Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(1): 95-101. **OPEN ACCESS**

The distribution of *FGFR3* mutations in bladder tumors of different stages

Sabah Bresam[1], Bashar Ouda Altae[2] and Abdul Hussein M. AlFaisal[3]

[1] Accommodation Department, University of Baghdad, Baghdad, **Iraq**.
[2] Hammurabi Medicine College, University of Babylon, Babylon, **Iraq**.
[3]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, **Iraq**.
.
***Correspondence:** kaiskassim@gmail.com **Accepted: 11Jan**. 2019 **Published online:** 20 Feb. 2019

Fibroblast growth factor receptor 3 (FGFR3) could represent a promising biomarker for bladder cancer. Current study consisted of 95 subjects with bladder cancer (Transitional cells carcinoma TCC) and 50 subjects control group. Patient age ranged from 30 to 86 years while control subjects ages ranged from 30 to 50 years. Mutations of FGFR3 genes were analyzed in 101 patients with transitional cell bladder cancer included 61(67%) male and 40(44%) female. 43 of 101 patients have mutations. 15(35.7%) of mutations shown in FGFR3 exon 7 and 27(64.3%) were in exon 10. These mutations affected codons g.13509, g.13501 of exon 7 and codons g.16021, g.16025 of exon 10. *FGFR3* mutations were observed in 13(%) Ta tumors, 10(%) T1, 8(%) T2 and 11(%) T3 of 42 mutations. The occurrence of *FGFR3* mutations with respect to tumor stage revealed the presence of a *FGFR3* mutation in low stage tumors than high-stage tumors. These results indicate that a significant correlation was found between *FGFR3* mutations and low grade.

Keywords: *FGFR3*, TCC, Ta,T1-T3, mutations

INTRODUCTION

Bladder cancer has a high recurrence rate of any cancer with the progress of the tumor to become more invasive (Jemal et al., 2013, Siegel et al., 2013). The major risk factors for bladder cancer varied from exposure to toxic chemicals, bladder inflammation to some adverse side-effects of medications (Burger et al., 2013, Chavan et al., 2014). The most important factors for bladder cancer comprise tobacco smoking, industrial exposure to potential carcinogens such as carbon black dust and aromatic amines, Drink for long periods of water contaminated with arsenic or chlorinated, and family history of concordant cancers (Mhawech-Fauceglia et al., 2006, Burger et al., 2013). Substantial evidence proved that exposing to toxic environmental factors could cause genetic alteration of some genes that play an important role in the regulation of cell division in bladder carcinoma initiation such as *FGFR3*, RB1, H-ras, K-ras,TP53, TSC1 genes (Guancial et al., 2014, Liu et al., 2014, AL-Faisal and Nafeh, 2015, AL-Faisal et al., 2015, Nicolas et al., 2015). Fibroblast growth factor receptor 3 (FGFR3) could represent a promising biomarker for bladder cancer (AL-Faisal and Bresam, 2015, AL-Faisal and Bresam, 2016). FGFR3 is a glycoprotein and belongs to the tyrosine kinase receptor family (Naski et al., 1996, Van Rhijn et al., 2001). Activation of FGFR3 by mutations leads to congenital anomalies such as a chondroplasia and thanatophoric dysplasia (Wilcox et al., 1998, Cappellen et al., 1999). Recently, it has been shown that somatic mutations of the *FGFR3* gene occur frequently in urothelial tumors of the bladder and less frequently in carcinomas of the cervix uteri, suggesting that *FGFR3* plays an oncogenic role

(Billerey et al., 2001). 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 (Hernandez et al., 2006, Mhawech-Fauceglia et al., 2006, Breyer et al., 2016).Mutations with gene FGFR3 have been shown to lead to activation of the receptor FGFR3, which encodes a tyrosine kinase-linked cell surface receptor, is mutated in up to 80% of low-grade Ta tumors (Alsheikh et al., 2001). FGFR3 mutati\ons are a common occurrence with low-grade non-invasive papillary urothelial bladder cancer, and it occurs at a much lower frequency in high-grade invasive bladder cancer (Mhawech-Fauceglia et al., 2006, Ji Yun et al., 2018). Somatic mutation gene FGFR3 with bladder tumors has been localized in exon 7, 9 and 15, occurring in about 75% of all cases and exist frequently in low grade and low stage tumors (Mhawech-Fauceglia et al., 2006). The aim of this study was to analyze the distribution of *FGFR3* mutations in bladder tumors of different grade and stage.

MATERIALS AND METHODS

The study consisted of 95 subjects with bladder cancer (Transitional cells carcinoma TCC) and 50 subjects control group. 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. Total genomic DNA was isolated from samples for molecular studies using genomic DNA purification kits (Bioneer-South Korea). 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 (Ji Yun et al., 2018)

Polymerase Chain Reaction-PCR for Exon 10 and exone 7

The exon 10 region of *FGFR3* was amplified by PCR using the primers, F 5' CAGGCCAGGCCTCAACGCCC '3 and R 5'AGGCCTGGCGGGCAGGCAGC '3 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 72^{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 2% agarose gel with a ladder (100 bp) and visualized. Exon 7 region of *FGFR3* was amplified by PCR 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) (Figure-1 and Figure-2) 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.

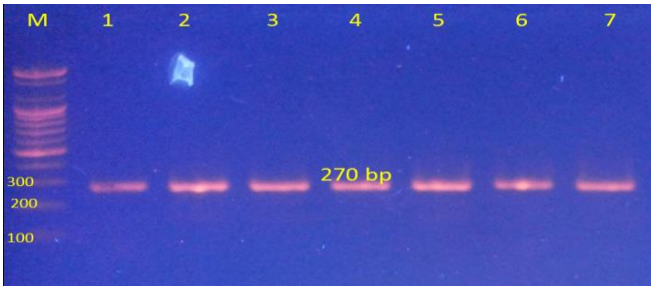

Figure 1. PCR products for FGFR3 exon 10 on agarose gel (2%) after electrophoresis for 1 hour at 100 volt. M: DNA ladder marker (100 bp) lane (1) to (5) for blood samples of bladder cancer patients.Lane (6) to (7) for blood samples of healthy controls.

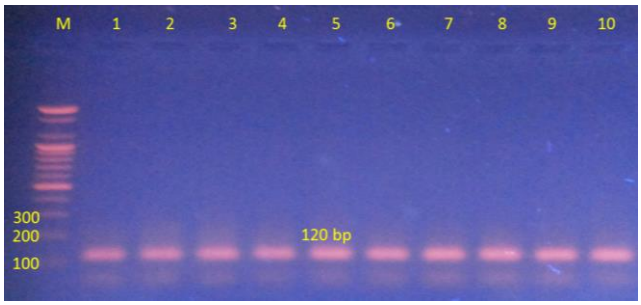

Figure 2. PCR products for FGFR3 exon 7 on agarose gel (2%) after electrophoresis for 1 hour at 100 volt. M: DNA ladder marker (100 bp) lane (1) to (7) for blood samples of bladder cancer patients. Lane (8) to (10) for blood samples of healthy controls.

RESULTS AND DISCUSSION

Mutations of FGFR3 genes were analyzed in 101 patients with transitional cell bladder cancer included 61(67%) male and 40(44%) female. 43 of 101 patients have mutations. 15(35.7%) of mutations shown in FGFR3 exon 7 and 27(64.3%) were in exon 10. These mutations affected codons g.13509, g.13501 of exon 7 and codons g.16021, g.16025 of exon 10 (Table 1)(Figure3).

The distribution of *FGFR3* mutations as a function of stage is shown in Table-2. *FGFR3* mutations were observed in 13(%) Ta tumors, 10(%) T1, 8(%) T2 and 11(%) T3 of 42 mutations. The occurrence of *FGFR3* mutations with respect to tumor stage revealed the presence of a *FGFR3* mutation in low stage tumors than high-stage tumors. This difference in mutation occurrence between tumor stages was significant and the difference between Ta and T1-T3 tumors was also significant (*P* , 0.001). While no difference in the occurrence of *FGFR3* mutations between pT1 and pT2–pT3 tumors. These results indicate that a significant correlation was found between *FGFR3* mutations and low grade. The distribution of *FGFR3* mutations according to histological grade and effect are shown in Table 2.

FGFR3 mutations were more frequently identified in low-grade tumors (62% *versus* 26% in high grade tumors), and non-invasive tumors (55% of Ta, 29% of T1 and 19% of T2) (Jebar et al., 2005). Lamy et al., (2006) reported the usefulness of *FGFR3* mutation analysis. A negative correlation of *FGFR3* mutation to tumor stage and cell grade was observed (Billerey et al., 2001).Van Rhijn et al., (2001) described a similar distribution of *FGFR3* mutation according to tumor stage and grade using the sequencing technique**.** Finally, in meta-analysis by Neuzillet et al., (2012) *FGFR3* mutation detection was performed using sequencing or SNaPshot with equivalent data: 65% of Ta and 70% of G1 tumors in contrast to 11% of T2 and 19% of G3 tumors.

The more frequent mutations among patients and among mutation types are mutations of exon 10 which represent 27(64.3%) of 42 mutations and mutation types g.16021 rs757013992 (14/42 (33.3%)) and g.16025 rs745683500 (13/42 (31%)) (Table 3). Analysis of the occurrence of *FGFR3* mutations with respect to tumor grade revealed the presence of a *FGFR3* mutation in 18 low grade tumors (62% of the cases) and in only 19 high-grade tumors (26% of the cases).

The high frequency of mutations that we found (42 of 101) confirmed that *FGFR3* mutations are a frequent event in bladder carcinomas. Clinical evidence and molecular studies have suggested that there are two different pathways of bladder carcinogenesis, generating two different non-invasive bladder tumors: carcinoma which often progress to T1 and T2–3 tumors, and non invasive bladder tumor-Ta, which rarely progress (Neuzillet et al.,2012, Elizabeth et al.,2014).The highly significant difference in the frequency of *FGFR3* mutations between Ta tumors and carcinoma T1-T3 (*P*, 0.0001) provides strong evidence that these two bladder cancers are different entities. The high occurrence of FGFR3 mutations in Ta tumors and their low occurrence in pT1 and pT2–3 tumors are identical to the model of bladder tumor progression in which the most common precursor of invasive tumors are T1-3. The mutated invasive tumors may arise from mutated Ta tumors that progress after acquire FGFR3 mutations during progression to invasive tumors. Specific point mutations in various domains of FGFR3 are associated with many autosomal dominant human disorders (Kaufman et al., 2009, Pouessel et al., 2018).

Table 1. Mutations, sex and histopathological Patients profile

Total (%)	Mutation %	*P* value	Stage	Mutations %	*P* value
	42/101 (41.6%)		Ta	13(30.9%)	0.097
Male	32/42(76.2%)	0.097	T1	10(23.8%)	
			T2	8 (19%)	
Female	10/ 42(23.8%)		T3	11(26.2%)	

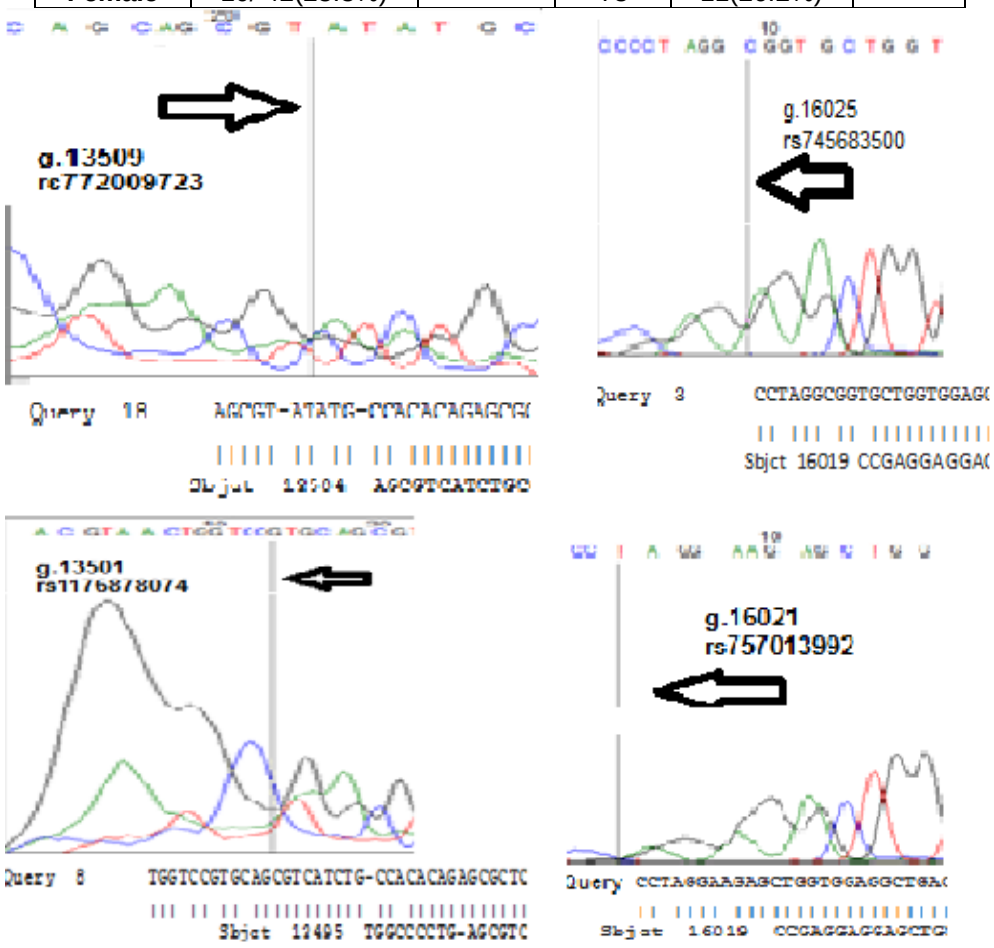

Figure 3. Location of *FGFR3* mutations.

Table 2. Mutations and their effect of the *FGFR3* gene exon 7 and 10 in bladder carcinoma

Exon	Codon	Mutation		Predicted effect	No. of tumor
7	g.13509	del C	TCA>TAT	Ser/Tyr	10
	g.13501	C>G	CCC>CCG	Pro/Pro	5
10	g.16021	G>T	GAG>TAG	Glu/Termination	15
	g.16025	A>C	GAG>GCG	Glu/Ala	12

Table 3. Distribution of exons 7 and 10 mutations of the *FGFR3* gene among bladder carcinoma stages.

Stage/ mutation	Exon 10 number+percentage		Exon 7 number+percentage	
	g.16021 rs 757013992	g.16025 rs 745683500	g.13509 rs772009723	g.13501 rs 1176878074
Ta	6/14 (42.9)	2/14 (14.3)	3/13(23.1)	2/16(12.5)
T1	2/9 (22.2)	5/11 (45.5)	2/14 (14.3)	1/13 (7.7)
T2	4/11 (36.3)	2/11 (18.2)	0	2/13(15.4)
T3	2/8 (25)	4/6 (66.7)	5/15(33.3)	0
Total	14/42 (33.3)	13/42 (31)	10/42 (23.8)	5/42 (11.9)

Several reports have demonstrated that these mutations lead to constitutive activation of the receptor (Kaufman et al., 2009,Pouessel et al., 2018). The identification in bladder and cervical carcinomas of somatic mutations of *FGFR3* identical to the activating mutations responsible for thanatophoric dysplasia and SADDAN8 (Neuzillet et al., 2012, Cappellen et al., 1999, Sethakorn and O'Donnell, 2016) suggested that FGFR3 plays an oncogenic role. This role of *FGFR3* mutations in development of bladder cancer was also proved by our results. This confirmed by others work who found that *FGFR3* genetic mutations were found in 62% of the studied tumors (Cappellen et al., 1999, Sethakorn and O'Donnell, 2016). In the present study, we identified *FGFR3* mutations in 41.6% of the 101 bladder tumors studied, confirming its implication in bladder carcinogenesis, as suggested by Pouessel et al., (2018) and confirmed more recently by Blanca et al., (2016).

CONCLUSION

The occurrence of *FGFR3* mutations with respect to tumor stage revealed the presence of a *FGFR3* mutation in low stage tumors than high-stage tumors. These results indicate that a significant correlation was found between *FGFR3* mutations and low grade**.**

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This work was supported by Genetic engineering department, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad.

AUTHOR CONTRIBUTIONS

AHMA contributed to the design of the experiments. All authors performed the experimental work, SB and BA carried out laboratory procedures. AHAA wrote the manuscript, all authors revised and approval the final version.

REFERENCES

AL-Faisal, A.H.M , Kradi, A.M. and Suleiman, A.A. 2015. Detection of codon 12/13 g.6262G>A mutation of *H-ras* gene in Iraqi bladder carcinoma patients. Iraqi J. of Biotechnology, 14(1), 44-52.

AL-Faisal, A.H.M. and Bresam, S. 2015. Detection of Three Novel Mutations in Exon 7 of FGFR3 Gene in Iraqi Patients with Bladder Cancer. Journal of Biology, Agriculture and Healthcare, 5, 218-225.

AL-Faisal, A.H.M. and Bresam, S. 2016. Association of Exon 9 *FGFR3* Mutations and Cancer Grads in Patients with Bladder Cancer. Iraqi Journal of Biotechnology, 15(2), 109-118.

AL-Faisal, A.H.M. and Nafeh, M.A. 2015.Detection of Five Novel Mutations in *K-ras* Gene for Iraqi Patients with Bladder Cancer. International Journal of Sciences: Basic and Applied Research, 24(5), 76-86.

Alsheikh A, Mohamedali Z. and Jones, E. 2001.

Comparison of the WHO/ISUP classification and cytokeratin 20 expression in predicting the behavior of low-grade papillary urothelial tumors, World/health organization/International society of urologic pathology. Mod. Pathol., 14, 267–72.

Billerey, C., Chopin, D., Aubriot-Lorton, M.H., Ricol, D., Gil, Diez de Medina, S. and Van Rhijn, B. 2001. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am. J. Pathol., 158, 1955–9.

Blanca, A., Requena, M.J., Alvarez, J., Cheng, L., Montironi, R. and Raspollini, M.R. 2016. FGFR3 and Cyclin D3 as urine biomarkers of bladder cancer recurrence. Biomark. Med., 10(3), 243–53.

Breyer, J., Gierth, M. and Shalekenov, S. 2016. Epithelial-mesenchymal transformation markers E cadherin and survivin predict progression of stage pTa urothelial bladder carcinoma. World J. Urol. 34, 709–716.

Burger, M., Catto, J.W. and Dalbagni, G. 2013. Epidemiology and risk factors of urothelial bladder cancer. Eur. Urol., 63, 234–41.

Cappellen, D., de Oliveira, C., Ricol, D. 1999. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat. Genet., 23, 18–20.

Chavan, S., Bray, F., Lortet-Tieulent, J. 2014. International variations in bladder cancer incidence and mortality. European Urology, 66, 59-73.

Guancial, E.A., Werner, L. and Bellmunt, J. 2014. FGFR3 expression in primary and metastatic urothelial carcinoma of the bladder. Cancer Med., 3(4), 835-44.

Hernandez, S., Lopez-Knowles, E., Lloreta, J., Kogevinas, M., Amoros, A., Tardon, A., Carrato, A., Serra, C., Malats, N. and Real, F.X. 2006. Prospective study of FGFR3 mutations as a prognostic factor in non muscle invasive urothelial bladder carcinomas. J. Clin. Oncol., 24, 3664–3671.

Jebar, A.H., Hurst, C.D. and Tomlinson, D.C. 2005. FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. Oncogene, 24, 5218–25.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., and Forman, D. 2011. Global cancer statistics. CA Cancer J. Clin., 61, 69–90.

Ji Yun, L., Kyung, K. and Hyun Hwan S. 2018. Molecular Characterization of Urothelial Carcinoma of the Bladder and Upper Urinary Tract. Transl. Oncol. J., 11(1), 37–42.

Kaufman, D.S., Shipley, W.U., Feldman, A.S. 2009. Bladder cancer. Lancet, 374, 239–49.

Lamy, A., Gobet, F., Laurent, M., Blanchard, F., Varin, C., Moulin, C., Andreou, A., Frebourg, T. and Pfister C. 2006. Molecular profiling of bladder tumors based on the detection of FGFR3 and TP53 mutations. J. Urol., 176, 2686-2689.

Liu, X., Zhang, W. Geng, D. and He, J. 2014. Clinical significance of fibroblast growth factor receptor-3 mutations in bladder cancer: a systematic review and meta-analysis. Genet. Mol. Res., 13, 1109–1120.

Mhawech-Fauceglia, P., Cheney, R.T. and Schwaller, J. 2006. Genetic alterations in urothelial bladder carcinoma: an updated review. Cancer, 106, 1205-1216.

Mhawech-Fauceglia, P., Cheney, R.T., Fischer, G., Beck, A. and Herrmann, F.R. 2006 b. FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer. Eur. J. Surg. Oncol., 32, 231-237.

Naski, M.C., Wang, Q., Xu, J., Ornitz, D.M. 1996. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Nat. Genet., 13, 233–237.

Neuzillet, Y., Paoletti, X., Ouerhani, S., Mongiat-Artus, P., Soliman, H., Sibony, M., Denoux, Y. *et al*. 2012. A Meta analysis of the relationship between *FGFR3* and *TP53* mutations in bladder cancer. PLos One, 7(12), e 48993.

Nicolas, N., Jerome, C., Geraldine, M. and Francoise G. 2015. *TP53* and *FGFR3* Gene Mutation Assessment in Urine: Pilot Study for Bladder Cancer Diagnosis., 35, 4915-4922.

Pouessel, D., Neuzillet, Y., Mertens, L.S. 2018. Tumor heterogeneity of fibroblast growth factor receptor3 (FGFR3) mutations in invasive bladder cancer: implications for perioperative anti-FGFR3 treatment. Annals of Oncology, 27, 1311–1316.

Sethakorn, N. and O'Donnell, P. H. 2016. Spectrum of genomic alterations in FGFR3: current appraisal of the potential role of FGFR3 in advanced urothelial carcinoma. BJU Int., 118, 681–691.

Siegel, R., Naishadham, D. and Jemal A. 2013. Cancer statistics, CA Cancer. J. Clin., 63, 11–30.

Van Rhijn, B.W., Lurkin, I., Radvanyi, F., Kirkels, W.J., Van der Kwast, T.H., Zwarthoff, E.C. 2001. The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of

superficial bladder cancer with low recurrence rate. Cancer Res., 61, 1265–1268.

Wilcox, W.R., Tavormina, P.L., Krakow, D., Kitoh, H., Lachman, R.S., Wasmuth, J.J., Thompson, L.M., Rimoin, D.L. 1998. Molecular, radiologic, and histopathologic correlations in thanatophoric dysplasia. Am. J. Med. Genet., 78, 274–281.