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Homozygous losses detected by array comparative genomic hybridization in multiplex urothelial carcinomas of the bladder

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Urothelial carcinomas (UCs) may present at first as a solitary or multifocal neoplasm. We applied high resolution array comparative genomic hybridization to 24 solitary and 32 multiplex UCs and used the hidden Markov model algorithm to identify the copy number changes at the probe level. Copy number losses and homozygous deletions at the chromosome 9p region affecting the *CDKN2A* and *MTAP* genes were the most frequent alterations in both groups of tumors. We have delineated two new tumor suppressor gene regions at chromosome 9p that harbor the *PTPRD* and *BNC2* genes. Copy number losses at chromosomal regions 2q, 8p, and 18p occurred preferentially in solitary UCs, whereas multiplex UCs displayed loss of large chromosomal regions at 9q, 10q, 11q, 18q, and 21q. Homozygous deletions harboring loci of cell adhesion genes such as claudins, desmocollins, and desmogleins were seen exclusively in multiplex UCs. Amplifications occurred only in invasive G3 UCs irrespective of staging. Our study suggests that solitary and multiplex UCs may have divergent genetic pathways. The biallelic inactivation of cellular adhesion genes by homozygous deletions in multiplex UCs may explain the frequent intravesical spreading of tumor cells.

Keywords urothelial carcinoma, multiplex tumors, array comparative genomic hybridization, homozygous loss, cell adhesion

Urothelial carcinomas (UCs) of the urinary bladder comprise biologically and morphologically heterogeneous groups of neoplasms. Approximately 75% of UCs are non–muscle-invasive (NMI) Ta, T1 tumors or carcinoma in situ with a good prognosis. The vast majority of NMI-UCs present as a solitary tumor, but occasionally multiplex cancers are observed at diagnosis. Frequent recurrence is a characteristic biological behavior of NMI-UCs and, in the case of multiplex growth, the probability of future recurrences increases to as much as 78% (1). Therefore, NMI-UCs, which make up 70–80% of UC cases, require repeated cystoscopic observation for detection and resection of recurrent tumors over many years. Although NMI-UCs generally have a good prognosis, up to 40% of them may progress to a muscle-invasive (MI) tumor during the course of the disease. Therefore, the synchronous or asynchronous growth of multiplex tumors remains a major problem in the clinical management of NMI-UCs. Histological analysis of tumors at the first observation may not help to estimate the rate of recurrences.

Genetic studies have indicated that most synchronous or asynchronous multiplex UCs are of monoclonal origin corresponding to intravesical homing of desquamated tumor cells (2). The development of polyclonal UCs has been explained by the field effect due to a high concentration of carcinogenic agents (3). Several studies have been performed on UCs to find genetic changes of biological importance (for a review, see the Knowles and Hurst article (4)). The most frequent genetic alterations include copy number losses of chromosome 9p (*CDKN2A* and *CDKN2B*) and of 9q (*PTCH, DBC1,* and *TSC1*) as well as mutation of the *TP53* and *FGFR3* genes (5). Based on the genetic data, molecular pathways of UC development indicating the biological behavior of tumors have been proposed (4–7). Why multiplex tumors arise

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synchronously or asynchronously in some patients and not in others is not yet known. To try to answer this question, we have analyzed 24 solitary and 32 multiplex UCs, including NMI and MI tumors, by 60K array comparative genomic hybridization (aCGH).

Materials and methods

Patients and tumor samples

Fresh tumor tissues were obtained by transurethral resection or radical cystectomy at the Departments of Urology, Philipps University of Marburg and Mannheim Clinic, Ruprecht-Karls University of Heidelberg. The use of material for this study was approved by the ethics commission of the University of Heidelberg. A part of the tumor tissue was immediately snap-frozen in liquid nitrogen and stored at –80°C. All histological slides were reevaluated according to the World Health Organization (WHO) classification by one of the authors (G.K.). Pertinent pathological data are presented in Table 1. Grading and staging was performed according to the WHO classification (8). Tumors having no preoperative clinical report and no recurrence within 3 years of follow-up were designated solitary UCs. Tumors that presented with multilocular appearance at first diagnosis or that showed recurrences during the course of follow-up were designated multiplex UCs. The monoclonal origin of multiplex UCs has been previously established by microsatellite analysis (not yet published).

DNA extraction and aCGH

Frozen tumor samples were placed in plastic Petri dishes, covered with 2 mL Tris-EDTA (TE)9 buffer, and allowed to thaw. The tumor cells were then carefully scraped or pushed out to separate them from stromal tissue under an inverted microscope by one of the authors (G.K.). Tumor cells were resuspended in 3–5 mL TE9 buffer with 1% SDS and 0.2 mg/mL proteinase K and were incubated for 5 hours at 55°C. DNA was precipitated with ethanol after phenol-chloroform extraction and dissolved in TE buffer. Normal control DNA was

Table 1 Pertinent clinicopathologic and genetic data of multiplex and solitary UCs

Patient	Age, y/Sex	No. of tumors	TNMG stage	Genomic change
1	75/F	Multiplex (6)	pTa, G1	L,G,HL,*
2	67/M	Multiplex (2)	pT1, G2	L,G
3a[a]	49/M	Multiplex (3)	pTa, G1	L,G
3b[b]	49/M	Multiplex (4)	pTa, G2 (+CIS)	L,G
4a[c]	88/M	Multiplex (4)	pTa, G1	L,G,HL,*
4b[d]	88/M	Multiplex (5)	pTa, G2	L,G,HL,*
5	66/M	Multiplex (8)	pT2, G3	L,G,HL,*
6	59/M	Solitary	pT3b, G3	L,G,A
7	68/M	Solitary	pT1, G2	G
8	64/F	Solitary	CIS	G
9	75/M	Solitary	pT2, G3	L,G,A
10	71/M	Solitary	pTa, G1	no
11	70/M	Solitary	pT3, G3	L,G,A,*
12	66/M	Solitary	pTa, G1	no
13	57/M	Solitary	pT3, G3	L,G,A,*
14	73/M	Solitary	pTa, G1	L,G
15	71/F	Solitary	pT2a, G3	L,G,A
16	81/M	Solitary	pTa, G2	no
17	61/M	Solitary	pT1, G3	L,G,A
18	77/M	Solitary	pT1, G2	no
19	74/M	Solitary	pT3b, G3	L,G,A
20	74/M	Solitary	pTa, G1	no
21	65/M	Solitary	pTa, G1	no
22	81/M	Solitary	pT1, G3	L,G,*
23	58/M	Solitary	pT1, G2	L
24	68/F	Solitary	pT1, G3	L,G,*
25	73/M	Solitary	pTa, G1	L,G,*
26	58/M	Solitary	pTa, G1	no
27	68/M	Solitary	pTa, G2	no
28	72/M	Solitary	pT1, G3	L,G,A
29	64/M	Solitary	pTa, G1	no

Abbreviations: A, amplification; CIS, carcinoma in situ; G, gain; HL, homozygous loss; L, heterozygous loss; *, homozygous loss at chromosome 9p.

[a] 3a, multifocal UC with recurrence.

[b] Patient 3 recurrence detected 2 mo later.

[c] 4a, multifocal UC with recurrence.

[d] Patient 4 recurrence detected 29 mo later.