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DETECTION OF COPY NUMBER CHANGES OF 20q13.2 IN TRANSITIONAL CELL CARCINOMAS OF THE URINARY BLADDER - TISSUE MICROARRAY ANALYSIS

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Summary. *Gains and amplifications in 20q have been reported in a number of malignancies including bladder cancer by comparative genomic hybridization.*

In order to study the relation of the increased copy number of 20q13.2 with tumor phenotype in bladder cancer, we applied FISH on a tissue microarray.

Overall, the frequency of 20q13.2 alterations was 14.9%, including 9.6% gains and 5.3% amplifications.

20q13.2 amplifications were more frequent in pT2-4 than in pT1 tumors but the difference was not significant. Amplifications were not associated with the tumor grade.

Gains were significantly more frequent in pT2-4 than in pT1 tumors and were strongly associated with the tumor grade. These associations were observed also if both amplifications and gains were considered together.

In conclusion, our results suggest that increased copy number of 20q13.2 is associated with unfavorable tumor phenotype in bladder cancer which is mostly due to genetic gains rather than amplifications.

Key words: *20q13.2, tissue microchips, bladder cancer, fluorescence in situ hybridization*

Introduction

Gains and amplifications in 20q have been reported in a number of malignancies such as breast (1-3), colon (4), and stomach cancer (5) as well as in osteosarcomas (6) and chondrosarcomas (7). In breast cancer these changes have been reported to correlate with poor prognosis (8). This chromosomal region is believed to contain one or more genes that are overexpressed in several types of epithelial cancer. *AIB1,* a steroid receptor coactivator, and *BTAK*, a serine/threonine kinase, have been shown to be amplified and overexpressed in breast cancer (9, 10). The PTPN1 gene on 20q12 is a nonreceptor tyrosine phosphatase involved in growth regulation (11) and has been reported to be overexpressed in 72% of breast carcinomas (12). Another candidate genes are *MYB12* at 20q13, which encodes a transcription factor and plays a role in the cell progression (13), and CAS (cellular apoptosis susceptibility gene)(14).

Amplifications and gains in 20q12-qter have been found also in bladder cancer by comparative genomic hybridization (CGH)(15, 16). 20q+ was shown to be associated with the metastatic process (15) but not with muscle invasion (17, 18), although being more frequent in muscle-invasive (stage pT2-4) than in minimally invasive (stage pT1) tumors. In our previous study by CGH, however, 20q+ was significantly more often detected in pT2-4 than in pT1 bladder carcinomas (19).

In order to study the relation of the increased copy number of 20q with muscle invasion in bladder cancer, we applied FISH on tissue microarray. The tissue microarray technology (TMA) is especially suitable for the detection and estimation of rare events because it allows simultaneous in situ analysis of a large number of minute tissue samples, arranged together in an array (20, 21).

Materials and methods

Tissue microarray. A preexisting TMA containing 207 samples from primary urinary tract tumors from 207 patients was used (22). The slides of all tumors were reviewed by a pathologist and tumor stage and grade were defined according to UICC and WHO criteria (23, 24). Stage pT1 was defined by the presence of both unequivocal tumor invasion of the suburothelial stroma and tumor-free fragments of the muscular bladder wall. Only transitional cell carcinomas of the urinary bladder were included in this study (159 samples). Of this group, consisting of 159 patients, 133 were males and 26 females. The average age was 62 years (range from 30 to 84 years).

Fluorescence in situ hybridization (FISH). Prior to hybridization the slides were treated with Paraffin Pretreatment Reagent Kit (Vysis). FISH was performed using a locus-specific probe for ZNF217 (20q13.2) labeled in Sectrum Orange (Vysis, Cat #32-190016). De-

naturation of the DNA was carried out at 75°C for 10 minutes (probe mixture) and 5 minutes (slides). The probe mixture was applied to the slides and hybridized overnight in a moist chamber at 37°C. The posthybridization washes were performed as described in "LSI procedure" (Vysis). Slides were counterstained with DAPI in antifade. The presence of six or more gene signals in at least 50% of tumor cells was considered as an amplification while the presence of 3 or more, but less than six signals was recorded a gain.

Statistics. Contingency table analysis and Chi-square tests were used to study the relationship between tumor grade and stage, and locus copy number increase.

Results

20q13.2 copy number changes. A TMA including 159 transitional cell bladder tumor samples were analyzed by FISH for 20q13.2 copy number changes (amplifications and gains). FISH was successful in 59.1% of the tumors (94 samples). The frequency of 20q13.2 alterations was 14.9%: amplifications were found in 5 tumors (5.3%) while gains - in another 9 tumors (9.6%) (Table 1). None of the cases with amplification showed cluster amplification.

20q13.2 copy number increase and tumor phenotype. The relationship between 20q13.2 copy number increase and tumor phenotype is summarized in table 1. There were only two successfully analyzed pTa tumors which were therefore excluded from the analysis. The frequency of amplifications was higher in muscle invasive (pT2-4) than in minimally invasive (pT1) tumors but the difference was not statistically significant. Amplifications were not associated with tumor grade since their frequency even increased in G2 compared to G1, did not reach significance and, besides, amplification was not detected in G3 tumors.

Gains were more frequent than amplifications and they were associated with muscle invasion being significantly more rare in pT1 than in pT2-4 tumors ($p<0.05$). Gains were strongly associated with the tumor grade as well ($p<0.001$).

If considered together, amplifications and gains were associated both with tumor stage and grade ($p<0.05$).

Discussion

Gene amplifications are generally rare events in bladder cancer, most of them occurring in less than 10% of cases (25, 26, 27). High numbers of tumors, therefore, have to be analyzed in order to determine the frequency of amplifications and their significance for the disease. Tissue microarrays (TMAs) are optimally suited to determine associations between molecular marker and tumor phenotype or prognosis. In the present study we used a progression TMA of urinary tract tumors in order to determine the frequency of amplifications and gains of 20q13.2 and to evaluate their distribution in transitional cell carcinomas of the bladder from different stage and grade.

The frequency of amplifications was 5.3%. Amplification at 20q was reported in studies by comparative genomic hybridization (CGH) - 3.8% (1/26) in the study of Kalllioniemi et al. (15) and 2.2% (2/90) in the study of Richter et al. (18). The frequencies determined for individual oncogene amplifications in the different studies are strongly dependent on the method used for detection, the definition of amplification and on the number of tumors analyzed. This study is the first one with FISH for 20q13.2 on TMAs. The discrepancy of our results with literature is maybe due to the fact that CGH is less specific method than FISH in the study of individual loci.

In the literature, the cases with amplifications were only pT2-4 tumors while our analysis showed amplifications also in pT1 tumors. The frequency of amplifications did not increase significantly form pT1 to pT2-4 and was not associated with the tumor grade.

Similarly to the results of other authors 20q13.2 gains were markedly more frequent than amplifications (9.6%). Results with the less specific method CGH showed gains in pT1-4 tumors with higher frequency (20-30%) (17, 18, 28, 29).

The frequency of gains increased significantly in pT2-4 compared to pT1 tumors as well as with advanced tumor grade. The association of 20q+ with muscle invasion is in agreement with our previous study by CGH contrary to the findings of other authors (17, 18).

In conclusion, our results suggest that increased

Table 1. Frequency of amplifications and gains in 20q13.2 and tumor phenotype

		Tumors with successful hybridization (n)	Normal copy number (%)	Ampllification (%)	P[a]	Gain (%)	P[b]	Increased copy number (%)	P[c]
all		94	80 (85.1%)	5 (5.3%)		9 (9.6%)		14 (14.9%)	
stage	pTa*	2	2 (100.0%)	0 (0.0%)		0 (0.0%)		0 (0.0%)	
	pT1	63	57 (90.4%)	3 (4.8%)	0.6748	3 (4.8%)	0.0169	6 (9.6%)	0.0250
	pT2-4	29	21 (72.4%)	2 (6.9%)		6 (20.7%)		8 (27.6%)	
grade	G1	37	34 (91.9%)	2 (5.4%)	0.6291	1 (2.7%)	0.0006	3 (8.1%)	0.0288
	G2	44	38 (86.4%)	3 (6.8%)		3 (6.8%)		6 (13.6%)	
	G3	13	8 (61.5%)	0 (0.0%)		5 (38.5%)		5 (38.5%)	

[a] amplified vs non-amplified tumors
[b] tumors with gains vs tumors with no gains
[c] tumors with increased copy number of the locus vs normal copy number tumors

copy number of 20q13.2 is associated with unfavorable tumor phenotype in bladder cancer which is mostly due to genetic gains rather than amplifications.

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