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High Frequency Novel Mutations Of FGFR3 Gene In Bladder Carcinoma Stages.

Bashar Ouda Jawad Altae[1]*, Sabah Bresam[2], and Abdul Hussein M Al Faisal[3].

[1]Hammurabi Medicine College, University of Babylon, Babylon, Iraq.
[2]Accommodation Department, University of Baghdad, Baghdad, Iraq.
[3]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

ABSTRACT

One hundred and one Transitional cells carcinoma TCC with different T-categories were included in this study and 50 subjects control group. Patient age ranged from 30 to 86 years while control subjects ages ranged from 30 to 50 years. The exon 7 and 10 regions of *FGFR3* was amplified by PCR. PCR products were sequenced and compared with the information in gene bank of the National Center for Biotechnology Information (NCBI) with reference *FGFR3* gene using (Mega -6) software. Two hot-spot novel mutations of *FGFR3* gene were identified. One was mutation g.16216 of exon 10 led to G>C GAG>GAC Glu→ Asp substitution. The other mutation, was a mutation in exon 7 nucleotide g.13517, led to a codon C >A CCC>CCA Pro→Thr substitution. These mutations of *FGFR3* gene have not been reported previously. Concerning T-category, the following mutation frequencies occurred: Ta, 59.4%; T1, 60.9%; T2, 64% and T3, 66.7%. The majority of mutations were found in low malignant tumors, Ta-T2, whereas mutations in T3 tumors were low and restricted to mutation g.13517. Therefore, novel mutation g.13517 of exon 7 of *FGFR3* gene represents a valuable prognostic marker of tumors with low malignant potential and can be used as marker for the detection of genetically stable bladder tumors.

Keywords: TCC, *FGFR3,* T-category, g.13517, g.16216, bladder carcinoma.

Corresponding author

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INTRODUCTION

Bladder carcinoma is representing the fifth most common cancer worldwide [1]. Most of these carcinomas are noninvasive, papillary tumors and transitional cells carcinoma [2]. However, up to 15-30% is characterized by tumor progression. It is necessary for an effective therapy to early detection of tumor. It is important to discover new prognostic markers for patients with bladder cancer. Therefore, identification of mutations of various genes and proteins involved in tumor development and progression are essential [3,4]. Fibroblast growth factor receptor 3 (*FGFR3*) gene has potential as a molecular marker for bladder cancer [5]. A previous study by Rieger-Christ *et al.* [6] showed an overall frequency of *FGFR3* mutations in bladder carcinoma of 43%. Several genetic changes may occur in bladder cancer, but a mutation in the fibroblast growth factor receptor 3 (*FGFR3*) gene is the most common and most specific genetic abnormality in bladder cancer [7,8,9]. Interestingly, these mutations were associated with bladder tumors of low stage and grade [10], which makes the *FGFR3* mutation a good marker that can be used for diagnosis of bladder tumors [11,12]. Further studies demonstrated that mutations in *FGFR3* occur frequently in bladder tumors and might correlate with favorable clinical outcome [13,14,15].

The fibroblast growth factor (FGF) receptor family consists of four transmembrane tyrosine kinase receptors (FGFR1-4), and 23 FGF ligands have been described [15]. The mitogenic activity of FGFs is not restricted to fibroblasts, but stimulates many cell types, including endothelial cells and chondrocytes [16]. *FGFR3* gene coded a glycoprotein belongs to tyrosine kinase receptor family. *FGFR3* gene found to associate with congenital anomalies such as achondroplasia and thanatophoric dysplasia [17,18] Recently, it has been shown that the *FGFR3* gene plays an oncogenic role in bladder carcinoma [19].

The aim of this study was to analyze *FGFR3* mutations of transitional cells carcinoma of the bladder in order to investigate any high frequency mutations that related to histopathological stages.

MATERIALS AND METHODS

One hundred and one Transitional cells carcinoma TCC with different T-categories were included in this study and 50 subjects control group. All bladder cancer samples were staged using histopathological sections according to WHO (World Health Organization) and ISUP (International Society of Urological Pathology) Grading of Urothelial (Transitional Cell) Tumors [20]. Patient samples were obtained from Ghazi Al Hariri Hospital in Baghdad. Patient age ranged from 30 to 86 years while control subjects ages ranged from 30 to 50 years. DNA was extracted using genomic DNA purification kits (Bioneer, South Korea). The exon 7 and 10 regions of *FGFR3* was amplified by PCR using the primers, F 5' CAGGCCAGGCCTCAACGCCC '3 and R 5'AGGCCTGGCGGGCAGGCAGC '3 for exon 10 region with a condition, initial denaturation 5 minutes at 95^{o}C, followed by 40 cycle each of denaturation 1 minute at 95^{o}C, annealing 1 minute at 72^{o}C, extension 1 minute at 72^{o}C and a final extension step at 72^{o}C for 10 minute and Exon 7 region was amplified using the primers, F 5' CGGCAGTGGCGGTGGTGGTG'3 and R 5' AGCACCGCCGTCTGGTTG '3 and the condition, initial denaturation 5 minutes at 95^{o}C, followed by 40 cycle each of denaturation 1 minute at 95^{o}C, annealing 1 minute at 67^{o}C, extension 1 minute at 72^{o}C and a final extension step at 72^{o}C for 10 minute. PCR products (3 µl) were then separated on 3% agarose gel with a ladder (100 bp) and visualized. PCR products of the *FGFR3* gene exon 10 and exone 7 regions (91 samples) and primers were sent to Macrogen Company (U.S.A) for sequencing. The sequences of these samples were compared with the information in gene bank of the National Center for Biotechnology Information (NCBI) with reference *FGFR3* gene using (Mega 6) software.

RESULTS AND DISCUSSION

Identification of somatic mutations is a key to understanding the molecular mechanism of bladder carcinoma and the development of novel therapeutics. It is also presumed that genomic data could be used for disease prognostication for patients with bladder carcinoma with different clinical outcomes since mutations of specific genes are known to correlate with distinct biological behaviors of tumors.

We initially screened for *FGFR3* gene mutations in exon 10 and exone 7 regions in 101 cases of bladder carcinoma samples using the direct genomic sequencing. Two hot-spot novel mutations of *FGFR3* gene were identified. One was mutation g.16216 of exon 10 led to G>C GAG>GAC Glu→ Asp substitution. The other mutation, was a mutation in exon 7 nucleotide g.13517, led to a codon C >A CCC>CCA Pro→Thr

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substitution (Figure-1). These mutations of *FGFR3* gene have not been reported previously. The results showed that 61(67%) of studied patients were males and 40(44%) were females. 59 of 101 patients have mutations in *FGFR3* gene exon 10 and exone 7 regions (Table 1). 46(45.5%) of mutations shown in FGFR3 exon 7 and 13(12.9%) were in exon 10. These mutations affected codon g.13517 of exon 7 and codon g.16216 of exon 10 (Table-2).

Table 1: Mutations and Patients profile

Total (%)	Mutation %	*P* value
	59/101 (58.4%)	
Male	61/101(67%)	0.097
Female	40/101(44%)	

Table 2: Frequency of the novel *FGFR3* gene mutations in bladder carcinoma stages

Stage/ mutation	Exon 10 number+percentage	Exon 7 number+percentage	Total Mutation
	g.16216 Novel mutation G>C GAG>GAC Glu/Asp	g.13517 Novel mutation C>A CCC>CCA Pro/Thr	
Ta	6/14 (43)	13/18(72)	19/32(59.4)
T1	2/9 (22)	12/14(86)	14/23(60.9)
T2	5/11 (46)	11/14(79)	16/25(64)
T3	0	10/15(67)	10/15(66.7)
Total	13/34(38)	46/51(90)	59/85(69.4)
P value	0.013		

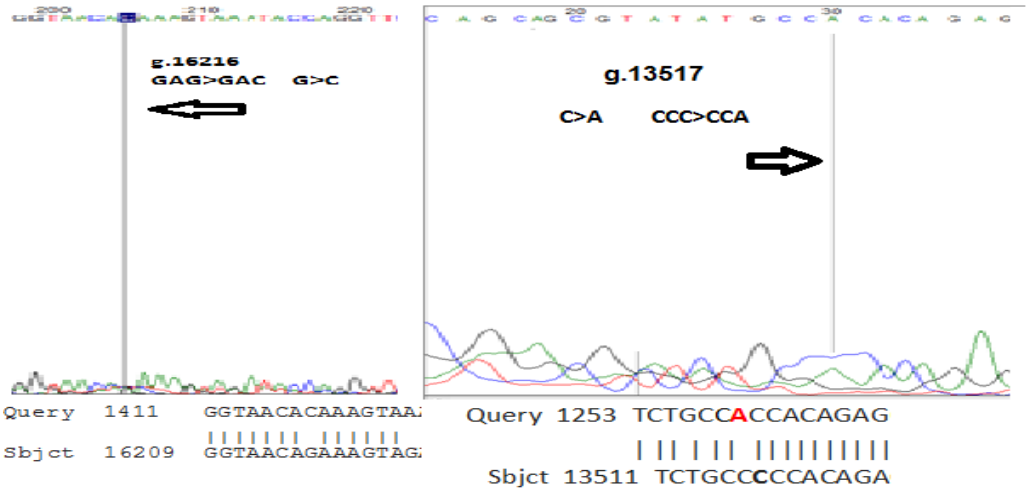

Figure1: Location of novel *FGFR3* gene mutations in exon 10 and exone 7.

Concerning T-category, the following mutation frequencies occurred: Ta, 59.4%; T1, 60.9%; T2, 64% and T3, 66.7%. The majority of mutations were found in low malignant tumors, Ta-T2, whereas mutations in T3 tumors were low and restricted to mutation g.13517. Mutation frequency was significantly associated with tumor stage which could represent a promising biomarker for bladder cancer. Results also showed that these two novel mutations (g.16216, g.13517) are more representative in Ta- T2 while novel mutation of exon 7, g.13517 is the only represented mutation in T3 which characterize a high risk tumor than exon 10 novel

mutation g.16216**.** Moreover, novel mutation g.13517 of exon 7 has a high frequency (54%) among all bladder carcinoma T-category comparing to 15.3% of the novel mutation g.16216 of exon 10**.** Therefore, novel mutation g.13517 of exon 7 of *FGFR3* gene represents a valuable prognostic marker of tumors with low malignant potential and can be used as marker for the detection of genetically stable bladder tumors [21,22]. These results confirmed findings of other studies. Whereas the frequency in Ta tumors was similar compared to these studies, the percentage of T1 tumors with mutations differs between the studies [13,14,23] This strong correlation with grade was striking in all studies. The majority of mutations were found in low malignant tumors, Ta-T2, whereas mutations in T3 tumors were very rare. These results from different studies underline that *FGFR3* mutations characterize tumors with favorable histological features [24,25]. Furthermore, in the study of van Rhijn *et al.* [13], it was shown that the presence of an *FGFR3* mutation is a strong indicator of superficial bladder tumors with a favorable clinical outcome. Recently, Hernandez *et al.* [26] found *FGFR3* mutations to be associated with a higher rate of recurrence but again with good clinical outcome. *FGFR3* mutations were detected in bladder cancer by several groups and described to be associated with stage-grade or with recurrence and progression rate [10,19]. Further studies demonstrated that mutations in *FGFR3* occur frequently in noninvasive urothelial tumors of the bladder, but not in invasive tumors, and might correlate with favorable clinical outcome [10,13,26]. Therefore, *FGFR3* mutations were associated with noninvasive low malignant tumors or tumors with limited invasive potential. These results confirmed findings of other studies [10,14]. While the frequency in Ta tumors was similar compared to these studies, the percentage of T1 tumors with mutations differs between the studies [13,14,23,27,28].

CONCLUSION

Two hot-spot novel mutations of *FGFR3* gene were identified. One was mutation g.16216 of exon 10 led to G>C GAG>GAC Glu→ Asp substitution. The other mutation, was a mutation in exon 7 nucleotide g.13517, led to a codon C >A CCC>CCA Pro→Thr substitution. These mutations of *FGFR3* gene have not been reported previously and represents a valuable prognostic marker of tumors with low malignant potential and can be used as marker for the detection of genetically stable bladder tumors.

Ethics approval and consent to participate

The current study was approved by the Institution of Genetic Engineering and Biotechnology Committee and under agreement of Iraqi MOH.

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