Increased Expression of FN1 in Head and Neck Squamous Cell Carcinoma Predicts Poor Prognosis

Hung-Sheng Shih  
National Taiwan University

Li-Yu Hung  
National Taiwan University

Ming-Yu Hsieh (✉️ 163024@cch.org.tw)  
Changhua Christian Hospital

Research Article

Keywords: Head and neck cancer, Fibronectin, Gene expression, gene set enrichment analysis, Immune Infiltration Analysis

Posted Date: November 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1033849/v1

License: 😊  This work is licensed under a Creative Commons Attribution 4.0 International License. 
Read Full License
Abstract

Background: A few recent studies have addressed the function of FN1 (Fibronectin 1) in head and neck cancer. The clinical information from 500 HNSCC (Head and neck squamous cell carcinoma) patients with FN1 gene expression data set was published by The Cancer Genome Atlas (TCGA). The correlation between clinicopathologic characteristics and FN1 expression was analyzed by Logistic regression and Wilcoxon signed rank test. Survival function was performed employing Kaplan-Meier estimator, and the relationship between clinicopathological characteristics, prognostic outcome, and FN1 expression were examined by using Cox regression analysis. As Gene set enrichment analysis (GSEA) was performed, we investigated the correlation between FN1 expression and immune cell infiltrates with single-sample gene set enrichment analysis (ssGSEA).

Results: Patients with high FN1 expression revealed a significantly decreased overall survival (OS), and disease-specific survival (DSS) than those with low FN1 expression in Kaplan-Meier survival analyses. According to the above results, univariate and multivariate analysis revealed that patients with high FN1 expression had lower OS than those with low FN1 expression.

Conclusions: The findings of this research provide insights for FN1 may be potential prognostic biomarkers for diagnosis as well as therapeutic targets in HNSCC patients.

Background

Head and neck cancer (HNC) is the sixth most common cancer in the world. Approximately 650,000 new cases and 350,000 deaths are reported worldwide each year, accounting for 6% of all cases. Despite advances in surgical techniques and chemoradiotherapy, the overall 5-year relative survival rate of head and neck squamous cell carcinoma patients has remained unchanged for decades.[1]

In consequence of high-throughput technologies, including microarray and RNA sequencing, gene expression has significantly improved our understanding of molecular mechanisms. [2] A series of published studies have proved that CD44, miR-608, and miR-21 are common biomarkers for head and neck cancer progression and prognosis. [3, 4] Nevertheless, in the aspect of predicting cancer metastasis, prognosis, and recurrence, these biomarkers still lack necessary specificity and sensitivity. Consequently, the role of early diagnosis of value cancer biomarkers is very crucial.

Fibronectin 1 (FN1) is an essential extracellular matrix (ECM) glycoprotein involved in cell adhesion and migration since it connects cells to the ECM via interacting with the cell surface β1 integer protein receptor and all ECM molecules except collagen IV. [5] Fibronectin is expressed by multiple cell types and is critically important in the development of vertebrates, as demonstrated by the early embryonic lethality in mice with targeted inactivation of the FN gene. [6] FN1 has been demonstrated to promote cell proliferation and migration of many malignant tumors, such as gastric cancer, lung cancer, and thyroid cancer. [7-9] In addition, FN1 can induce macrophage migration via the Src family kinases and focal adhesion kinase (SFK-FAK/CSF-1R) pathway, which plays an important role in cancer invasion and...
metastasis. [10] FN1 could down-regulate P53 and inhibit cell growth and increase apoptosis in colon cancer. [11] Although FN1 overexpression is regarded as a poor prognosis factor in radiotherapy resistance in oral squamous cell carcinoma, the potential functions of the FN1 mechanism have not been defined in HNSCC yet.

The goal of the current study was to use a comprehensive expression analysis of the FN1 and its relationship with the prognosis of patients with head and neck squamous cell carcinoma (HNSCC) by The Cancer Genome Atlas (TCGA), Kaplan–Meier plotter, and gene-set enrichment analysis (GSEA). Further, we estimated the correlation between FN1 and infiltration abundances of immune cells by single-sample Gene Set Enrichment (ssGSEA) analysis. In addition, the limma package administers the removeBatchEffect function, which performs batch effect correction on the TCGA dataset [12]. Our results purport the prognostic value of FN1 in HNSCC and explore the underlying mechanism of FN1 in regulating HNSCC patients.

**Results**

**Characteristics of patients**

Gene expression data and clinical data of 500 cases of head and neck cancers were collected from the TCGA database in August 2021 (Table 1).

In this study, under the current eighth edition of the TNM staging system, the total size of most patients was stage IV (272 cases; 55.7%), followed by stage III (102 cases; 20.9%), and the least patients were stage II ( 95 cases; 19.5%) and stage I disease (19 cases; 3.9%). The primary therapy outcomes included complete response (CR) 87.3%, partial response (PR) 1.4%, stable disease (SD) 1.4%, and disease progression (PD) 9.9%. The median follow-up time of overall survival (OS) in the FN1 high expression group and FN1 low expression group at last contact were 57.3 months and 40.1 months, respectively. Among 441 patients who were assessed for lymphovascular invasion (LI), 122 (35.8%) patients had LI-positive, while 219 (64.2%) patients had LI-negative. In this study, there were 244 patients under 60 years old, accounting for 48.9% of the total number of patients, and 255 patients 60 years or older, accounting for 51.1%.

**Association with FN1 gene expression and clinical pathological Variable**

To better determine the relevance and clinical significance of FN1 expression in HNSCC, the association of FN1 expression with clinical characteristics was investigated of 500 HNSCC samples. Our result are plotted in Figure 1 and showed increased FN1 expression was enriched in advanced stage III and IV (p = 0.979), objective clinical response (CR and PR, p = 0.415), high histological grade G3 and G4 (p = 0.027), and lymphovascular invasion-positive (p = 0.114).
To identify which poor prognostic clinicopathologic and characteristics were independently associated with FN1 expression, we performed a univariate analysis. Specifically, poor clinicopathological did not associate with co-pathology in any group via logistic regression analysis (Table 2).

**Survival Outcomes and Multivariate Analysis**

Kaplan–Meier survival plots showed a significant correlation between high expression FN1 levels and revealing a poor prognostic value for FN1. In univariable analysis (Figure 2), low FN1 expression was associated with a better OS, and DSS (p = 0.046, 0.041, respectively).

When other clinicopathologic features that were significant with poor OS in primary therapy outcome, lymphovascular invasion and metastasis stage in univariate analysis were adopted as covariates (Table 3). Multivariate analysis revealed that primary therapy outcome was an prognostic factor for OS (HR = 0.161; p < 0.001) but not for lymphovascular invasion and metastasis stage (HR = 1.446, 2.107 respectively; p = 0.065, 0.337, respectively). FN1 was still independently associated with the OS.

When other clinicopathologic features that were significant with poor DSS in primary therapy outcome, degree of spread to regional lymph nodes, metastasis stage, and lymphovascular invasion in univariate analysis were adopted as covariates (Table 4). Multivariate analysis revealed that primary therapy outcome was an prognostic factor for DSS (HR = 0.091; p < 0.001) but not for degree of spread to regional lymph nodes, metastasis stage, and lymphovascular invasion (HR =1.386, 2.374, 1.292 ; p = 0.136, 0.243, 0.310, respectively ). Associations with DSS were independent of risk factors and persisted even after additional adjustment.

**GSEA Related Signaling Pathway**

To explore FN1-related catalogue signaling pathways in HNSCC, we utilized Gene Set Enrichment Analysis (GSEA) between low- and high-expression FN1. We used the median score as a cut-off point to divide HNSCC samples into either high-expression or low-expression FN1. GSEA demonstrated significant enrichment differences (false discovery rate (FDR)-adjusted pooled p-values < 0.05) of the MSigDB molecular signatures databases Collection (c2.cp.v7.2 symbols.gmt). To investigate the function of the target gene, we then selected the most enriching signaling pathways via their normalized enrichment score (NES) value (Table 5 and Figure 3). The high expression of FN1 revealed the differential enrichment of amino acid and its derivatives metabolism and olfactory signaling pathway categories.

**Statistical Correlations between the Expression of FN1 and Immune Cells**

To identify immune cell-types and genes related to the anti-tumour function that might be a key factor to immunotherapy, we focused on the correlations between FN1 and immune cells of HNSCC in the
databases of Bindea et al to clarify their relation to each other [16]. Finally, this analysis demonstrated FN1 expression was correlated with 24 immune cell subsets. For example, T helper (Th)1, Th2, Th17, effector memory (Tem), γδ, central memory, regulatory, and cytotoxic T cells, three dendritic cells types (immature, activated, and plasmacytoid), two subtypes of natural killer cells, as well as B cells, neutrophils, and mast cells (Figure 4). The finding showed a significant positive association between FN1 and macrophages. Significant correlations and moderately positive were found between FN1 and NK cells, Eosinophils, immature dendritic cells, and Tem cells. An insignificant relationship between FN1 and the other helper T cell-types was shown in Figure 4.

**Discussion**

Fibronectin 1 (FN1), the key component of the extracellular matrix, is a glycoprotein mainly found in the plasma membranes. It is involved in cell adhesion, migration, and simultaneously binds to a diversity of extracellular matrix proteins including collagen, fibrin, and integrins. Plasma fibronectin is synthesized via liver cells and expression in bloodstream stages is density-dependent, inactive conformation. Fibronectin matrix formation begins at cellular surfaces; integrin binds fibronectin to an extended conformation complexes and exposes its functional domain to activated extracellular matrix (ECM).[17] Fibronectin monomers are divided into three types according to their repeating units-type I, type II, and Type III. The expression isoforms are named in line with the splice site position in the type III repeat unit: fibronectin and the type III connecting segment (IIICS-FN), extra domain A (EDA-FN), extra domain B (EDB-FN).[18, 19] Among them, the extra domain A (EDA) and extra domain B (EDB) are expressed well during chronic inflammation and cancer. Thus, it is a potential diagnosis and treatment target.[20-22] One interesting finding is that EDB overexpression appears in various human cancers, including Hodgkin's lymphoma, non-small cell lung cancer, and prostate cancer.[23-25] Very little was found in the literature on the association between Fibronectin and head and neck cancer (HNC). Herein, we validated the high-throughput RNA sequencing data downloaded from TCGA showing the increase of FN1 expression in head and neck squamous cell carcinoma (HNSCC) is associated with poor prognosis, short survival time, and poor clinicopathological characteristics. Besides, our analysis revealed that the expression level of FN1 in HNSCC is related to inflammatory infiltrates in diverse immune cell types. Thus, this study confirms, and supports evidence from our understanding of the potential target of FN1 in HNSCC immunity and as a diagnostic marker of this malignancy or potential prognostic.

This study explored the expression level of FN1 and visualizes the prognostic outcome in head and neck cancer (HNSCC) using TCGA datasets. Fibronectin is one of the defining features of EMT, it alters the composition of the ECM and is believed to contribute to the invasive properties of cancers. Indeed, previous studies provided evidence that fibronectin has malignant effects and is carcinogenic in several malignant neoplasms. [23, 26, 27] As mentioned in the literature review, it can be hypothesized that FN1 is an oncogenic factor for developing squamous cell carcinoma, but there is no sufficient research on the role of FN1 in head and neck squamous cell carcinoma after reviewing the literature.
Based on the TCGA cohort, FN1-related gene expression was negatively correlated with OS and DSS, and there was significant statistical interaction between the parameters. Univariate and multivariate Cox regression analysis indicated that high FN1 expression was associated with poor prognosis of HNSCC. Moreover, when FN1 was highly expressed in HNSCC, the current study have found that higher expression levels of FN1 were strongly correlated with worse primary outcome and lymphovascular invasion for poorer OS and DSS.

Increasing evidence demonstrated that HNSCC was considered as an immunogenic tumor.[28] Through the regulation of the balance of tumor immunogenicity to immune response, host cells aid in apoptotic-escape of cancer cells, thereby avoiding immune recognition and switching-off persistent immune stimulation. Our current study has found a strong correlation between FN1 expression and a variety of immune infiltrating cells, especially macrophages. Macrophage polarization may exacerbate inflammation and accelerate cancer progression.[29] The other researchers have described a new pathway related to FN-induced macrophage migration, as the SFK-FAK/CSF-1R binding site. [10, 30] On the other hand, iDC, pDC, aDC, and FN1 expression are significantly correlated. Dendritic cells are typical antigen-presenting cells. Mature DCs make a huge production of cytokines and conduct core regulatory functions, while immature DCs have the ability to phagocytose. [31] These results indicate that FN1 may potentially activate DCs. Moreover, the correlation interaction between NK cells and Tem cells must play a significant role in HNSCC and FN1 expression. After the response to target recognition, NK cells and Tem cells secrete interferon-γ, which has enhanced cytotoxic activity against cancer cells directly. [32] This also accords with the fact, which showed that FN-induced FAK activation depends on the integrity of the cytoskeleton Macrophages have been activated to extend lamellipodia and pseudopods, and migrate up a chemotaxis gradients. [33] This correlation may tip a potential mechanism for FN1 to regulate the inflammation of macrophages and infiltrated by immune cell in HNSCC.

TGF-β1 regulates migration and cell proliferation which depends on higher fibronectin levels. Experimental results of studying intracellular signaling molecules showed that fibronectin coordinates cell structure and enhances migration, which has a direct role on differentiation by locally activating integrin via the small guanosine triphosphatases (GTPases), Src, and FAK. [34] In addition, it is clear that Src and Akt have pleiotropic effects on gene expression. Utilizing a drug to inhibit the TGF-β1 signaling is being considered as an important target for lung cancer. [35] Thus, we hypothesized that this could be a potential therapeutic target for HNSCC, and FN1 may regulate the expression of TGF-β1 via different signaling pathways.

Some limitations must be accounted for. Firstly, for FN1 genes, mRNA expression and protein expression may not be well correlated. This viewpoint supports evidence from previous observations (Guo et al., 2008) [36] Secondly, the function of the factor is therefore needed to develop an experimental verification, which requires co-verification of multiple-site studies, verification of in vitro and in vivo experiments, or cross-validation of multiple databases. These differences induced an increase in variability for biological replicates which contributed to fewer data integration. In order to solve the problem, ComBat- and limma-
corrected methods have been performed. Though we used the removeBatchEffect function (limma package in R) to minimize batch effects, the sequencing may still cause some batch effects.

**Conclusion**

This study found that increased FN1 expression indicated poor long-term prognosis of HNSCC with increased abundance of macrophages and immune cells subsets proportion. In addition, the expression of FN1 in HNSCC regulated memory T cells, NK cells, and neutrophils, by activating different signal cascades to activate the level of TGF-β1 receptor associated with the growth of HNSCC. Therefore, FN1 may be a prognosis predictive biomarker, and plays a significant role in immune cell infiltration for HNSCC patients.

**Materials And Methods**

**Data from TCGA**

Analysis of mRNA SeqV2 expression in head and neck squamous cancer were profiling from The Cancer Genome Atlas (TCGA) database. ([https://tcga-data.nci.nih.gov/tcga/](https://tcga-data.nci.nih.gov/tcga/)) [13]. Gene expression profiling of FN1 was obtained from TCGA RNA-seq data of 500 Head and neck squamous cell carcinoma (HNSCC) patients. Clinical performance of overall survival (OS) and disease-specific survival (DSS) endpoints were derived from the TCGA pan-cancer Clinical Data Resource (CDR) [14]. HTSeq-FPKM was used to calculate fragments per kilobase of transcript per million mapped reads, normalized gene expression. We analyzed the clinical data of level 3 of expression level in HNSCC patients simultaneously. According to the CDR, the clinical endpoints used for HNSCC were selected as OS and DSS. Patients who were off study or died were counted as nonresponses in this measurement.

**Gene enrichment analysis**

Gene set enrichment analysis (GSEA) was used to determine gene sets significance and concordant differences between two ranked gene states. ([http://software.broadinstitute.org/gsea/index.jsp](http://software.broadinstitute.org/gsea/index.jsp)) [15] In this analysis, GSEA was the first to the rank-ordered list based on the Spearmen's correlation with FN1 expression. GSEA was used to determine significance of survival differences between high and low FN1 expression leve. Normalized Enrichment Score (NES), and nominal p-value were ranked by log2FC with permutations 1000 times for each analysis from FN1 enrichment of variant effects on each phenotype.

**Immune Infiltration Analysis**

The Single-Sample GSEA (ssGSEA) marker genes were extracted from the research of Bindea et al. and it included 24 types of immune cells. [16] Spearman's correlation has analyzed the correlation between the FN1 and these 24 immune cell types. The analysis describes the association as the strength of the correlation between immune infiltrating cells and FN1 (absolute values: 0.00–0.05 “very weak,” 0.06–0.10
P-values < 0.05 were considered statistically significant.

**Statistical Analysis**

Statistical analyses were performed with R version 4.1.1 software [http://www.Rproject.org](http://www.Rproject.org).

The collected data had analyzed the relationship between clinicopathological features and FN1 by logistic regression test and Wilcoxon sign-rank test. Uni- and multivariate binary regression analysis were performed to evaluate FN1 expression scores. Survival curves were constructed using Kaplan–Meier method, and differences between survival curves were analyzed using the log-rank test. Hazard ratios (HRs) of the operating system were estimated by univariate Cox proportional hazards regression models. The p values were two-sided and p values less than 0.05 indicating significance.

**Abbreviations**
| Abbreviations | Description |
|---------------|-------------|
| FN1           | Fibronectin 1 |
| HNSCC         | Head and neck squamous cell carcinoma |
| TCGA          | The Cancer Genome Atlas |
| GSEA          | Gene set enrichment analysis |
| ssGSEA        | single-sample gene set enrichment analysis |
| OS            | overall survival |
| DSS           | disease-specific survival |
| ECM           | extracellular matrix |
| SFK-FAK       | Src family kinases and focal adhesion kinase |
| CSF-1R        | colony-stimulating factor-1 receptor |
| CR            | complete response |
| PR            | partial response |
| SD            | stable disease |
| PD            | disease progression |
| LI            | lymphovascular invasion |
| NES           | normalized enrichment score |
| IIICS-FN      | type III connecting segment of Fibronectin |
| EDA-FN        | extra domain A of Fibronectin |
| EDB-FN        | extra domain B of Fibronectin |
| GTPases       | guanosine triphosphatases |
| CDR           | Clinical Data Resource |
| HRs           | Hazard ratios |

**Declarations**

**Authors contributions:**

Conceptualization, H.-S.S.; methodology, H.-S.S.; formal analysis, H.-S.S.; resources, H.-S.S.; writing, H.-S.S.; supervision, M.-Y.H.; project administration, L.-Y.H. All authors have read and agreed to the published version of the manuscript.
Ethics approval and consent to participate:

Not applicable

Consent for publication:

Not applicable

Availability of data and materials:

Data generated are included within the manuscript and supplementary materials.

Competing interests:

The authors declare no conflict of interest.

Funding:

This research received no external funding.

References

1. Ferlay, J., et al., Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer, 2019. 144(8): p. 1941-1953.
2. Zhao, S., et al., Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. PLoS One, 2014. 9(1): p. e78644.
3. Kerk, S.A., et al., 5T4-Targeted Therapy Ablates Cancer Stem Cells and Prevents Recurrence of Head and Neck Squamous Cell Carcinoma. Clin Cancer Res, 2017. 23(10): p. 2516-2527.
4. Ge, Y., et al., Strand-specific in vivo screen of cancer-associated miRNAs unveils a role for miR-21(*) in SCC progression. Nat Cell Biol, 2016. 18(1): p. 111-21.
5. Wang, K., et al., Fibronectin Mechanobiology Regulates Tumorigenesis. Cell Mol Bioeng, 2016. 9: p. 1-11.
6. George, E.L., et al., Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. Development, 1993. 119(4): p. 1079-91.
7. Lou, X., et al., SOX2 targets fibronectin 1 to promote cell migration and invasion in ovarian cancer: new molecular leads for therapeutic intervention. Omics, 2013. 17(10): p. 510-8.
8. Waalkes, S., et al., Fibronectin 1 mRNA expression correlates with advanced disease in renal cancer. BMC Cancer, 2010. 10: p. 503.
9. Sponziello, M., et al., *Fibronectin-1 expression is increased in aggressive thyroid cancer and favors the migration and invasion of cancer cells*. Mol Cell Endocrinol, 2016. 431: p. 123-32.

10. DIGiacomo, G., et al., *Fibronectin induces macrophage migration through a SFK-FAK/CSF-1R pathway*. Cell Adh Migr, 2017. 11(4): p. 327-337.

11. YI, W., et al., *High expression of fibronectin is associated with poor prognosis, cell proliferation and malignancy via the NF-κB/p53-apoptosis signaling pathway in colorectal cancer*. Oncol Rep, 2016. 36(6): p. 3145-3153.

12. SMYTH, G.K. and T. Speed, *Normalization of cDNA microarray data*. Methods, 2003. 31(4): p. 265-73.

13. WANG, Z., M.A. Jensen, and J.C. Zenklusen, *A Practical Guide to The Cancer Genome Atlas (TCGA)*. Methods Mol Biol, 2016. 1418: p. 111-41.

14. LIU, J., et al., *An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics*. Cell, 2018. 173(2): p. 400-416.e11.

15. SUBRAMANIAN, A., et al., *Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles*. Proc Natl Acad Sci U S A, 2005. 102(43): p. 15545-50.

16. BINDEA, G., et al., *Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer*. Immunity, 2013. 39(4): p. 782-95.

17. TO, W.S. and K.S. Midwood, *Plasma and cellular fibronectin: distinct and independent functions during tissue repair*. Fibrogenesis Tissue Repair, 2011. 4: p. 21.

18. RONCA, R., et al., *Delivering cytokines at tumor site: The immunocytokine-conjugated anti-EDB-fibronectin antibody case*. Immunobiology, 2009. 214(9-10): p. 800-10.

19. VENTURA, E., et al., *Alternative splicing of the angiogenesis associated extra-domain B of fibronectin regulates the accessibility of the B-C loop of the type III repeat 8*. PLoS One, 2010. 5(2): p. e9145.

20. KASPAR, M., L. Zardi, and D. Neri, *Fibronectin as target for tumor therapy*. Int J Cancer, 2006. 118(6): p. 1331-9.

21. WANG, J.P. and A. Hielscher, *Fibronectin: How Its Aberrant Expression in Tumors May Improve Therapeutic Targeting*. J Cancer, 2017. 8(4): p. 674-682.

22. ZENT, J. and L.W. Guo, *Signaling Mechanisms of Myofibroblastic Activation: Outside-in and Inside-Out*. Cell Physiol Biochem, 2018. 49(3): p. 848-868.

23. KHAN, Z.A., et al., *ED-B fibronectin in non-small cell lung carcinoma*. Exp Lung Res, 2005. 31(7): p. 701-11.

24. SAUER, S., et al., *Expression of the oncofetal ED-B-containing fibronectin isoform in hematologic tumors enables ED-B-targeted 131I-L19SIP radioimmunotherapy in Hodgkin lymphoma patients*. Blood, 2009. 113(10): p. 2265-74.

25. LOCKER, R., et al., *Abundant in vitro expression of the oncofetal ED-B-containing fibronectin translates into selective pharmacodelivery of (131)I-L19SIP in a prostate cancer patient*. J Cancer Res Clin Oncol, 2014. 140(1): p. 35-43.
26. Wang, W.Y., et al., *Fibronectin promotes nasopharyngeal cancer cell motility and proliferation*. Biomed Pharmacother, 2019. 109: p. 1772-1784.

27. Albrecht, V., et al., *Anticalins directed against the fibronectin extra domain B as diagnostic tracers for glioblastomas*. Int J Cancer, 2016. 138(5): p. 1269-80.

28. Cavalieri, S., et al., *Immuno-oncology in head and neck squamous cell cancers: News from clinical trials, emerging predictive factors and unmet needs*. Cancer Treat Rev, 2018. 65: p. 78-86.

29. Weinberger, T., et al., *Ontogeny of arterial macrophages defines their functions in homeostasis and inflammation*. Nat Commun, 2020. 11(1): p. 4549.

30. Salvagno, C., et al., *Therapeutic targeting of macrophages enhances chemotherapy efficacy by unleashing type I interferon response*. Nat Cell Biol, 2019. 21(4): p. 511-521.

31. Huang, C.T., et al., *Insulin-like growth factors inhibit dendritic cell-mediated anti-tumor immunity through regulating ERK1/2 phosphorylation and p38 dephosphorylation*. Cancer Lett, 2015. 359(1): p. 117-26.

32. Wall, L., et al., *The anti-proliferative activity of interferon-gamma on ovarian cancer: in vitro and in vivo*. Gynecol Oncol, 2003. 88(1 Pt 2): p. S149-51.

33. Kuninty, P.R., et al., *ITGA5 inhibition in pancreatic stellate cells attenuates desmoplasia and potentiates efficacy of chemotherapy in pancreatic cancer*. Sci Adv, 2019. 5(9): p. eaax2770.

34. Roman, W., J.P. Martins, and E.R. Gomes, *Local Arrangement of Fibronectin by Myofibroblasts Governs Peripheral Nuclear Positioning in Muscle Cells*. Dev Cell, 2018. 46(1): p. 102-111.e6.

35. Wang, H., et al., *TGF-β1-induced epithelial-mesenchymal transition in lung cancer cells involves upregulation of miR-9 and downregulation of its target, E-cadherin*. Cell Mol Biol Lett, 2017. 22: p. 22.

36. Guo, Y., et al., *How is mRNA expression predictive for protein expression? A correlation study on human circulating monocytes*. Acta Biochim Biophys Sin (Shanghai), 2008. 40(5): p. 426-36.

**Tables**

Table 1

The Cancer Genome Atlas (TCGA) head and neck squamous cell carcinoma (HNSCC) patient characteristics.
| Characteristic | Number of cases | Percentages |
|---------------|----------------|-------------|
| **T stage, n (%)** | | |
| T1            | 33             | 6.8%        |
| T2            | 143            | 29.5%       |
| T3            | 130            | 26.8%       |
| T4            | 179            | 36.9%       |
| **N stage, n (%)** | | |
| N0            | 239            | 50%         |
| N1            | 80             | 16.7%       |
| N2            | 152            | 31.8%       |
| N3            | 7              | 1.5%        |
| **M stage, n (%)** | | |
| M0            | 470            | 98.9%       |
| M1            | 5              | 1.1%        |
| **Clinical stage** | | |
| Stage I       | 19             | 3.9%        |
| Stage II      | 95             | 19.5%       |
| Stage III     | 102            | 20.9%       |
| Stage IV      | 272            | 55.7%       |
| **Primary therapy outcome, n (%)** | | |
| PD            | 41             | 9.9%        |
| SD            | 6              | 1.4%        |
| PR            | 6              | 1.4%        |
| CR            | 363            | 87.3%       |
| **OS event, n (%)** | | |
| Alive         | 283            | 56.6%       |
| Dead          | 217            | 43.4%       |
| **DSS event, n (%)** | | |
| Alive         | 346            | 72.8%       |
| Characteristics                              | Total(N) | Odds Ratio in FN1 expression | P value |
|---------------------------------------------|----------|------------------------------|---------|
| T stage (T3/T4 vs. T1/T2)                  | 485      | 0.928 (0.640-1.344)          | 0.693   |
| N stage (N1/N2/N3 vs. N0)                  | 478      | 1.000 (0.698-1.432)          | 1.000   |
| M stage (M1 vs. M0)                        | 475      | 0.244 (0.012-1.662)          | 0.208   |
| Clinical stage (Stage III/Stage IV vs. Stage I/Stage II) | 488 | 0.707 (0.462-1.077)          | 0.108   |
| Primary therapy outcome (PR/CR vs. PD/SD)  | 416      | 1.343 (0.730-2.506)          | 0.346   |
| Histologic grade (G3/G4 vs. G1/G2)         | 481      | 1.269 (0.840-1.922)          | 0.259   |
| Lymphovascular invasion (Yes vs. No)       | 341      | 0.840 (0.539-1.310)          | 0.442   |
| Age (>60 vs. <=60)                         | 499      | 1.008 (0.709-1.432)          | 0.965   |

SD: stable disease; PD: progressive disease; PR: partial remission; CR: complete remission.
Table 3

Univariate and multivariate Cox proportional hazard analysis of FN1 expression and overall survival (OS) for patients with head and neck squamous cell carcinoma (HNSCC) in the validation cohort.

| Characteristics                     | Univariate analysis | Multivariate analysis |
|-------------------------------------|---------------------|-----------------------|
|                                     | Hazard ratio (95% CI) | P value | Hazard ratio (95% CI) | P value |
| T stage (T1/T2 vs. T3/T4)           | 1.245 (0.932-1.661)  | 0.137                |                        |         |
| N stage (N0 vs. N1/N2/N3)           | 1.257 (0.960-1.647)  | 0.097                | 1.316 (0.945-1.832)    | 0.104   |
| M stage (M0 vs. M1)                 | 4.794 (1.765-13.016) | 0.002                | 2.017 (0.482-8.445)    | 0.337   |
| Clinical stage (stage I/II vs. stage III/IV) | 1.217 (0.878-1.688)  | 0.238                |                        |         |
| Primary therapy outcome (PD-SD vs. PR-CR) | 0.181 (0.122-0.269)  | <0.001               | 0.160 (0.101-0.255)    | <0.001  |
| Histologic grade (G1/G2 vs. G3/G4)  | 0.939 (0.688-1.282)  | 0.692                |                        |         |
| Lymphovascular invasion (negative vs. positive) | 1.699 (1.211-2.384)  | 0.002                | 1.446 (0.977-2.142)    | 0.065   |
| Age (<=60 vs. >60)                  | 1.252 (0.956-1.639)  | 0.102                |                        |         |
| FN1 (low vs. high)                  | 1.333 (1.018-1.746)  | 0.037                | 1.605 (1.075-2.395)    | 0.021   |

SD: stable disease; PD: progressive disease; PR: partial remission; CR: complete remission; HR: hazard ratio; CI: confidence interval.

Table 4

Univariate and multivariate Cox proportional hazard analysis of FN1 expression and disease-specific survival (DSS) for patients with head and neck squamous cell carcinoma (HNSCC) in the validation cohort.
| Characteristics                                      | Univariate analysis |            | Multivariate analysis |            |
|------------------------------------------------------|---------------------|------------|-----------------------|------------|
|                                                      | Hazard ratio (95% CI) | P value | Hazard ratio (95% CI) | P value |
| T stage (T1/T2 vs. T3/T4)                           | 1.459 (0.988-2.153) | 0.057     | 1.386 (0.903-2.128) | 0.136     |
| N stage (N0 vs. N1/N2/N3)                           | 1.472 (1.034-2.096) | 0.032     | 1.386 (0.903-2.128) | 0.136     |
| M stage (M0 vs. M1)                                 | 8.231 (2.581-26.248) | <0.001    | 2.374 (0.556-10.130) | 0.243     |
| Clinical stage (stage I/II vs. stage III/IV)        | 1.151 (0.753-1.760) | 0.517     |                       |           |
| Primary therapy outcome (PD-SD vs. PR-CR)           | 0.097 (0.062-0.150) | <0.001    | 0.091 (0.054-0.153) | <0.001    |
| Histologic grade (G1/G2 vs. G3/G4)                  | 1.051 (0.712-1.552) | 0.801     |                       |           |
| Lymphovascular invasion (negative vs. positive)      | 1.658 (1.079-2.546) | 0.021     | 1.292 (0.788-2.118) | 0.310     |
| Age (<=60 vs. >60)                                  | 1.078 (0.763-1.524) | 0.670     |                       |           |
| FN1 (low vs. high)                                  | 1.446 (1.016-2.058) | 0.041     | 1.767 (1.153-2.710) | 0.009     |

SD: stable disease; PD: progressive disease; PR: partial remission; CR: complete remission; HR: hazard ratio; CI: confidence interval.

Table 5

Gene sets enriched in high phenotype
| Description                                      | Enrichment score | NES     | $p$ adjust | FDR $q$ values |
|-------------------------------------------------|------------------|---------|------------|----------------|
| Reactome_metabolism_of_amino_acids_and_derivatives | -0.437           | -2.055  | 0.015      | 0.009          |
| Reactome_olfactory_signaling_pathway             | -0.329           | -1.546  | 0.015      | 0.009          |

NES: normalized enrichment score; FDR: false discovery rate. Gene sets with NOM $p$ value < 0.05 and FDR $q$ value < 0.05 are considered as significant.

Figures
Figure 1

Association with FN1 expression and clinicopathological characteristics including (a) T stage, N stage (b), M stage (c), clinical stage (d), primary therapy outcome (e), histologic grade (f), and lymphovascular invasion (g) in patients with head and neck squamous cell carcinoma (HNSCC) in The Cancer Genome Atlas (TCGA) cohort.
Figure 2

Impact of FN1 expression on overall survival (OS) and disease-specific survival (DSS) in head and neck squamous cell carcinoma (HNSCC) in The Cancer Genome Atlas (TCGA) cohort.
Enrichment plots from set enrichment analysis (GSEA). GSEA results showing metabolism of amino acids and derivates, and olfactory signal pathway are enriched in FN1-related head and neck cancer.