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Compound FGFR3 Mutations Associated With Grads Of Bladder Carcinoma.

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ABSTRACT

Activating mutations of fibroblast growth factor receptor-3 (FGFR3) have been observed in up to 70% of bladder carcinoma and implicated in the tumorigenesis of many other malignancies. The current study aimed to detect the FGFR3 mutations and their correlation with stages of bladder carcinoma. The results revealed that FGFR3 compound mutations in about 42.11% of all bladder carcinoma patients and 60% of them were detected in male patients and constitute as a risk factor (OR=1.194). The most frequent compound mutations were exist between g.13535 of the exon 7 and g.16137 of the exon 10 which represent 17(42%) of 40 compound mutations followed by tripled compound mutations between g.13518, g.13516 of exon 7 and g.16026 of exon 10 which represented as 12(30%) of compound mutations. Most of compound mutations were detected in G3 (18/40(45%)) and the most frequent mutations exist in G3 are the compound mutation between g.13535 of exon 7 and g.16137 of exon 10 and the triple compound mutation between g.13518, g.13516 of exon 7 and g.16026 of exon 10 which represented as 8/18(44.4%) and 5/18(27.8%) respectively. These results indicated that these two types of compound mutations could be have a role in transition of bladder carcinoma to stage 3 and could be useful as biomarker for this stage. The results also revealed that mutations g.13518+g.16024, g.13518+g.13516+g.16026 and g.13535+g.16137 were constitute as a risk factor in G3 than G2 (OR=1.364, 1.071 and 1.307 respectively).

Keywords: Bladder carcinoma, FGFR3, Compound mutation, cancer grads, Risk factor

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INTRODUCTION

Bladder cancer (BC) is the seventh most frequently diagnosed malignancy worldwide in men [1] and the most common cancer of the urinary tract [2]. Mutations in human TP53, FGFR3, erb-B, Myc and ras genes have been proposed as potential molecular markers in bladder tumor [3,4]. Among them, Fibroblast growth factor receptor 3 (FGFR3) has received much attention after their strong association with malignant proliferation due to many type of mutations [5,6]. Mutations with gene FGFR3 have been shown to lead to activation of the receptor FGFR3, which encodes a tyrosine kinase-linked cell surface receptor [7]. Activating FGFR3 mutations are more frequently identified in bladder carcinoma patients. The most common type of FGFR3 mutations are those in exons 9 and 10 [8]. Compound FGFR3 mutations have been detected within the same tumor tissues in some patients [9].

Compound FGFR3 mutations, defined as double or multiple mutations in the FGFR3 are frequently detected. Oncogenic mutations such as compound mutations are critical for cancer development and maintenance but its clinical significance is unclear [10]. The identification of such mutations has improved clinical outcomes of bladder carcinoma patients by incorporating tumor genetics into therapeutic decision making [11].

Activate mutations of FGFR3 were first described over 18 years ago [12, 13]. Subsequent larger studies established that FGFR3 mutations occur in around 50% of both lower and upper urinary tract tumors and these cluster in exons 7, 10, and 15 [14,15]. Mutations in exon 7 and 10 create a cysteine or glutamic acid residue in the proximal extracellular region of the receptor. The abnormal residues form either disulfide or hydrogen bonds between adjacent monomer receptors, favoring ligand-independent dimerization, trans activation, and signaling [16, 17]. These mutations could be induce a conformational change in the kinase domain resulting in ligand-independent receptor activation and signaling [18]. They have also been shown to alter FGFR3 cellular localization, inducing aberrant signaling from the endoplasmic reticulum [19].

There was a strong correlation between FGFR3 mutations and stage or grade. Mutations were restricted to Ta and T1 tumors with high or moderate differentiation and therefore, FGFR3 mutations are associated with noninvasive low malignant tumors or tumors with limited invasive potential [13,20,21]. The majority of mutations were found in low malignant tumors, whereas mutations in G3 tumors were very rare [22]. These results from different studies underline that FGFR3 mutations characterize tumors with favorable histological features [21,23,24].The aim of our study was to search simultaneously for mutations in FGFR3 gene in the BC patients and to evaluate their role as predictors of recurrence, progression, and survival.

MATERIALS AND METHODS

Ninety five Transitional cells carcinoma TCC and 50 apparently healthy subjects as control were included in this study. Patients and healthy samples were collected from Ghazi Al Hariri Hospital in Baghdad-Iraq. Patient age ranged from 30 to 86 years while control subjects ages ranged from 30 to 50 years. 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 [25]. DNA was extracted and purified using kits (Bioneer-South Korea). Regions from exon 7 and 10 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 primers, F 5' CGGCAGTGGCGGTGGTGGTG'3 and R 5' AGCACCGCCGTCTGGTTG '3 for exon 7 with 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 and primers were sent then 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.

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Statistical Analysis

The statistical analysis system- SAS (2012) [26] program was used to evaluate effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and in this study.

RESULTS

The present study examined the utility of FGFR3 compound mutations as prognostic markers in bladder carcinoma stages. FGFR3 mutations were detected in bladder cancer by several groups and described to be associated with recurrence and progression [3,4,6,7,11]. In our study, we found FGFR3 compound mutations in about 42.11% of all bladder carcinoma patients and 60% of them were detected in male patients. The results listed in table-1 revealed that the incidence rate of the FGFR3 mutations was significant (P> 0.05) in male patients than in females and constitute as a risk factor (OR=1.194). Such results were also detected by other research groups [1,2,7,27].

Table1: Patients and Mutations profile

Sex	No (%) of mutation	Chi-Square	O.R
Total mutation (%)	90/95 (94.74%)		
Male	57/95 (60%)	7.240 **	1.194
Female	38/95 (40%)		
* (P<0.05), ** (P<0.01).			

Table2 showed that among 90 bladder carcinoma patients with FGFR3 mutations, 40 (42.11%) of them were with compound mutations. The most frequent compound mutations were exist between g.13535 of the exon 7 and g.16137 of the exon 10 which represent 17(42%) of 40 compound mutations followed by tripled compound mutations between g.13518, g.13516 of exon 7 and g.16026 of exon 10 which represented as 12(30%) of compound mutations. The results also showed that 6 types of mutations from both exons were seen in these compound mutations (3 from exon 7 and 3 from exon 10) and the most frequent mutations exist in these 40 compound mutations were g.13518 of exon 7 which represent as 23(57.5%) and g.16026 of exon 10 which represent as 19(47.5%) (Table-2) which indicate that these FGFR3 two mutations are hotspot mutations associated with bladder carcinoma.

According to stage of carcinoma, the results of table-2 showed that most of compound mutations were detected in G3 (18/40(45%)) and the most frequent mutations exist in G3 are the compound mutation between g.13535 of exon 7 and g.16137 of exon 10 and the triple compound mutation between g.13518, g.13516 of exon 7 and g.16026 of exon 10 which represented as 8/18(44.4%) and 5/18(27.8%) respectively. These results indicated that these two types of compound mutations could be have a role in transition of bladder carcinoma to stage 3 and could be useful as biomarker for this stage.

The statistical analysis of these data revealed that no significant correlation between stages G1, G2 and mutations g.13518+g.16024, g.13535+g.16137 and between stages G2, G3 and mutation g.13518+g.16026. The incidence rate of g.13518+g.13516+16026 triple mutation was significant (P> 0.05) in G1 than G2 and in G3 than G2 but not constitute a risk factor (OR=0.663) while mutation g.13518+g.16026 constitute a risk factor (OR=1.644) in G2 than G1. Moreover, mutations g.13518+g.16024, g.13518+g.16026 and g.13535+g.16137 were constitute as a risk factor in G3 than G1 (OR=1.364, 1.644 and 1.095 respectively). The results also revealed that mutations g.13518+g.16024, g.13518+g.13516+g.16026 and g.13535+g.16137 were constitute as a risk factor in G3 than G2 (OR=1.364, 1.071 and 1.307 respectively).

The results of table-3 revealed that the involvement of exon 7 in compound mutations was significant (5/9(55.6%) than exon 10 (4/9(44.4%) and the incident rate of exon 7 mutation g.13518 in compound mutation was significant (23/40(57.5%)) than mutation g.16026 of exon 10 (19/40(47.5%)) with no significant risk factor of both mutations (OR=0.611, 0.608 respectively).

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Table 2: Profile of FGFR3 compound mutations and stages of bladder carcinoma

Number Cases	Exon 7	Exon 10	Stage		Chi-Square	O.R
			G1	G2		
4/40(10%)	g.13518	g.16024	1/4 (25%)	1/4 (25%)	0.00 NS	0.00
12/40(30%)	g.13518 g.13516	g.16026	4/12 (33%)	3/12 (25%)	4.29 *	0.663
7/40(18%)	g.13518	g.16026	1/7 (14%)	3/7 (43%)	9.17 **	1.644
17/40(42%)	g.13535	g.16137	5/17 (29%)	4/17 (24%)	1.82 NS	0.279
Total	5/9(55.6%)	4/9(44.4%)	11/40 (27.5%)	11/40 (27.5%)	0.00 NS	0.00
Number Cases	Exon 7	Exon 10	Stage		Chi-Square	O.R
			G1	G3		
4/40(10%)	g.13518	g.16024	1/4 (25%)	2/4 (50%)	8.63 **	1.364
12/40(30%)	g.13518 g.13516	g.16026	4/12 (33%)	5/12 (42%)	4.52 *	0.682
7/40(18%)	g.13518	g.16026	1/7 (14%)	3/7 (43%)	9.17 **	1.644
17/40(42%)	g.13535	g.16137	5/17 (29%)	8/17 (47%)	6.47 **	1.095
Total	5/9(55.6%)	4/9(44.4%)	11/40 (27.5%)	18/40 (45%)	6.68 **	1.178
Number Cases	Exon 7	Exon 10	Stage		Chi-Square	O.R
			G2	G3		
4/40(10%)	g.13518	g.16024	1/4 (25%)	2/4 (50%)	8.63 **	1.364
12/40(30%)	g.13518 g.13516	g.16026	3/12 (25%)	5/12 (42%)	6.25 **	1.071
7/40(18%)	g.13518	g.16026	3/7 (43%)	3/7 (43%)	0.00 NS	0.00
17/40(42%)	g.13535	g.16137	4/17 (24%)	8/17 (47%)	8.59 **	1.307
Total	5/9(55.6%)	4/9(44.4%)	11/40 (27.5%)	18/40 (45%)	6.68 **	1.178
* (P<0.05), ** (P<0.01).						

Table 3: The incident rate and involvement of FGFR3 exons in compound mutations

Mutation	No (%)	Chi-Square	O.R
% of exon 7 mutations exist in compound mutations	5/9(55.6%)	0.044 *	0.611
% of exon 10 mutations exist in compound mutations	4/9(44.4%)		
The incident rate of g.13518 mutation of exon 7 in compound mutations	23/40(57.5%)	0.043 *	0.608

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The incident rate of g.16026 mutation of exon 10 in compound mutations	**19/40(47.5%)**		
*** (P<0.05), ** (P<0.01)**			

DISCUSSION

The first study examining FGFR3 involvement in bladder tumors was conducted by Cappellen et al [12]. Since then, mutations in FGFR3 have been identified in several tumor types including bladder carcinoma, cervical carcinoma, and multiple myeloma [12,14,7]. Moreover, numerous studies have been performed to improve understand the potential role of mutant FGFR3 as an oncogenic factor [4,6,8] and as potential molecular markers in bladder tumor. Activating mutations of FGFR3 have been observed in up to 70% of non-muscle-invasive bladder tumors, while overexpression of a wild-type receptor, found in approximately 40% of tumors, has been correlated with more invasive disease [28].

In the current work, FGFR3 compound mutations were detected in 42.11% of all bladder carcinoma samples. The significant involvement of FGFR3 exon 7 in compound mutations in the current study was similar with that detected by previous studies which demonstrated that mutations in exon 7 revealed significant high FGFR3 than other exons [27,29].

Our results revealed that the involvement of exon 7 in compound mutations was significant than exon 10 and the incident rate of exon 7 mutation g.13518 in compound mutation was significant. FGFR3 point mutations are found almost exclusively in exons 7, 10 and 15 [29]. The most frequent and significant extracellular domain-activating mutations in the current study are g.13518 of exon 7 (57.5%) and g.16026 of exon 10 (47.5%), and other mutations occur at low frequencies. Occurrence of more than one mutation for FGFR3 has been found before [4,10,30,31] and is probably due to either tumor heterogeneity or the presence of multiple tumor clones in the bladder. The incidence rate of FGFR3 double and triple mutations which detected in the current study were significant (P> 0.05) in G3 than G2 and G1 and mostly constitute a risk factor. These results indicated that the four types of FGFR3 compound mutations were highly correlated with the development of bladder carcinoma. Previous studies also demonstrated that FGFR3 mutations are associated with grades and stages of bladder carcinoma [13,30,32]. This strong correlation with stages and grads were striking in all studies. The majority of mutations were found in low malignant tumors, whereas mutations in G3 tumors were very rare. These results from different studies underline that FGFR3 mutations characterize tumors with favorable histological features. Furthermore, in a study from van Rhijn et al. it was shown that the presence of an FGFR3 mutation is a strong indicator of superficial bladder tumors [13,14,20]. Hernandez et al. found FGFR3 mutations to be associated with a higher rate of recurrence [22]. Moreover, studies clearly showed that bladder cancer cases can be separated in genetically stable low malignant tumors with few genetic alterations and genetically unstable highly malignant tumors with multiple genomic aberrations [33]. Activating mutations in the FGFR3 gene have been reported in ≤75% of low-grade and low-stage BC, but are absent or rare in carcinoma in situ and MIBC [30,32]. The association between FGFR3 mutations and pathological phenotype has been well established, but the prognostic significance of FGFR3mutations in BC remains poorly defined [34]. The results presented in the present study in contrast with previous studies demonstrating that the presence of FGFR3mutations is significantly associated with low tumor grade [13,14,20,30].

CONCLUSION

FGFR3 compound mutations were detected in about 42.11% of all bladder carcinoma patients and 60% of them were detected in male patients. Mutations in exon 7 revealed significant high FGFR3 than exon 10. The incidence rate of FGFR3 double and triple mutations which detected in the current study were significant (P> 0.05) in G3 than G2 and G1 and mostly constitute a risk factor.

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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