HNSC will increase in the future correlated with these risk factors worldwide (2,3).

IGFL gene family, which encodes a protein consisting of approximately 100 amino acids, contains 11 conserved cysteine residues, 2 of which are the CC motif. This family, located on chromosome 19, consists of four genes and two pseudogenes clustered at 35 kb intervals (IGFL1-IGFL4, IGFL1P1 and IGFL1P2). It has been determined that IGFL1 is expressed in the ovaries and spinal cord, IGFL2 is expressed in the cerebellum, heart, placenta, spleen, stomach, testicle and thymus, and IGFL3 and IGFL4 is expressed in the cerebellum (4).

IGFL genes have been determined to have structural homology with IGF family (4). IGF signaling system consists of IGF1 and IGF2 ligands. These ligands interact with cell surface receptors IGF-1R, IR and IGF-2R. These activated receptors then bind to SHC and IRS proteins, exerting proliferative and apoptotic effects via pathways such as MAP kinase and PI3 kinase. IGF signals play a role not only in growth and development but also in pathological conditions such as tumor formation (5,6). Many types of cancer, such as prostate cancer, breast cancer, lung cancer, colon cancer, and osteosarcoma, have been found to be associated with IGF signals (5-11).

Although it has been shown that IGF family plays a crucial role in many types of cancer, studies on the IGFL family, which has similar functions, are limited. Studies on the IGFL family have showed that IGFL2 is the member of this family which is

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widely expressed in human tissues and it was determined that IGFL2 gene expression increased in various cancer types such as bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA) and kidney renal clear cell carcinoma (KIRC). This study also showed that the IGFL2 gene is associated with survival in KIRC and BLCA and IGFL2 expression leads to poor prognosis (12). However, IGFL2 needs to be examined comprehensively for each type of cancer. Therefore, we examined the expression and functions of IGFL2 in HNSC comprehensively using bioinformatical approaches.

## Material and Methods

**IGFL2 Gene Expression Profiling in HNSC: GEPIA2 database:** To determine the IGFL2 gene expression in HNSC, GEPIA2 database was used. GEPIA2 is an updated database that allows the analysis of RNA sequencing expression data for 84 cancer types and 198,619 isoforms, obtained from The Cancer Genome Atlas (TCGA) project and the Genotype-Tissue Expression (GTEx) project. Using this database, it is possible to perform comparisons of expression levels at the protein and transcript levels in normal and tumor tissues for different cancer types, as well as to conduct pathological staging and survival analyses (13).

**Relationship Between IGFL2 Expression and the Pathological and Clinical Features of HNSC: UALCAN database:** The relationship between IGFL2 and the pathological and clinical features of HNSC was examined using the UALCAN database. UALCAN enables users to conduct detailed analyses with OMICS data. It provides easy access to publicly available cancer OMIC data (TCGA, MET500, CPTAC, and CBTTC), and is capable of correlating gene expressions with cancer clinico-pathological features, presenting pan-cancer gene expressions graphically, and matching gene methylation characteristics with pathological data. To provide additional information on selected genes, it can direct users to GeneCards, PubMed, TargetScan, the Human Protein Atlas, DRUGBANK, Open Targets, and GTEx (14,15).

**Relationship Between IGFL2 Expression and Functional Cancer Stages in HNSC: Cancersea**: To understand the relationship between IGFL2 gene expression and the functional stages of cancer, Cancer Single-cell State Atlas (CANCERSEA) database was used. CANCERSEA is a database capable of presenting expression changes in 18,895 genes through single-cell analysis in a total of 93,475 cancer cells across 27 cancer types and their related cancer types. Additionally, this database allows for the statistical analysis of 14 functional states active in carcinogenesis by correlating these gene expressions (16).

**Determination of IGFL2 Methylation Profile in HNSC: Mexpress:** The MEXPRESS database was used to determine the methylation profile of the IGFL2 gene in head and neck cancers. MEXPRESS is a database that allows for the acquisition and visualization of single-gene expression profiles, DNA methylation, and clinical data from TCGA (17,18).

**Survival Analyses of Patients Based on IGFL2 Expression in HNSC: Kaplan-Meier Plotter:** Survival analyses of patients based on IGFL2 expression in HNSC were conducted using the Kaplan-Meier Plotter. The Kaplan-Meier Plotter is a database that evaluates the correlation between the expression of all genes (mRNA, miRNA, protein, and DNA) and survival outcomes across over 35,000 samples from 21 tumor types, obtained from GEO, EGA, and TCGA (19,20).

**Gene Correlation Analyses in HNSC: OncoDB:** Gene correlation analyses were performed using OncoDB database which is a database that presents data on RNA expression, DNA methylation, oncoviral infections, and the clinical characteristics of cancer patients obtained from more than 10,000 cancer patients in TCGA and normal tissues from the GTEx study (21,22).

**Statistical Analyses:** All statistical analyses were performed using the GEPIA2, UALCAN, CancerSea, MEXPRESS, Kaplan-Meier Plotter, and OncoDB databases, and p-values of less than 0.05 were considered significant.

## Results

**Expression Profile of IGFL2 in HNSC:** Transcriptional expression levels of the IGFL2 in normal and tumor tissues in HNSC were determined using the GEPIA2 database. Our results indicate that expression of IGFL2 gene increased by an average of 10-fold in tumor tissue compared to normal tissue in HNSC ($p<0.05$) (Figure 1)

**Relationship of IGFL2 Expression with Clinicopathological Features of HNSC**: Correlation of IGFL2 expression with

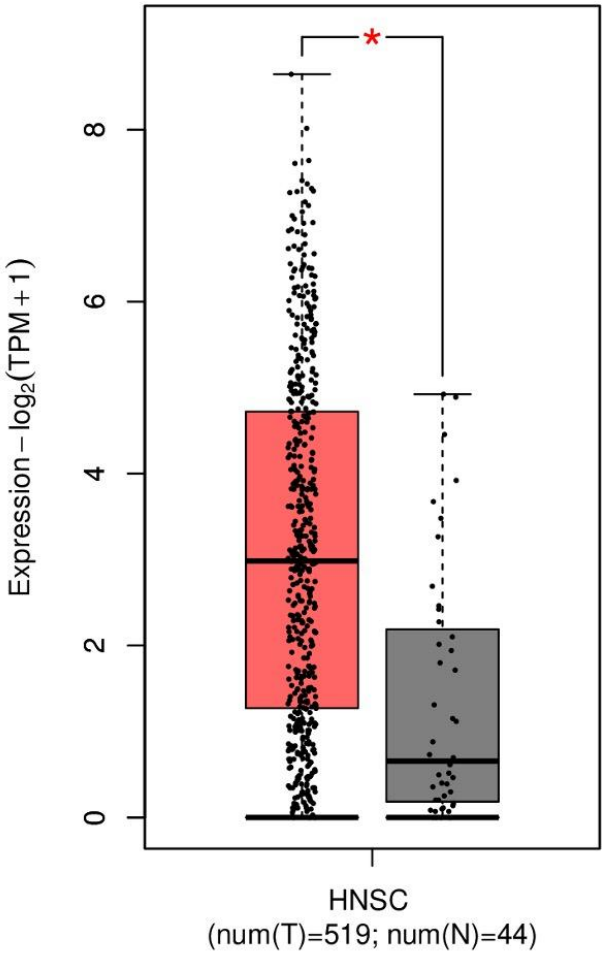


**Fig.1.** Expression profile of IGFL2 in HNSC and normal tissues (*<0.05)

clinicopathological features such as tumor stage, grade, and nodal metastasis of HNSC was examined using UALCAN database.

Firstly, expression profile of IGFL2 was classified according to tumor stages. Results indicate that IGFL2 expression is increased by an average of 27.39-fold in Stage 1 ($p<0.001$), 19.75-fold in Stage 2 ($p<0.0001$), 20.67-fold in Stage 3 ($p<0.0001$), and 20.91-fold in Stage 4 tumors compared to normal tissue ($p<0.0001$) (Figure 2a).

The distribution of IGFL2 expression according to tumor grades in HNSC was also determined. Compared to normal tissue, IGFL2 expression levels are increased by an average of 53.05-fold in Grade 1 tumors ($p<0.0001$), 22.92-fold in Grade 2 tumors ($p<0.0001$), 5.09-fold in Grade 3 tumors ($p<0.001$), and 2.13-fold in Grade 4 tumors. However, we did not observe statistically significant difference in expression profiles of IGFL2 between normal and Grade 4 tumors. Additionally, when tumor grades were compared among themselves, it was found that IGFL2 expression showed statistically significant

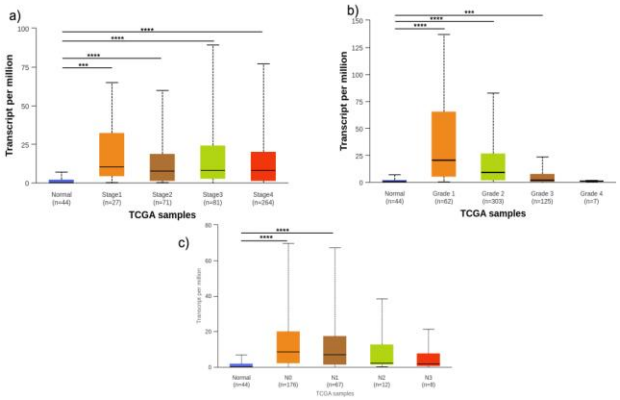


**Fig. 2.** IGFL2 Expression based on stages (a), grades (b) and nodal status (c) of HNSC (***<0.001; ****<0.0001)

decrease stepwise from Grade 1 to Grade 4 tumors, with an average decrease of 24.82-fold between Grade 1 and Grade 4 (Figure 2b).

As another clinicopathological feature, IGFL2 expression was related to level of lymph node metastasis of tumors. In N0 tumor tissue, which has no lymph node metastasis, IGFL2 expression was found to be increased by an average of 22.62-fold compared to normal tissue ($p<0.0001$). In N1 level tumors with 1-3 axillary node metastasis, IGFL2 expression increased by an average of 18.16-fold ($p<0.0001$). No statistically significant change was observed in IGFL2 expression levels between N2 and N3 level tumors compared to N0 and normal tissue (Figure 2c).

Finally, IGFL2 expression was correlated with HPV infection, a significant risk factor in the development of head and neck cancer. It was found that IGFL2 expression was on average 12.46-fold higher in HPV-negative cases compared to HPV-positive ones ($p<0.0001$) (Figure 3).

**The Relationship of IGFL2 Expression with Functional Cancer Stages**: When IGFL2 expression at the single-cell level is associated with 14 functional stages of cancer, analysis results indicate that an increase in IGFL2 expression is positively correlated with DNA repair mechanisms, DNA damage, cell cycle and invasion ($p<0.001$), while it is negatively correlated with stemness ($p<0.001$). (Figure 4)

**Promoter Methylation Status of IGFL2:** Promoter methylation profile analyses of IGFL2 showed that the methylation rate in the promoter region of IGFL2 is significantly decreased in tumor tissues compared to normal tissues (Figure 5). Additionally, according to METEXPRESS data, significant hypomethylation was observed in some CpG dinucleotide repeat regions within the IGFL2 transcript in tumor tissues (Figure 5).

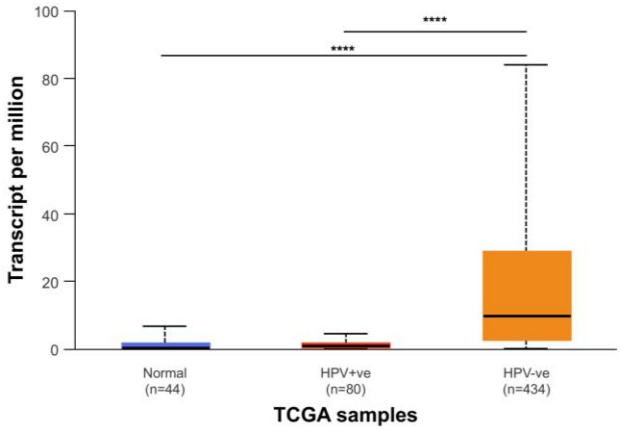


**Fig. 3.** Expression profile of IGFL2 depending on HPV status of HNSC tissues (****<0.0001)

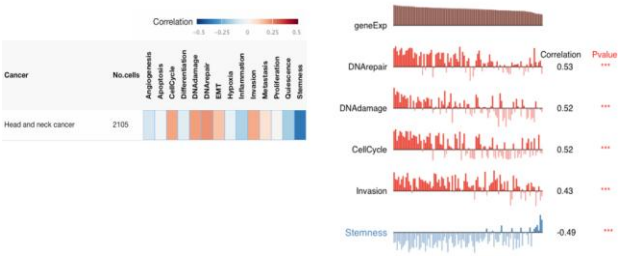


**Fig. 4.** IGFL2 Expression with functional cancer stages

IGFL2 promoter methylation profile was decreased in primary tumors compared to normal tissues (p<0.0001) (Figure 6). In further analyses, IGFL2 promoter methylation profile was examined according to the stages of HNSC and we observed that there is a significant decrease in promoter methylation in Stage 1, 2, 3, and 4 tissues compared to normal tissues, while no significant difference was detected between the stages (Figure 7a).

When the changes in the IGFL2 promoter methylation profile were examined according to the grades of tumors, it is observed that promoter methylation is significantly reduced in grade 1, 2, and 3 tumors compared to normal, while no significant change is observed in grade 4 tumors. Additionally, IGFL2 promoter methylation in grade 1, 2, and 3 tumors is significantly reduced compared to grade 4 tumors (Figure 7b).

**Relationship Between IGFL2 Expression and Survival in HNSC:** KM Plotter was used to evaluate the effect of IGFL2 gene expression on survival in head and neck cancer. The obtained data revealed that IGFL2 gene expression does not have a significant impact on survival in head and neck cancer (Figure 8).

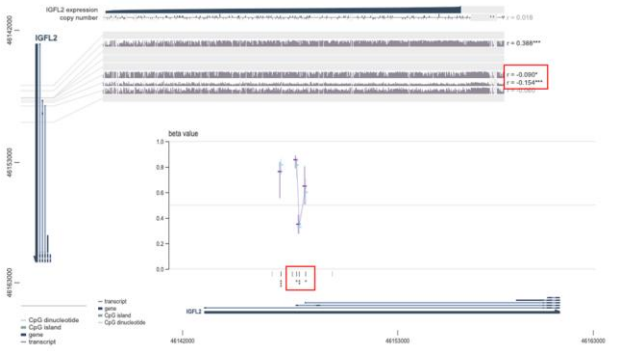


**Fig. 5.** Promoter methylation status of IGFL2 according to MEXPRESS result

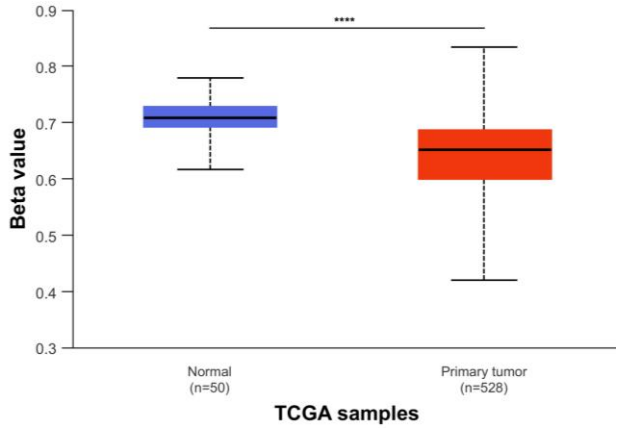


**Fig. 6.** Promoter methylation status of IGFL2 in normal and HNSC tissues (****<0.0001)

**Correlation Between IGFL2 and Gene Products Involved in HNSC Development:** In HNSC tumor tissues, correlation analyses between IGFL2 expression and gene products associated with HNSC development showed a statistically significant positive correlation with the expressions of IGF2, IRS1, EGFR, CDK6, and CCND1, while a statistically significant negative correlation was observed with the expression of CDKN2A (Figure 9).

## Discussion

In this study, the expression and methylation profiles of IGFL2 in HNSC were comprehensively examined through bioinformatics analyses for their functional implications such as survival, gene correlation, and prognosis. Currently, studies on IGFL2 is quite limited, and a pan-cancer study aimed at elucidating the role of IGFL2 in cancer has provided a general profile of IGFL2's role in different cancer types (12). However, this study focuses on the detailed analysis of the role of IGFL2 in the development of HNSC through bioinformatics.

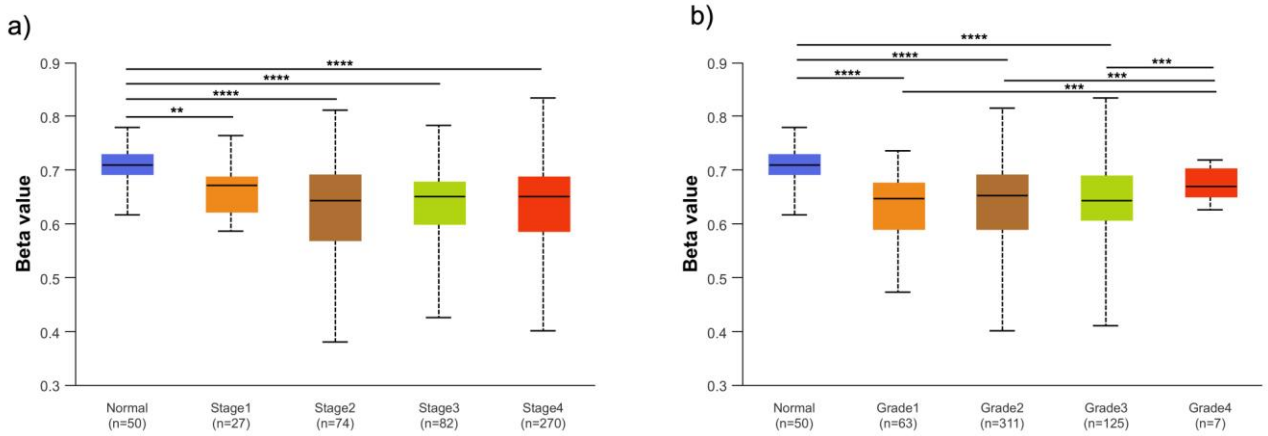


**Fig. 7.** Promoter methylation status of IGFL2 based on stages (a) and grades (b) of HNSC tissues (**<0.01; ***<0.001; ****<0.0001)

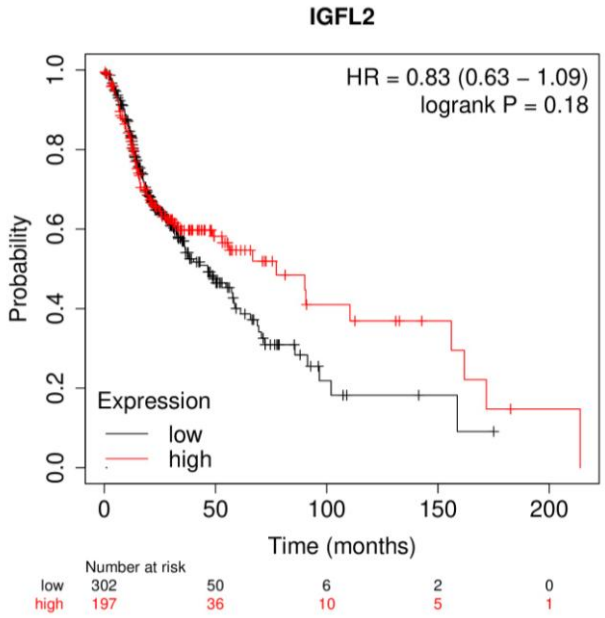


**Fig. 8.** Effects of IGFL2 expression on survival rate of HNSC patients

Our results indicate that IGFL2 expression was increased in HNSC compared to normal tissues. The pan-cancer study, which aimed to determine the increase in IGFL2 expression in different cancer types, also identified a positive correlation of increased IGFL2 expression with several cancer types, including BLCA, BRCA, CHOL, COAD, ESCA and KIRC, while a negative correlation was found with skin cutaneous melanoma (SKCM), testicular germ cell tumors (TGCT), uterine corpus endometrial carcinoma (UCEC) and uterine carcinosarcoma (UCS) cancers (12).

Comparison analyses of the relationship between IGFL2 expression and tumor stages and grades showed that a significant increase was detected in IGFL2 expression, especially in Stage 1 and Grade 1 tumors, which are the early stages of cancer. This result suggested that IGFL2 expression can be significant factor in oncogenic activation. Although increased IGFL2 expressions were observed in various stages and grades of the tumors compared to normal tissues, the significant decrease in expression of IGFL2 was detected in Grade 1 and Grade 4 tumors. These results assumed that increased IGFL2 expression is more likely responsible for transformation of normal cells to tumor cells and oncogenic activations. Additionally, the analysis results, aimed at uncovering the relationship between IGFL2 expression and nodal status, particularly observing an increase in IGFL2 expression in N0 and N1 stages, also supported this hypothesis. Moreover, the lack of relationship between IGFL2 expression and survival rate of the patients suggested that IGFL2 may be an effective protein that induces the oncogenesis in the early stages of cancer.

In HNSC, a decrease in the methylation profile in tumor tissues and CpG dinucleotide repeats was found in the IGFL2 promoter. Decreased promoter methylation status correlated with the increased IGFL2 expression in tumor tissues (12,17). Additionally, the IGFL2 methylation profiles observed in tumor stages and grades are consistent with IGFL2 expression, this is another supportive data that IGFL2 may play a significant role in the transition from normal tissue to cancer tissue.

Single-cell RNA seq data which makes possible to uncover the relationship between IGFL2 expression and 14 functional stages occuring in carcinogenesis showed that that IGFL2's positive correlation with oncogenic activation, DNA damage, DNA repair, and cell cycle stages results

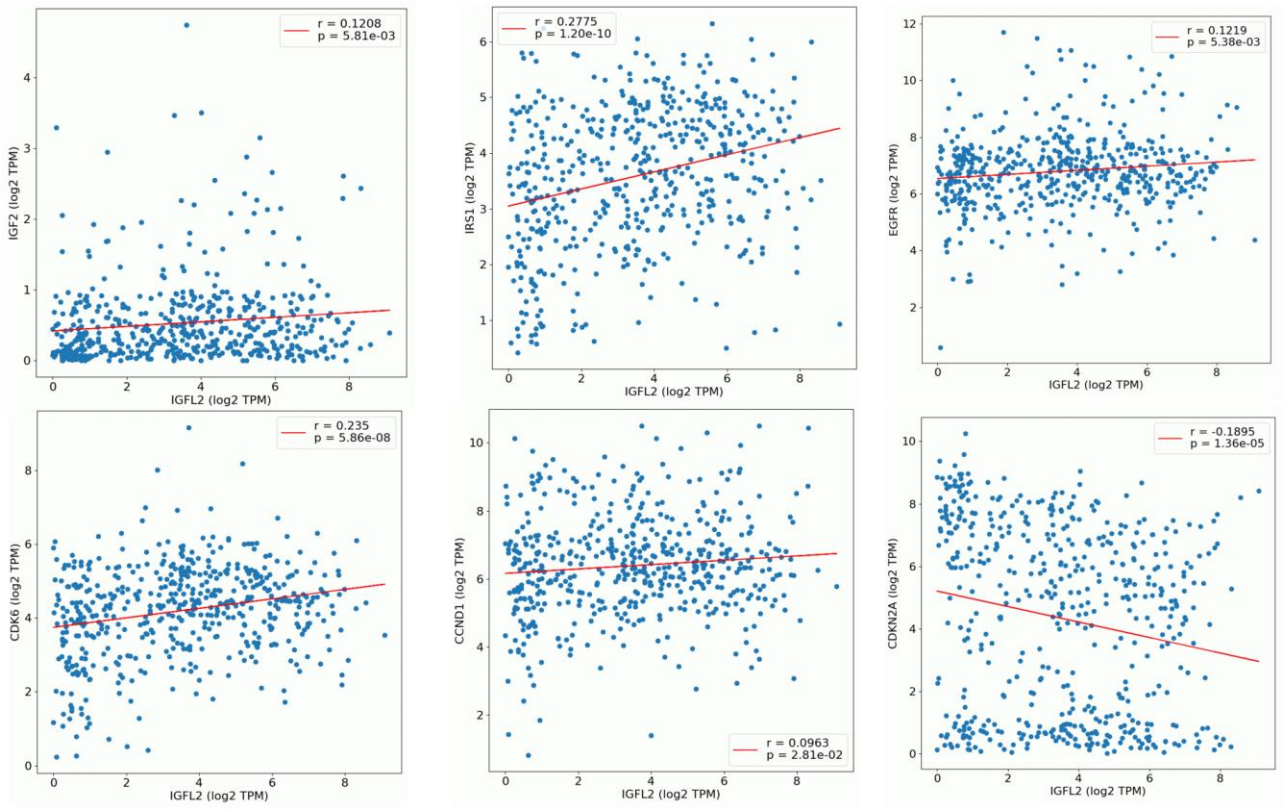


**Fig. 9.** Gene correlation analyses of IGFL2

in its activation (16). To further analyses we determined the gene correlations of the IGFL2 protein with proteins involved in the development of HNSC were examined. In this context, IGFL2 was found to show a positive correlation with proteins such as IGF2, IRS1, EGFR, CDK6, and CCND1, which are involved in cell proliferation, survival, and the cell cycle, particularly in the early stages of cancer (23).

As a conclusion, all our results indicate that IGFL2 plays an oncogenic role in HNSC and it has a potential to induce tumorigenesis in the early stages of cancer, highlighting its prognostic significance.

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