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Increased Expression of *ITGA6* as a Predictor for Poor Prognosis in Head and Neck Squamous Cell Carcinoma

Research Article

Suvarna K[1], Gheena S[1], Paramasivam A[2*]

[1] Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India.
[2] Department of Dental Research Cell, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India.

Abstract

Background and Aim: *ITGA6* expression has significant impact on angiogenesis, tumour metastasis and stemness of cancer stem cells. Several studies have found the increased expression of *ITGA6* associated with tumorigenesis and poor prognosis in patients with cancer. However, the expression and prognostic value of *ITGA6* remain largely unknown in HNSCC.
Objectives: The aim of the present study was to analyze the expression and prognostic value of *ITGA6* in HNSCC.
Materials and Methods: In the present study, we used the large TCGA (The Cancer Genome Atlas) RNA sequencing (RNAseq) dataset to explore the *ITGA6* expression level in HNSCC. This study included a total of 564 tissue samples (520 HNSCC and 44 control tissues). The mRNA expression level of *ITGA6* in various kinds of cancers, including HNSCC, was analysed via the ONCOMINE and GEPIA databases.
Results: We observed that the mRNA expression level of *ITGA6* was increased in most cancers compared with normal tissues, especially in HNSCC. In addition, we also used Kaplan-Meier plotter to evaluate the prognostic value of *ITGA6* in HNSCC patients. It showed highly expressed *ITGA6* was significantly related with poor overall survival (OS) in HNSCC patients.
Conclusion: The *ITGA6* highly expressed in HNSCC and associated with poor prognosis in HNSCC patients. Therefore, *ITGA6* could be a promising prognostic biomarker for HNSCC.

Keywords: *ITGA6*; mRNA Expression; HNSCC; Prognostic Value; TCGA Database.

Introduction

Head and neck cancer is the 6th most common cancer worldwide with an annual increase of approximately 6,30,000 patients and a mortality rate of 3,50,000 deaths every year [1, 2]. The head and neck cancer include malignant tumours arising from various sites in the upper aerodigestive tract [3, 4]. Among head and neck cancer types, head and neck squamous cell carcinoma (HNSCC) is the most common variety and accounts for 90% of the head and neck cancers [3, 5, 6].

Progression of a suspicious lesion into cancer depends on the progression of epithelial dysplasia, which does not follow a predictable sequential progression from mild to moderate to severe dysplasia and in rare cases may revert to normal [7, 8]. The etiological factors for HNSCC include tobacco chewing, smoking, alcohol consumption, virus etiologies like HPV and genetic factors [9-11]. Recent molecular genetic studies provided evidence that the majority of head and neck squamous cell carcinomas (HNSCCs) develop within a contiguous field of preneoplastic cells [12]. Based on several studies conducted, it can be inferred that these alterations in several cellular molecules including DNA, RNA, and proteins play a significant role in tumor progression and the overall survival of the malignant cells [13]. Hence these markers can assist in early diagnosis and prediction of prognosis. The diagnosis of carcinoma at an early stage can prevent extensive treatment and thus biomarkers can serve as a tool for diagnosis [14-16].

The ITGAG gene is located on chromosome 2q.31.1, codes for

***Corresponding Author**:
Paramasivam A,
Department of Dental Research Cell, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Poonamallee High Road, Chennai, Tamilnadu - 600077, India.
Tel: 879025024
E-mail: paramasivama.sdc@saveetha.com

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Integrins [17]. Integrins are a family of transmebrane heterodimeric glycoprotein receptors which mainly functions in cell to cell adhesion [17]. Integrins are made of alpha and beta subunits bound together by Covalent bond [17, 18]. 18 alpha and 8 beta subunits are known till now which can form 24 distinct integrin heterodimers [19]. Integrins can bind to extracellular materials like collagens, fibronectins, laminins, receptors such as vascular cell adhesion molecules (VCAM-1) and intercellular cell adhesion molecules [20, 21]. Integrins are bidirectional in signalling functions, which transmit signals from extracellular to intracellular and vice versa [17]. This indicates their major role in immune response, homeostasis and overall cell development [17, 22]. Through various signalling pathways integrins can cross talk with growth factor receptors and it is required for many growth factor receptors to function [23].

ITGAG expression has significant impact on angiogenesis, tumor metastasis and self renewal and other properties of cancer stem cells [23, 24]. There are several studies showing increased levels of *ITGA6* can promote tumour susceptibility and progression, or SNP/ mutations in *ITGA6* genes may be responsible for cancer progression [25, 26]. But some of the researchers also stated that decreased levels of *ITGA6* can also lead to cancer progression [27]. The expression of *ITGA6* and its association with cancer susceptibility and progression is a controversial topic. Hence more research in the area of *ITGA6* gene expression and analysis of association between this *ITGA6* gene expression and tumour susceptibility and progression of cancer should be promoted. Aim of this present study was to analyse the *ITGA6* gene expression in HNSCC which is an aggressive malignancy with high morbidity and mortality rates.

Materials and Methods

Gene Expression Analysis

The present study initially analyzed the *ITGA6* expression in HNSCC (n=520) and normal tissues (n=44) using data from TCGA dataset. The data regarding the samples were collected and analysed during April 2020. We used the ONCOMINE (https://www.oncomine.org/) and GEPIA (http://gepia.cancer-pku.cn/) used to analyse the *ITGA6* expression in primary HNSCC and normal tissues.

Survival analysis by Kaplan-Meier plotter

In the present study, the prognostic values of *ITGA6* at mRNA level in HNSCC was analyzed using Kaplan-Meier Plotter (http://kmplot.com/analysis/) which is an online database containing gene expression profiles and survival information of cancer patients.

Results and Discussion

The ITGA6 gene encodes the integrin alpha Chain family of proteins which are proteolytically processed to form light and heavy chains that comprises alpha 6 subunits of integrin [26]. The functions of ITGA6 includes roles in cell survival, migration, and angiogenesis. This integrin also plays a role in tumour invasion and metastases [23]. Integrins can interact with extracellular materials. The lack of cell adhesion leads to disordered integrin signalling pathways including PI3K/AKT, MEK/ERK, FAK and NF-KB [24]. Through these signalling pathways integrins can cross talk with growth factor receptors and it is required for many growth factor receptors to function [21]. Integrin ligation was found to suppress apoptosis by activating suppressors of apoptosis [27, 28]. Stupack et al., in 2001, Kim et al. in 2002 found that integrin can inhibit caspase activation [29, 30]. Integrins also stimulate cell migration by activating Rho and Rac GTPase [31]. Integrins promote cell cycle entry by stimulating cyclins expression [32].

Predilection of epidemiological and clinical factors are also very important in pathologies [28]. In the present study, the *ITGA6* expressionin HNSCC was first determined using the Oncomine and GEPIA database. We found that *ITGA6* was highly expressed in various types of cancer including HNSCC (Figure-1A,1B). In addition, the GEPIA database used to evaluate the exact *ITGA6* mRNA expression in HNSCC and normal tissues. We found that the mRNA level of *ITGA6* was significantly up-regulated in HNSCC compared to normal tissues ($p<0.01$) (Figure-2A). Among the selected samples of HNSCC, males were found to be more affected than females. This was also in accordance with an epidemiological study by Nadarajah Vigneswaran et al, 2015. Males were found to be more affected because the HNSCC development can be associated with habit history and habits are more prevalent in male population [29]. Hence HNSCC can be prevalent in males (67.3%).

The age group most affected in our study was 21-40 years and 81-100 years. Many studies showed that HNSCC was also prevalent in younger age groups [30]. Several studies showed that there is an increase in incidence of HNSCC after 50 years [21, 22] This can be in concordance with our study, even though some variation from the most prevalent age group was also observed by some researchers [Muir et al – 24% of HNSCC are found in patients older than 70 years].

Most of the HNSCC patients were of grade 2 tumour and stage 4 cancer and most of the affected population showed no nodal metastasis. Our result also showed that *ITGA6* was highly expressed in HPV negative patients compared with HPV positive patients. This is in line with study by Bratman SV et al, identified the presence of HPV transcripts in 14% of HNSCC samples [23].

ITGA6 expression analysis using the GEPIA and Oncomine datasets showed the overexpression of *ITGA6* in various types of cancer including HNSCC than normal ($p<0.01$). Similar results were observed in the study by Shauntell et al., and In his study he concluded that increased *ITGA6* expression especially the cleaved *ITGA6* expression allows cells to develop cancerous abilities such as aggressiveness, motility through ECM, invasion and metastasis. Shauntell stated in his study that *ITGA6* gene expression can be used as a biomarker in HNSCC. Bo Yang et al., in 2017 also showed that one of the 4 highly expressed genes in HNSCC cases was *ITGA6* and it can be used as a biomarker in HNSCC [31, 32]. Apart from this Ting hu et al in 2016 also showed that ITGA6 gene expression is increased in breast cancer cell lines and causes radiation resistance *in vitro* and interferes with radiation induced cell apoptosis [33].

In the present study, high *ITGA6* expression was found related to poor survival rate in HNSCC patients ($p=1e-04$, Figure 2B). Yang et al., in 2017 stated that high expression of *ITGA6* gene can

Figure 1. *ITGA6* expression levels in human cancers. (A) *ITGA6* in data sets of different cancers in the Oncomine database (red, overexpression; blue, downexpression). (B) *ITGA6* expression levels in different tumor types from TCGA database were determined by GEPIA (*P < 0.01).

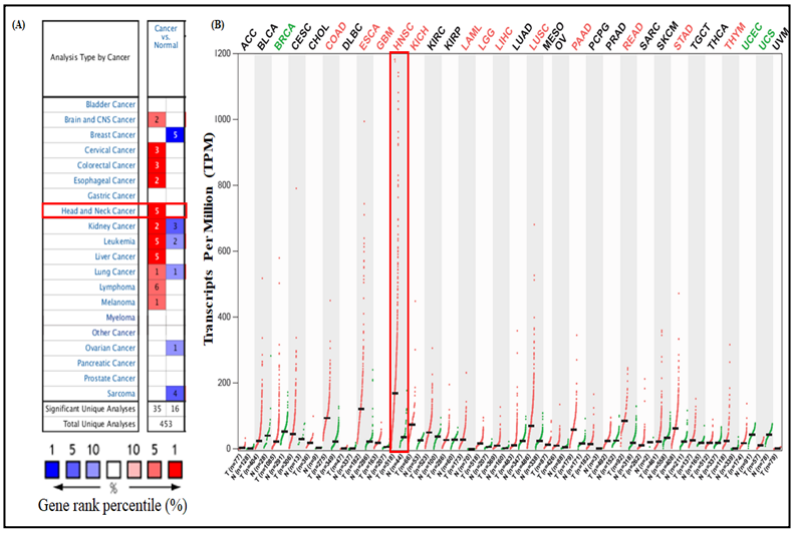
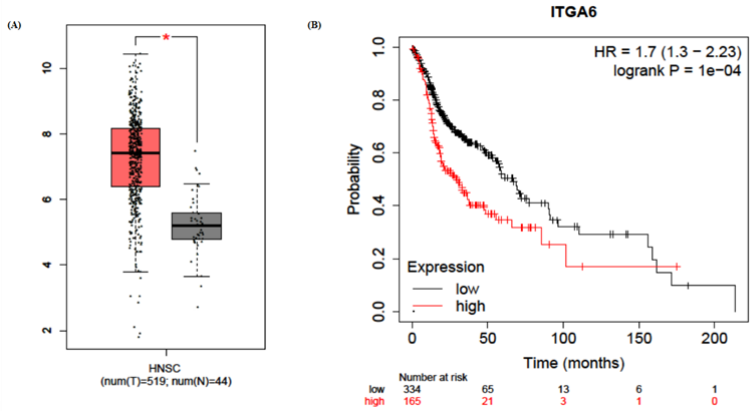


Figure 2. (A) Boxplot showing *ITGA6* expression in patients with HNSCC and normal tissues (GEPIA), *P < 0.01. (B) Kaplan-Meier curves indicated that HNSCC patients had poorer overall survival with high expression of *ITGA6* mRNA (P =1e-04).



be associated with poor overall survival in HNSCC patients [32]. Apart from HNSCC, gall bladder carcinoma, breast cancer also showed decreased overall survival rate associated with increased expression of ITGA6 gene [34, 35]. The present study results were also in accordance with previous literature in the case of increased ITGA6 expression and decreased overall survival rate of HNSCC patients. However, large scale studies are required to substantiate the findings obtained in this study [36].

Conclusion

In conclusion, *ITGA6* mRNA level was overexpressed in HNSCC. In addition, high *ITGA6* expression was significantly related to poor survival in HNSCC patients. Hence *ITGA6* can be used as a potential prognostic biomarker for HNSCC.

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References

[1]. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr;55(2):74-108. Pubmed PMID: 15761078.

[2]. van der Waal I, Snow GB. Oral Oncology. Springer Science & Business Media; 2012.

[3]. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res. 2007 Feb;86(2):104-14. Pubmed PMID: 17251508.

[4]. Jayaraj G, Sherlin HJ, Ramani P, Premkumar P, Natesan A. Stromal myofibroblasts in oral squamous cell carcinoma and potentially malignant disorders. Indian J Cancer. 2015 Jan-Mar;52(1):87-92. Pubmed PMID: 26837985.

[5]. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am. 2014 May;26(2):123-41. Pubmed PMID: 24794262.

[6]. Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthuswami M, et al. Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations. PLoS One. 2016;11:e0156582.

[7]. Jayaraj G, Ramani P, Sherlin HJ, Premkumar P, Anuja N. Inter-observer agreement in grading oral epithelial dysplasia–A systematic review. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2015 Jan 1;27(1):112-6.

[8]. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral

Pathol Med. 2019;48:299-306.
[9]. Jayaraj G, Sherlin HJ, Ramani P, Premkumar P, Anuja N. Cytomegalovirus and Mucoepidermoid carcinoma: A possible causal relationship? A pilot study. J Oral Maxillofac Pathol. 2015;19:319-24.
[10]. Kumar A, Sherlin HJ, Ramani P, Natesan A, Premkumar P. Expression of CD 68, CD 45 and human leukocyte antigen-DR in central and peripheral giant cell granuloma, giant cell tumor of long bones, and tuberculous granuloma: An immunohistochemical study. Indian J Dent Res. 2015 May-Jun;26(3):295-303. Pubmed PMID: 26275199.
[11]. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. Hum Exp Toxicol. 2019 Jun;38(6):694-702. Pubmed PMID: 30924378.
[12]. Gupta V, Ramani P. Histologic and immunohistochemical evaluation of mirror image biopsies in oral squamous cell carcinoma. J Oral Biol Craniofac Res. 2016 Sep-Dec;6(3):194-197.Pubmed . PMID: 27761383.
[13]. Sridharan G, Ramani P, Patankar S. Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Cancer Res Ther. 2017;13(3):556-561.
[14]. Hema Shree K, Ramani P, Sherlin H, Sukumaran G, Jeyaraj G, Don KR, et al. Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma - a Systematic Review with Meta Analysis. Pathol Oncol Res. 2019;25:447-53.
[15]. Viveka TS, Shyamsundar V, Krishnamurthy A, Ramani P, Ramshankar V. p53 Expression Helps Identify High Risk Oral Tongue Premalignant Lesions and Correlates with Patterns of Invasive Tumour Front and Tumour Depth in Oral Tongue Squamous Cell Carcinoma Cases. Asian Pacific Journal of Cancer Prevention. 2016;17:189-95.
[16]. Hannah R, Ramani P, Sherlin HJ, Ranjith G, Ramasubramanian A, Jayaraj G, et al. Awareness about the use, ethics and scope of dental photography among undergraduate dental students dentist behind the lens. Research Journal of Pharmacy and Technology. 2018;11:1012-6.
[17]. Srichai MB, Zent R. Integrin Structure and Function. Cell-Extracellular Matrix Interactions in Cancer. 2010:19-41.
[18]. Robert J. Integrins. Textbook of Cell Signalling in Cancer 2015:117-26.
[19]. Hynes RO. Integrins: bidirectional, allosteric signaling machines. cell. 2002 Sep 20;110(6):673-87.
[20]. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell. 1992;69:11-25.
[21]. Plow EF, Haas TA, Zhang L, Loftus J, Smith JW. Ligand Binding to Integrins. Journal of Biological Chemistry. 2000;275:21785-8.
[22]. Sivaramakrishnan SM, Ramani P. Study on the Prevalence of Eruption Status of Third Molars in South Indian Population. Early Pregnancy. 2015;7:1.
[23]. Legate KR, Wickström SA, Fässler R. Genetic and cell biological analysis of integrin outside-in signaling. Genes Dev. 2009 Feb 15;23(4):397-418. Pubmed PMID: 19240129.
[24]. Sroka IC, Sandoval CP, Chopra H, Gard JMC, Pawar SC, Cress AE. Macrophage-Dependent Cleavage of the Laminin Receptor 6 1 in Prostate Cancer. Molecular Cancer Research. 2011;9:1319-28.
[25]. Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. Br J Cancer. 2004 Feb 9;90(3):561-5. Pubmed PMID: 14760364.
[26]. Wei L, Yin F, Chen C, Li L. Expression of integrin α-6 is associated with multi drug resistance and prognosis in ovarian cancer. Oncol Lett. 2019;17(4):3974-3980.
[27]. Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle. 2009;8:3274-84.
[28]. Jangid K, Alexander AJ, Jayakumar ND, Varghese S, Ramani P. Ankyloglossia with cleft lip: A rare case report. J Indian Soc Periodontol. 2015;19:690-3.Pubmed PMID: 26941523.
[29]. Assoian RK, Schwartz MA. Coordinate signaling by integrins and receptor tyrosine kinases in the regulation of G1 phase cell-cycle progression. Current Opinion in Genetics & Development. 2001;11:48-53.
[30]. Toner M, O'Regan EM. Head and Neck Squamous Cell Carcinoma in the Young: A Spectrum or a Distinct Group? Part 2. Head and Neck Pathology. 2009;3:249-51.Pubmed PMID: 20596980.
[31]. Kalita C, Sharma J, Baishya N, Kataki A, Das A, Rahman T. Head and neck squamous cell carcinoma in young adults: A hospital-based study. Indian Journal of Medical and Paediatric Oncology. 2019;40:18.
[32]. Yang B, Chen Z, Huang Y, Han G, Li W. Identification of potential biomarkers and analysis of prognostic values in head and neck squamous cell carcinoma by bioinformatics analysis. OncoTargets and Therapy. 2017;10:2315-21.
[33]. Hu T, Zhou R, Zhao Y, Wu G. Integrin α6/Akt/Erk signaling is essential for human breast cancer resistance to radiotherapy. Sci Rep. 2016;6:33376.
[34]. Zhang D-H, Yang Z-L, Zhou E-X, Miao X-Y, Zou Q, Li J-H, et al. Overexpression of Thy1 and ITGA6 is associated with invasion, metastasis and poor prognosis in human gallbladder carcinoma. Oncology Letters 2016;12:5136-44. PubmedPMID: 28105220.
[35]. Brooks DL, Schwab LP, Krutilina R, Parke DN, Sethuraman A, Hoogewijs D, et al. ITGA6 is directly regulated by hypoxia-inducible factors and enriches for cancer stem cell activity and invasion in metastatic breast cancer models. Mol Cancer. 2016;15:26.PMID: 27001172.
[36]. Swathy S, Gheena S, Varsha SL. Prevalence of pulp stones in patients with history of cardiac diseases. Research Journal of Pharmacy and Technology. 2015;8:1625-8.