Decoding the Genetic Alterations in Genes of Fibroblast Growth Factor Family and Their Possible Association with HNSCC

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Authors’ contributions
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ABSTRACT

Introduction: HNSCC is a type of cancer in the oral and pharynx region. Several mutations/variations are observed in these cancer phenotypes. Fibroblast growth factor belongs to the family of heparin binding growth factors. FGFs are multifunctional proteins with a wide variety of effects; they are most commonly mitogens. Their expression pattern correlates with invasion of HNSCC.

Aim: To assess the genetic alterations in genes of the fibroblast growth factor family and their association with HNSCC.

Materials and Methods: The demographic data and samples of 528 HNSCC patients was collected from the cBioportal database. Oncoprint analysis was done to assess the amplification and genetic alterations of the members of the FGF gene family. String analysis was performed to evaluate the protein-protein interaction. The information about previous reported mutation and correlation with novel and reported mutation was obtained using GnomAD analysis.

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Results and Discussion: FGF3,4 and 19 genes showed maximum variation (25%). FGF4 and FGF19 genes showed maximum amplification in addition to deletion mutation. Excitingly FGF3, FGF4 and FGF19 genes showed similar amplification patterns in most of the HNSCC patients. Statistical significant difference in the gene expression of FGF3 9.578 x 10^-3 observed between normal and primary tumour. S. Findings showed many novel mutations and also 4 reported mutations ie:FGF1, FGF12, FGF20, FGF21

Conclusion: Our present study concludes that more evidence is required to confirm their association with HNSCC.

Keywords: Amplification; fibroblast growth factor; head and neck squamous cell carcinoma; novel; variants.

1. INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) includes cancer in lip, oral cavity, oropharynx, nasopharyngeal and laryngeal regions. Incidence is nearly 7,00,000 new cases and more than 3,80,000 deaths per annum worldwide [1]. Squamous cells are protective cells which are seen in the outermost layers of skin and mucous membranes [2]. Head and squamous cell carcinoma mostly develops in mouth and throat regions. Survey conducted in 2018 by GLOBOCAN concludes that higher prevalence rates are grouped in asian countries [3]. Head and squamous cell carcinoma mainly caused due to alterations in DNA or specific genes. Other exogenous factors like heavy alcohol consumption, tobacco chewing and smoking [4]. Neoplasm can be treated by surgery, radiation, chemotherapy and hormonal therapy. Long term treatment causes major side effects like nausea, vomiting, bone-marrow suppression, paraesthesias, and reversible hair loss [5]. Incidence of carcinoma is more among male than females [6].

Fibroblast growth factor (FGFs) belongs to the family of heparin binding growth factors. These growth factors are generally extracellular in origin, but they will activate the cell surface receptors [7]. Mostly they will bind heparin and its metabolites. They are released only upon injury or tissue remodelling. Fibroblast growth factors are a vast family which contains nearly 22 individual members. They are structurally designed as signaling molecules. Members include FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF20, FGF2, FGF22, FGF23. There is no human FGF15. Fibroblast growth factor has 4 receptors FGFR1, FGFR2, FGFR3, FGFR4. FGF 1-10 binds to the receptors. FGF1 is known as acidic fibroblast growth factor and FGF2 is known as basic fibroblast growth factor [8]. FGF18 is identified to possess cell development and morphogenic properties. Fibroblast growth factor helps in mesoderm induction, limb development and wound healing processes. FGFs secreted by hypoblasts play a role in stimulating a Wnt signalling pathway during avian gastrulation. Our team has extensive knowledge and research experience that has translate into high quality publications[9–13].

Previous studies have concluded in the diagnosis of subtypes of RCC, FGFR2 isoform testing can be used. The tissue-specific discovery of a prevalent isoform switch of FGFR2 holds promise for the potential development of FGFR2-IIIc as a separate biomarker of early detection and therapeutic target for ccRCC [14]. Formalin fixed, paraffin embedded tumours compared with frozen neoplasm, described the feasibility of comprehensive gene profiling [15]. Various studies have been done regarding the etiology of HNSCC, but none of them is precise. Our aim of the study is to decoding the genetic alterations in fibroblast growth factor and assessing its interrelationship with HNSCC.

2. MATERIALS AND METHODS

2.1 Data Source

The present study is in retrospective study design Source of information (ie): patients data were procured from cBioportal database [16]; [17]. cBioportal genomics integrates an exhaustive collection of molecular lines. The database is user friendly. Epigenetic and proteomic collections were registered. Information about different cohorts was collected. The sample data set contains 528 tumour samples of head and squamous cell carcinoma. The samples harboured copy number variations and mutation data. The demographic details of patients with head and squamous cell carcinoma
dataset were tabulated (Table 1). User defined questions queries regarding genes submitted on cBioportal database.

2.2 Oncoprint Analysis

The oncoprint data provides information on the probabilities of specific gene alterations, the type of alterations, changes in protein that codes for amino acids, and genes of amplification, deletion, insertion, frameshift, mutation, and so on. This information is used to derive a putative relation between the disease phenotype and genotype, assess the variation in less understood pathways or genes, differentiate the novel and previously reported variations associated with disease phenotype.

2.3 Protein Network Analysis

STRING is one of the first projects, and it focuses on high coverage, ease of use, and a consistent scoring system to set itself apart. The STRING database incorporates data from a variety of sources, including experimental data, numerical prediction methods, and publicly available text collections. It is open to the public and is maintained on a regular basis. Using a variety of functional classification schemes such as GO, Pfam, and KEGG, the resource also highlights functional enrichments in user-provided protein lists. The advanced version of string contains approximately 24.5 million proteins from 5000 organisms [18].

2.4 GNOMAD Analysis

A resource created by an international consortium of researchers, the genome aggregation database(GnomAD) aims to collect and harmonise both exome and genome sequencing data from a wide range of large scale sequencing projects and to make summary data accessible to the broader scientific community. The data set provided on this website unrelated individuals sequenced as part of various different diseases. These sample sequences were collected based on public consent, consortium, permission, exome data quality and lack of relatedness with other samples [19].

2.5 Gene Expression and Survival Analysis

The expression of the gene presenting with highest frequency of gene alteration in HNSCC was analysed using the UALCANC (http://ualcan.path.uab.edu/cgi-bin/TCGA-survival) database. Survival curve analysis based on the tumor grade and expression profile was performed to demonstrate the putative role of FGF family of genes with HNSCC. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using log-rank test that generated a p value which was further used to indicate statistical significance of survival correlation between groups [20].

3. RESULTS

Primary database used from cBioportal. Several dataset collected from TCGA, firehouse legacy. Only selected samples were used in the present study. TCGA gave information about 528 HNSCC patients. From the (Table:1) demographic data, male and female ratio was found to be 193:71 of diagnostic age ranging from 19-90 years. The number of individuals having a history of smoking was found to be 97% (515 individuals). The percentage of patients with HNSCC who consume alcohol is 66% (352 individuals). The data sets were collected from different geographic populations, white 85% (452 individuals), africans 9% (48 individuals), asian 2% (11 individuals), american indian 0.3% (2 individuals). The distribution of patients based on histological grading of tumour 58% of patients had grade-II tumour.

Oncoprint analysis performed to analyse the genetic mutation and alterations of the fibroblast growth factor family. Here gene FGF3,FGF4 and FGF19 are observed to have higher percentages of alteration (Fig. 1). Oncoprint analysis reveals that 14 genes underwent amplification, In which genes like FGF3, FGF4 and FGF19 showed 25% of gene alteration. Genes FGF1, FGF2, FGF4, FGF5, FGF7, FGF8, FGF9, FGF10, FGF11, FGF13, FGF14, FGF19, FGF21 and FGF23 have undergone genetic alteration. A shift in DNA that causes a protein to terminate or terminate its translation earlier than expected is stop mutation. This is a common type of mutation that causes a shortened or non-functional protein to be expressed in humans and other animals. Genes have undergone a stop mutation or termination mutation ie: FGF1, FGF12. Genes like FGF3, FGF4 and FGF19 harboured the highest number of variations/ mutations among the gene identified alteration (Table 2). Several splice mutations and missense mutations of unknown significance were documented (Fig 1). Genetic alterations in the genes associated with
Fibroblast growth factor families were documented. Alteration of each gene is noted and checked whether it is a novel mutation or previously reported mutation. Interestingly patients with FGF19 mutation also had FGF3 and FGF4 genes mutated. Arranging the gene based on high degree of amplification FGF3, FGF4, FGF19 followed by FGF12, FGF10, FGF6 and FGF23.

Oncoprint analysis of samples showed mostly no alterations in most of the genes. More than 20% of the gene has undergone amplification. Some genes showed truncating mutation, as a change in DNA that can truncate or shorten protein seen in FGF1, FGF2, FGF12, FGF21, FGF22 and FGF23. Splice mutation is also genetic mutation that inserts, deletes, changes the number of nucleotides at specific sites, splicing occurs during processing from precursor messenger RNA to mature messenger RNA. Only gene showing splice mutation is FGF11. statistically significant difference in the gene expression of FGF3 9.578 x 10^{-3} observed between normal and primary tumour. Total of 4 reported variant have been identified by GnomAD analysis viz., FGF1 (rs770592235), FGF12 (rs7685097171), FGF20 (rs765043985) and FGF21 (rs781670890). Differential expression of gene in normal versus primary tumour for FGF3 only showed statistical significance value. Protein network interaction of FGF3 (Fig. 2).

Differential expression of genes in Normal vs Primary tumor

|                      | FGF3    | FGF4    | FGF19   |
|----------------------|---------|---------|---------|
|                      | 9.578 x 10^{-3} | 1.172 x 10^{-1} | 5.165 x 10^{-2} |

4. DISCUSSION

Fibroblast growth factors (FGFs) are generally secreted small proteins which act by binding to receptors of transmembrane tyrosine kinase (FGFRs). Activation of FGFRs will activate the multiple cytoplasmic cascades that lead to cell behaviour changes [21]. In a number of developmental and physiological processes, FGFs play a critical role [22]. FGF gene family composed of eight subfamilies. Evolutionary history of the FGF gene family is characterized by gene duplication and gene losses [23]. Humans have 18 FGF ligands in the whole body and four FGFR receptors (FGFR1, FGFR2, FGFR3, FGFR4). FGF ligands are growth factors of polypeptides that influence several processes of development including cell proliferation, differentiation, migration, morphogenesis and patterning [24]. In order to build an axial and craniofacial skeleton, FGF-FGFR signalling is also important [25]. In particular, intramembranous ossification of cranial bone and suture has been implicated in signalling cascade [26]. In adults FGF and FGFRs are used in tissue repair. FGF signalling follows one of the three signalling pathways of transduction: RAS/MAP kinase, P13/AKT or PLCY [27].

Table 1. Represents the demographics details of the HNSCC patients collected from cBioportal

| Gender                  | Male (n = 386) | Female (n = 142) |
|-------------------------|---------------|-----------------|
| Mutation count          | 6-3181        |                 |
| Diagnosis age           | 19-90 years   |                 |
| Smoking status          | Smokers: 515  | Data not available: 12 |
|                         | Unknown: 1    |                 |
| Alcohol history         | Yes – 352     | Data not available: 11 |
|                         | No – 165      |                 |
| Neoplasm Histologic grade | Grade 1: 63 | Grade 2: 311 |
|                         | Grade 3: 125  | Grade 4: 7     |
|                         | Grade GX: 18  | Data not available: 4 |
| Race category           | White: 452    | African: 48    |
|                         | Asian: 11     | American Indian or Alaska native: 2 |
|                         | Data not available: 15 |
| Gene       | Protein encoded     | Cytogenetic loci | Alteration          | % of alteration | Variant allele frequency | gnomAD frequency    |
|------------|---------------------|------------------|---------------------|-----------------|--------------------------|---------------------|
| FGF1       | Fibroblast growth factor 1 | 5q31.3 | Deep deletion | 0.8             | 0.12                     | Novel               |
|            |                     |                  | GI45*               |                 | 0.27                     | rs770590235         |
|            |                     |                  | R137W               |                 | 0.74                     | rs764725960         |
| FGF2       | Fibroblast growth factor 2 | 4q28.1 | Deep deletion | 0.8             | 0.27                     | Novel               |
|            |                     |                  | I176F               |                 | 0.33                     | Novel               |
| FGF3       | Fibroblast growth factor 3 | 11q13.3 | Amplification | 25              | 0.43                     | Novel               |
|            |                     |                  | V113E               |                 |                          |                     |
| FGF4       | Fibroblast growth factor 4 | 11q13.3 | Amplification | 25              | -                        | -                   |
|            |                     |                  | Deep deletion       |                 |                          |                     |
| FGF5       | Fibroblast growth factor 5 | 4q21.21 | Amplification | 1.4             | 0.04                     | Novel               |
|            |                     |                  | Deep deletion       |                 | 0.43                     | Novel               |
|            |                     |                  | L9F                 |                 | 0.30                     | Novel               |
| FGF6       | Fibroblast growth factor 6 | 12p13.32 | Amplification | 3               | 0.21                     | Novel               |
|            |                     |                  | G64V                |                 |                          |                     |
| FGF7       | Fibroblast growth factor 7 | 15q21.2 | Amplification | 0.6             | 0.06                     | Novel               |
|            |                     |                  | Deep deletion       |                 |                          |                     |
|            |                     |                  | N149S               |                 |                          |                     |
| FGF8       | Fibroblast growth factor 8 | 10q24.32 | Deep deletion | 0.8             | 0.20                     | Novel               |
|            |                     |                  | A170V               |                 | 0.14                     | Novel               |
|            |                     |                  | T183M               |                 | 0.17                     | Novel               |
| FGF9       | Fibroblast growth factor 9 | 13q12.11 | Amplification | 1.6             |                          | Novel               |
|            |                     |                  | Deep deletion       |                 |                          |                     |
|            |                     |                  | T81S                |                 |                          |                     |
| FGF10      | Fibroblast growth factor 10 | 5p12  | Amplification | 6               |                          | Novel               |
|            |                     |                  | Deep deletion       |                 |                          |                     |
|            |                     |                  | K137E               |                 | 0.19                     | Novel               |
|            |                     |                  | D149N               |                 | 0.12                     | Novel               |
| FGF11      | Fibroblast growth factor 11 | 17p13.1 | Amplification | 1.4             | 0.45                     | Novel               |
|            |                     |                  | Deep deletion       |                 |                          |                     |
|            |                     |                  | V203=               |                 |                          |                     |
| FGF12      | Fibroblast growth factor 12 | 3q28-q29 | Amplification | 20              | 0.24                     | Novel               |
|            |                     |                  | P89T                |                 | 0.04                     | rs768509717         |
|            |                     |                  | R185H               |                 | 0.12                     | Novel               |
|            |                     |                  | R64W                |                 |                          |                     |
| Gene   | Protein encoded   | Cytogenetic loci | Alteration       | % of alteration | Variant allele frequency | gnomAD frequency |
|--------|------------------|------------------|------------------|-----------------|--------------------------|-----------------|
| FGFA1  | Fibroblast growth factor 10 | 5p14.3            | Amplification    | 0.01            | Novel                     | Novel           |
| FGFB1  | Fibroblast growth factor 11 | 5q31.3            | Deletion         | 0.00            | Novel                     | Novel           |
| FGFC1  | Fibroblast growth factor 12 | 8q24.23           | Deep deletion    | 0.67            | Novel                     | Novel           |
| FGFD1  | Fibroblast growth factor 13 | 11p15             | Amplication      | 0.02            | Novel                     | Novel           |
| FGFE1  | Fibroblast growth factor 14 | 2p13.33           | Deep deletion    | 0.05            | Novel                     | Novel           |
| FGFF1  | Fibroblast growth factor 15 | 19q13.32          | Deletion         | 0.45            | Novel                     | Novel           |
| FGFG1  | Fibroblast growth factor 16 | 22q13.32          | Amplification    | 0.03            | Novel                     | Novel           |
| FGFH1  | Fibroblast growth factor 17 | 8q24.22            | Deletion         | 0.29            | Novel                     | Novel           |
| FGFI1  | Fibroblast growth factor 18 | 19q13.33          | Deep deletion    | 0.06            | Novel                     | Novel           |
| FGFJ1  | Fibroblast growth factor 19 | 22q13.32          | Amplification    | 0.05            | Novel                     | Novel           |
| FGFK1  | Fibroblast growth factor 20 | 11p15             | Deletion         | 0.15            | Novel                     | Novel           |
| FGFL1  | Fibroblast growth factor 21 | 19p13.3            | Deep deletion    | 0.06            | Novel                     | Novel           |
| FGFM1  | Fibroblast growth factor 22 | 19q13.33          | Amplication      | 0.03            | Novel                     | Novel           |
| FGFN1  | Fibroblast growth factor 23 | 22q13.32          | Deep deletion    | 0.45            | Novel                     | Novel           |
| FGFO1  | Fibroblast growth factor 24 | 11p15             | Amplification    | 0.05            | Novel                     | Novel           |
| FGFP1  | Fibroblast growth factor 25 | 19q13.33          | Deletion         | 0.06            | Novel                     | Novel           |

**FGF13**
- Fibroblast growth factor 13
- Xq26.3-q27.1
- Amplification
- Deep deletion
- H88Y
- R114H
- *244Lex*53
- *244Lext*53
- % of alteration: 0.06
- Variant allele frequency: 0.34
- gnomAD frequency: 0.10
- Novel

**FGF14**
- Fibroblast growth factor 14
- 13q33.1
- Amplification
- Deep deletion
- G91D
- H115Y
- P139T
- % of alteration: 1.6
- Variant allele frequency: 0.25
- gnomAD frequency: Novel

**FGF15**
- Fibroblast growth factor 15
- Xq21.1
- Deep deletion
- L12V
- R184H
- % of alteration: 0.6
- Variant allele frequency: 0.41
- gnomAD frequency: Novel

**FGF16**
- Fibroblast growth factor 16
- 8p21.3
- Deep deletion
- G95Efs*100
- % of alteration: 0.01
- Variant allele frequency: 0.19
- gnomAD frequency: Novel

**FGF17**
- Fibroblast growth factor 17
- 11q13.3
- Amplification
- Deep deletion
- A86T
- D129V
- % of alteration: 25
- Variant allele frequency: 0.21
- gnomAD frequency: Novel

**FGF18**
- Fibroblast growth factor 18
- 7p15.1
- Deletion
- R150Gfs*?
- % of alteration: 1
- Variant allele frequency: 0.29
- gnomAD frequency: Novel

**FGF19**
- Fibroblast growth factor 19
- 5q35.1
- Amplification
- Deep deletion
- H88Y
- R114H
- % of alteration: 3
- Variant allele frequency: 0.6
- gnomAD frequency: Novel

**FGF20**
- Fibroblast growth factor 20
- 19q13.33
- Amplification
- G95Efs*100
- % of alteration: 0.6
- Variant allele frequency: 0.19
- gnomAD frequency: Novel

**FGF21**
- Fibroblast growth factor 21
- 1p31.3
- Deletion
- R150Gfs*?
- % of alteration: 1
- Variant allele frequency: 0.29
- gnomAD frequency: Novel

**FGF22**
- Fibroblast growth factor 22
- 11p15
- Amplification
- Deep deletion
- A86T
- D129V
- % of alteration: 25
- Variant allele frequency: 0.21
- gnomAD frequency: Novel

**FGF23**
- Fibroblast growth factor 23
- 22q13.32
- Deletion
- R150Gfs*?
- % of alteration: 4
- Variant allele frequency: 0.19
- gnomAD frequency: Novel
Fig. 1. Oncoprint data depicting the genetic alterations in fibroblast growth factor family 1
Fig. 2. Protein network interaction of \textit{FGF3} as assessed using STRING database

More than 35\% of head and squamous cell carcinoma occurs due to amplification and rearrangement [28]. Higher incidence of HNSCC was observed in African and American populations [4]. Saliva serves as a diagnostic tool/medium for early detection of HNSCC [29][30]. Until now smoking seems to be a major etiology of head and neck squamous cell carcinoma. Hence saliva can be used as a diagnostic marker for smoking related cancer other than gene markers [31]. Fibroblast growth factor (FGFR1) receptor 1 serves as a putative driver for tumour progression. It was identified in patients by targeted sequencing [32]. Previous studies suggest that FGFR1 receptor amplification and overexpression was around (9-17\%) and (8-12\%). Former study reported that a high degree of polymorphism (35-54\%) was observed in \textit{FGFR4} in which glycine was replaced by arginine at 388 position. But amplification and overexpression of individual genes have no role in determining the survival rate of infected individuals [33]. High amplification of \textit{FGF19} may lead to head and neck squamous cell carcinoma due to increase in autocrine \textit{FGF19} secretion [34]. Amplification of \textit{FGF19} will cause constitutive activation of FGF receptor (FGF9)-dependent activation. \textit{FGF19} proved to promote cell proliferation [35]. Similar to our present study, previously many articles about genetic alteration in the family of \textit{SPARC} gene [36] and regarding the antioxidant gene association with HNSCC have been done [37].

Multiple studies have reported that proliferation, differentiation, mineralisation may depend on the \textit{FGF2} signalling pathway. But a unique inhibitor will inhibit the whole lineage from FGFR1, FGFR2, FGFR3 and FGFR4. On an interesting note, \textit{FGF2} plays a major role in differentiation of oligodendrocytes and inhibits the formation of myelin [38]. From Fig 2 \textit{FGF3} gene has been connected to variety of tumour related proteins like HRAS, MAPK, MAPK3, FGFR1, FGFR2, FRS2, FGFR1, FGFBP11 and PTPN11. \textit{HRAS} protein is involved in activation of Ras protein signalling transduction pathway. \textit{HRAS} and \textit{FGF3} gene correlation with bladder cancer has been proved. Increases or decreases in \textit{HRAS} gene cause huge variation in \textit{FGF3} gene [39]. Mitogen activated protein kinase 3 (\textit{MAPK3}) acts as essential components of the MAP kinase signaling transduction pathway. Reduction in ERK1/2(P44/P42) phosphorylation has relation with tumour growth in oral cell carcinoma[40]. Phosphatidylinositol 4,5 bisphosphate 3-kinase catalytic subunit alpha isoform, plays a key role by recruiting its domain-containing protein to the membrane including AKT1 and PDPK1, involved in cell growth, proliferation., motility and morphology. Expression of \textit{PIK3CA} was increased in case of oral cell carcinoma [41]. Limitations of this study, sampling is done in particular populations. Several studies conducted by the authors employing computational methods had aided us in developing the study design for the present study [42-52]. The in silico methods can be used to identify mutations which can act as potential drivers of the tumorigenesis process [53], [54][23,55], [56]. Our research team has conducted several studies related to identification of molecular targets and variants in genes which show a direct or indirect association with cancer [57,58,59,60,42,49,50,52,61].
5. CONCLUSION

In our present study, brief genetic alterations of the members of fibroblast growth factors was discussed. Not all the genes showed significant mutations. Only a few of the genes showed higher amplifications. To derive an association between genetic alterations and HNSCC, markers found in this study screened in specific populations. This will pave the way for upcoming research in pathogenesis, early diagnosis and therapy. Further experimental confirmation is required for the results to confirm the correlation factor.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline Patient’s consent and ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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