

SHORT REPORT

HRAS mutations in bladder cancer at an early age and the possible association with the Costello Syndrome

Willemien Beukers¹, Aleksander Hercegovic¹ and Ellen C Zwarthoff¹

Bladder tumours of patients <20 years have a low incidence of genetic aberrations typically found in tumours in older patients. In this study, we investigated oncogene mutations in patients with bladder cancer (BC) <20 years and compared them to older age groups. Interestingly, we observed a relatively high number of *HRAS* mutations in tumour from young patients. These mutations were also highly uncommon in BCs of older patients, ie, p.(Gly12Ser) and p.(Gly12Ala). Germline mutations in the *HRAS* gene, especially p.(Gly12Ser/Ala), cause Costello Syndrome (CS), a severe congenital disorder. Indeed, one of the patients had been diagnosed with CS. We hypothesized that some of the other patients might be mosaic for the *HRAS* mutation and therefore could express some of the clinical features of CS, like tumour predisposition. Hence, we isolated DNA from microdissected stroma and analysed it for *HRAS* mutations. In the CS patient and in patient X, the mutation was also highly expressed in normal stroma. We conclude that patient X is possibly mosaic for the *HRAS* mutation. These results suggest that mosaicism for oncogenic *HRAS* mutations may increase the risk for developing BC at a young age.

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INTRODUCTION

Bladder cancer (BC) is a disease of the elderly with a peak incidence in the sixth decade of life and only 1–2.4% of all cases present under the age of 40 years. BC in patients younger than 20 years is even more uncommon with reported incidence rates of only 0.1–0.4%.¹ Previous studies demonstrated that tumours of patients <20 years lack or have a much lower incidence of epigenetic and genetic aberrations typical of BC in elderly patients.²

Together with mutations in *FGFR3* and *PIK3CA*, mutations in the *RAS* genes are the most common mutations found in BC and up to 13% of all bladder tumours harbour a mutation in *HRAS*, *KRAS* or *NRAS*.³ There is limited data available regarding these oncogenic mutations in BC of patients <20 years. Therefore, we aimed to investigate *FGFR3*, *PIK3CA*, and *RAS* mutations in BC of patients <20 years and compared the results with the mutation status of a control group consisting of patients with BC >20 years.

MATERIALS AND METHODS

Fourteen patients <20 years (median 16.5; range 11–19 years) were included. In addition, three different control groups were investigated, ie, patients diagnosed with BC at 20–40 years ($n=43$), 40–60 years ($n=45$), and >60 years ($n=58$). Tumour tissue was obtained from a previous study.⁴ Normal surrounding stroma was microdissected ensuring >90% normal cells. Samples were used in accordance with the Dutch Code for 'Proper secondary use of human tissues' (<http://www.federa.org>). Tumour DNA was analysed for mutations in *HRAS*, *KRAS*, and *NRAS* with a multiplex Snapshot assay as described previously.⁵ This assay identifies 96% of the *RAS* mutations in BC, according to the Sanger Institute. In addition, the DNA was screened using the Snapshot analysis for mutations in *PIK3CA* and *FGFR3*.⁵ Reference sequences are shown in Supplementary File 1. Sequences and conditions of primers and probes are depicted in Supplementary Tables 1 and 2.

RESULTS

Table 1 shows the mutations found in the different age groups. *FGFR3* and *PIK3Ca* mutations are more abundant in tumours from patients over 40 years of age. Overall, the *FGFR3* mutation co-occurred with a mutation in *PIK3CA* in 24 cases, similar to previous observations. A mutation in the *FGFR3* gene together with a mutation in *KRAS* was found in only one patient. In the youngest age group, no mutations were found in *PIK3CA* and only one mutation was observed in the *FGFR3* gene. However, 4/14 (29%) of the young patients harboured a mutation in *HRAS*, a much higher percentage than in the older age groups (Table 1). Interestingly, not only did we observe this high number of *HRAS* mutations, but also 3/4 of these mutations were highly uncommon in BC, ie, c.34G>A (p.(Gly12Ser)) and c.35C>G (p.(Gly12Ala)). In addition, another p.(Gly12Ala) mutation was found in a bladder tumour of a 29-year-old patient from the control group. This patient was a known Costello Syndrome (CS) patient. The four patients with the uncommon p.(Gly12Ser/Ala) mutation all had a solitary TaG1 bladder tumour. In these four patients, *RAS* mutation analysis was performed on DNA isolated from microdissected tumour surrounding stroma. The areas of stroma used as normal surrounding tissue are depicted in Figure 1a. For both tumour and normal tissue, the results of the *RAS* mutation analysis are shown in Figure 1b. The stroma of patient #1, a 16-year-old boy with a p.(Gly12Ala) *HRAS* mutation in his bladder tumour, did also display this *HRAS* mutation. Likewise, although the signal was low, a p.(Gly12Ser) *HRAS* mutation was found in the normal stroma of patient #2, an 18-year-old boy with a p.(Gly12Ser) *HRAS* mutation in his bladder tumour. There was no mutation found in the normal tissue of patient #3, a 16-year-old male. In the 29-year-old female CS patient (Patient #4) from the control group, the p.(Gly12Ser) *HRAS* mutation was also found in the surrounding stroma.

¹Department of Pathology, Erasmus Medical Centre, Rotterdam, The Netherlands

*Correspondence: Dr EC Zwarthoff, Department of Pathology, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands. Tel: +31107043929; Fax: +31107044762; E-mail: e.zwarthoff@erasmusmc.nl

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Table 1 Distribution of oncogene mutations in bladder tumours of different age categories

Mutation	Age categories			
	<20 Years n = 14	20–40 Years n = 43	40–60 Years n = 45	> 60 Years n = 58
<i>Mutation</i>				
<i>HRAS</i>				
p.(Gly12Ala)	1			
p.(Gly12Ser)	2	1		
p.(Gly12Cys)			1	
p.(Gly13Arg)				1
p.(Gln61Leu)		2		1
p.(Gln61Arg)	1	1	1	1
<i>KRAS</i>				
p.(Gly12Cys)		1	2	1
p.(Gly12Asp)		1	1	1
p.(Gly12Val)		1	1	1
Wild type	10	36	39	52
<i>FGFR3</i>				
p.(Arg248Cys)		1	2	4
p.(Gly372Cys)			4	1
p.(Ser249Cys)	1	12	18	26
p.(Tyr375Cys)			8	5
p.(Arg248Cys) + p.(Ser249Cys)				1
Wild type	13	29	13	21
Unknown		1		
<i>PIK3CA</i>				
p.(Glu542Lys)		2	2	3
p.(Glu545Gly)			1	1
p.(Glu545Lys)		1	6	8
p.(His1047Arg)			2	2
p.(His1047Leu)				2
Wild type	13	40	34	42
Unknown	1			
<i>Co-occurrence of mutations</i>				
<i>FGFR3</i> and <i>PIK3CA</i> mutation		2	8	14
<i>FGFR3</i> and <i>KRAS</i> mutation			1	
<i>KRAS</i> and <i>PIK3CA</i> mutation		1	1	
<i>HRAS</i> and <i>PIK3CA</i> mutation				1
<i>FGFR3</i> , <i>KRAS</i> , and <i>PIK3CA</i> mutation			1	1

DISCUSSION

BC of patients <20 years seem to form a molecular distinct group compared with BC of the elderly. In this study, we screened BC of patients <20 years for oncogenic mutations that are typically found in BC of the elderly. As expected, no mutations were found in the *PIK3CA* gene, and only one mutation in the *FGFR3* gene. However, a high amount of uncommon p.(Gly12Ser/Ala) *HRAS* mutations were observed in the youngest age category.

Germline mutations in the *HRAS* gene are found in several developmental disorders, such as the CS. CS is a rare syndrome with only 300–400 reported cases worldwide. CS is caused by point mutations in the *HRAS* gene, mostly resulting in p.(Gly12Ser) or p.(Gly12Ala). This multisystem disorder causes mental retardation, distinctive facial appearance, cardiovascular abnormalities, skin and musculoskeletal abnormalities, and tumour predisposition. Most frequent malignancy found is the embryonal rhabdomyosarcoma, but also cases with BC have been described.⁶ It appeared that the 29-year-old patient with a p.(Gly12Ser) *HRAS* mutation in the bladder

tumour was known to have CS and therefore the p.(Gly12Ser) *HRAS* mutation could also be observed in the normal surrounding stroma. The mutation found in the normal tissue of patient #2 could be due to contamination of tumour cells, as the signal is mutant peak is low compared with the wild-type peak. However, the mutation signal in the normal tissue of patient #1 is too high to be caused by contamination of tumour cells during microdissection. This suggests that this patient could be mosaic for the p.(Gly12Ala) *HRAS* mutation and therefore might have some of the clinical features of CS, such as tumour predisposition. Blood or other patients' tissue samples would be necessary to strengthen this hypothesis. Unfortunately, because of ethical limitations in accessing clinical data, we were unable to obtain additional tissue or information on possible other CS-like features of patients #1 (and patient #2).

Mosaicism of *HRAS* mutations in relation with CS was described previously in a small number of patients.^{7,8} The phenotype of patients with mosaicism may differ and the clinical manifestations could occur along a spectrum depending on the affected tissue compartments.⁹

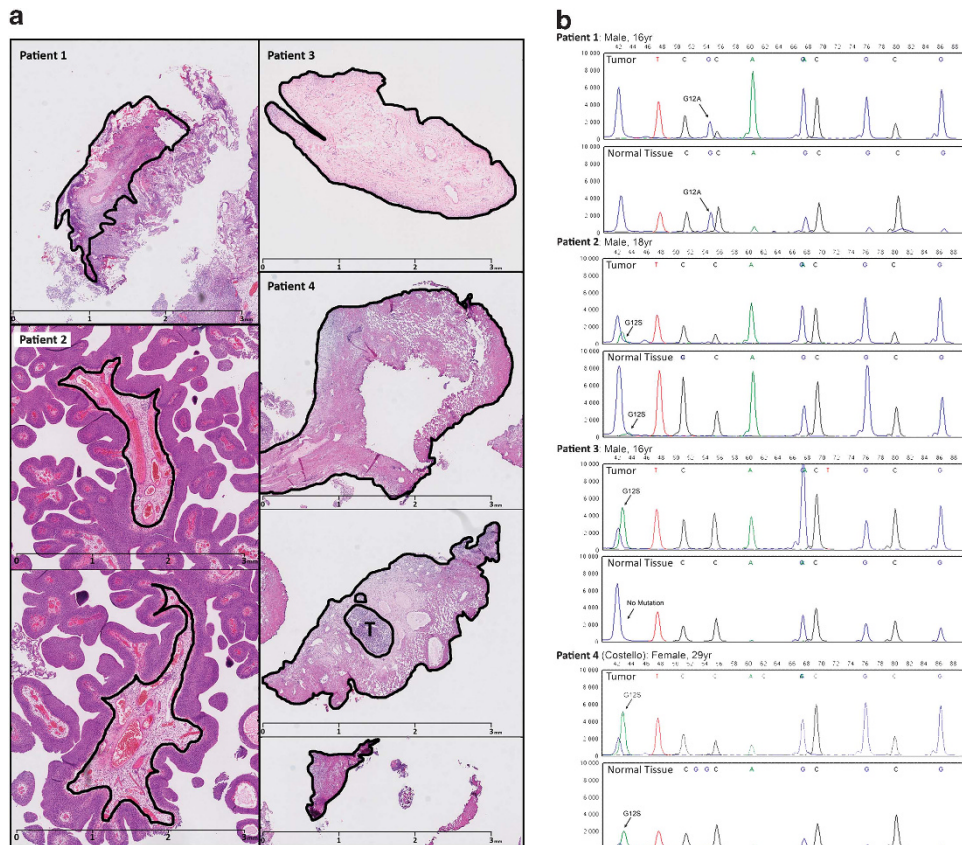


Figure 1 (a) Microdissected areas used as normal surrounding tissue in the Snapshot analysis. Microdissection ensured >90% normal stromal cells. Stroma from patient #1, 2, and 4 was derived from the same side as the bladder tumour. Stroma from patient #3 was obtained from random bladder biopsies. (b) RAS Snapshot analysis of tumour tissue and normal surrounding stroma of four patients. T, tumour tissue.

In a recently published case report, mutant *HRAS* mosaicism was also described in relation to BC. The publication described a patient with an extensive mosaicism for a *HRAS* p.(Gly12Ser) mutation. This 49-year-old man presented with widespread congenital epidermal nevi, multiple bladder tumours, and a BC lung metastasis. The *HRAS* p.(Gly12Ser) mutation was found in the epidermal nevi as well as in the multiple bladder tumours and lung metastasis.¹⁰ The authors concluded that these findings corroborate the theory that *RAS* mutations may occur during early embryogenesis and could contribute to carcinogenesis at later age. Our results contribute to this hypothesis and suggest that mosaicism for oncogenic *HRAS* mutations may even increase the risk for developing BC at very young age. Thus, *RAS* mutation analysis should be performed on bladder tumours of young patients and if positive, the patient should be screened for possible manifestations of CS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)