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Original Article

Comparison of structural genetics of non-schistosoma-associated squamous cell carcinoma of the urinary bladder

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Abstract: Little is known about genetic changes in squamous differentiation of non-schistosomiasis-associated bladder cancer. Therefore, we investigated pure squamous cell carcinomas (SqCC), squamous parts of mixed urothelial carcinomas with squamous differentiation (MIX) and mere urothelial cancers (UC) for structural genetic differences. Tissue microarray slides (n = 29 SqCC, n = 35 MIX and n = 23 UC) were analyzed by ZytoLight SPEC p16/CEN3/7/17 Quadruple Color Probe fluorescence-in-situ-hybridization (FISH) and DNA was investigated by comparative genomic hybridization (CGH) (n = 35 SqCCs, n = 40 MIX and n = 36 UC). By FISH the mean number of polysomic cells was lowest in SqCC (CEN3 P = 0.0498, CEN17 P = 0.0009). A slight tendency of lower copy numbers of chromosomes 3, 7 and 17 and higher numbers of the p16-locus in SqCC (P = 0.45) indicated less aneuploid tumor cells in SqCC compared to MIX and UC. In CGH SqCC showed the lowest mean number of aberrations per tumor (SqCC 5.37 changes, MIX 6.75 and UC 7.64; P = 0.1754). Significant differences between the three groups were found for loss of chromosome 3p (P = 0.004), 6q (P = 0.028), 11p (P = 0.024) and gains of 5p (P = 0.020). Loss of 3p was more frequent in SqCC (51.4%) than in MIX (37.5%) or UC (13.9%). To conclude, SqCCs show less polysomy and genetic alterations than MIX and UC. Loss of 3p is more frequent in SqCC but there are no absolute specific alterations for each tumor group. Squamous parts of mixed tumors show similar alterations than UC and should be considered as further development of UC, while pure SqCC seem to be a separate tumor group.

Keywords: Bladder cancer, urothelial carcinoma, urothelial carcinoma with squamous differentiation, squamous cell carcinoma, FISH, CGH

Introduction

Pure squamous cell carcinoma (SqCC) of the urinary bladder comprises less than 5% of all bladder cancers [1-3]. However, it is more frequent in certain subgroups like patients with chronic inflammation, urinary tract calculi or chronic bladder outlet obstruction, and in patients with spinal cord injury [1, 2, 4]. Additionally, higher numbers of SqCC were reported in regions with endemic schistosomiasis [5, 6]. According to the 2004 World Health Organization classification of bladder cancer the diagnosis of squamous cell carcinoma is reserved for tumors with complete squamous differentiation without any other tumor component including urothelial carcinoma in situ [3]. Indeed, histologically identifiable focal squamous differentiation in high grade urothelial carcinoma is a quite frequent finding reported in literature in up to 50% of tumors [7]. Additionally, recent advances in whole genome expression profiling revealed a subtyping of muscle invasive bladder cancer (MIBC) into breast cancer-like basal and luminal types, with identification of a “basal/squamous-like” subtype [8-10]. These cancers express higher levels of high molecular weight keratins (KRT5, KRT6, KRT14) and EGFR [8, 9], show an invasive/metastatic phenotype with shorter surviv-

Table 1. Characteristics of the multicentric tumor cohort (classified according to the results of Gaisa et al., 2011 [15])

ID	classification	center	year of sampling	sex	age	tumor grade	pT	pN	L	V	R
1	MIX	Aachen	2004	M	77	3	2	0	0	0	0
2	UC	Aachen	2005	M	72	3	2	0	0	0	0
3	SqCC	Aachen	2003	F	47	2	3	0	N/A	N/A	0
5	SqCC	Aachen	2005	M	78	3	3	N/A	N/A	N/A	N/A
7	MIX	Aachen	2006	F	66	3	3	0	N/A	1	0
10	SqCC	Aachen	2005	F	81	3	4	0	N/A	1	1
11	SqCC	Aachen	2006	F	63	3	3	N/A	N/A	N/A	0
12	MIX	Aachen	2006	M	53	3	4b	2	1	N/A	0
22	UC	Aachen	2004	M	64	2	3	0	1	0	0
24	UC	Aachen	2006	M	77	3	3b	2	1	1	0
29	SqCC	Aachen	2004	F	82	3	2	N/A	N/A	N/A	N/A
30	UC	Aachen	2005	M	58	3	2	0	0	0	0
32	UC	Aachen	2006	F	84	3	2b	0	1	1	0
33	UC	Aachen	2005	M	60	3	3	0	0	0	0
34	UC	Aachen	2008	F	56	3	4a	1	0	0	x
35	UC	Aachen	2008	M	82	3	2	N/A	N/A	N/A	N/A
36	MIX	Aachen	2004	F	35	3	3	N/A	0	0	1
39	SqCC	Aachen	2006	F	57	2	3	0	0	0	0
41	SqCC	München	2008	F	63	3	3a	0	1	1	0
43	UC	Aachen	2003	M	77	2	3	0	1	0	0
44	UC	Aachen	2006	M	76	3	3a	2	1	1	1
45	UC	Aachen	2009	M	78	3	4a	1	1	1	1
46	UC	Aachen	2006	M	94	3	3a	N/A	0	1	0
47	UC	Aachen	2004	M	48	3	3	0	0	0	0
56	MIX	München	2008	F	47	2	2	0	0	0	0
62	MIX	Aachen	2005	M	58	3	3	0	0	0	0
66	UC	Aachen	2007	F	76	3	3	N/A	0	1	0
70	UC	Aachen	2009	M	72	3	2b	0	0	0	0
71	UC	Aachen	2005	M	74	3	2b	0	0	0	0
77	MIX	Aachen	2005	M	60	3	3	0	0	0	0
38	UC	Aachen	2005	M	60	3	3	0	0	0	0
78	UC	Aachen	2006	M	70	3	4	3	1	0	1
80	MIX	Aachen	2003	M	77	2	3	0	1	0	0
88	MIX	Aachen	2007	F	76	3	3	N/A	0	1	0
89	MIX	München	2007	F	71	3	3	N/A	0	0	0
90	MIX	Aachen	2008	F	70	3	3	0	0	0	0
91	SqCC	Aachen	2003	F	77	3	3	N/A	N/A	N/A	N/A
98	SqCC	Regensburg	2007	F	79	3	2	N/A	N/A	N/A	N/A
102	SqCC	Regensburg	2007	F	53	3	2	N/A	N/A	N/A	N/A
107	SqCC	Regensburg	1999	F	76	2	2	N/A	N/A	N/A	0
110	MIX	Regensburg	2007	F	76	3	2	N/A	N/A	N/A	N/A
113	MIX	Regensburg	1994	F	71	2	2	N/A	N/A	N/A	N/A
117	SqCC	Regensburg	2005	M	49	3	3b	0	0	0	0
120	MIX	Regensburg	2004	M	75	3	2	N/A	N/A	N/A	N/A
121	SqCC	Regensburg	2007	M	53	3	3	0	0	0	0

125	MIX	Regensburg	2002	F	76	3	3	0	N/A	N/A	0
129	MIX	Regensburg	2007	F	80	3	3a	1	1	1	0
132	SqCC	Regensburg	2001	M	80	3	4	N/A	N/A	N/A	N/A
135	SqCC	Regensburg	2001	F	57	3	3	0	N/A	N/A	1
136	SqCC	Dresden	2003	M	82	3	3a	0	N/A	N/A	0
138	MIX	Dresden	2006	F	81	3	3b	0	0	0	1
141	MIX	Dresden	2006	F	87	3	2a	0	N/A	N/A	0
143	UC	Dresden	2005	M	71	3	3b	0	1	1	0
146	MIX	Dresden	2004	F	68	3	3a	0	N/A	1	0
148	MIX	Dresden	2004	F	68	3	3b	2	1	0	0
150	MIX	Dresden	2005	M	68	2	3a	0	1	0	N/A
152	MIX	Dresden	2005	M	82	3	3b	N/A	1	1	0
154	MIX	Dresden	2006	F	84	3	3b	1	1	0	0
156	MIX	Dresden	2006	M	52	3	3a	0	0	0	0
158	MIX	Dresden	2001	F	80	2	3a	0	N/A	N/A	0
160	MIX	Dresden	2002	M	78	3	3b	0	N/A	N/A	0
162	MIX	Dresden	2002	M	61	3	3a	0	N/A	N/A	0
163	MIX	Dresden	2004	F	80	3	3b	2	1	0	0
167	SqCC	Münster	2002	F	65	2	4	0	N/A	N/A	0
170	SqCC	Münster	2003	M	58	2	3	2	N/A	N/A	1
173	MIX	Münster	2003	F	62	3	3	N/A	N/A	N/A	N/A
179	UC	Münster	2003	M	68	2	2	0	N/A	N/A	N/A
180	MIX	Münster	2003	M	68	2	2	0	N/A	N/A	N/A
182	SqCC	Münster	2003	M	75	2	4	0	N/A	1	0
186	MIX	Münster	2003	F	34	3	3b	0	N/A	N/A	0
189	SqCC	Münster	2003	F	43	2	3a	0	N/A	N/A	0
195	MIX	Münster	2003	M	65	3	3a	1	N/A	N/A	0
197	SqCC	Münster	1993	M	68	2	3b	0	N/A	N/A	N/A
199	SqCC	Münster	1995	M	60	3	3b	N/A	N/A	N/A	0
201	MIX	Münster	1996	M	60	3	3b	0	1	N/A	0
206	SqCC	Münster	1998	M	50	3	4b	N/A	N/A	N/A	1
208	SqCC	Münster	1999	M	69	3	3b	0	N/A	N/A	N/A
210	SqCC	Münster	1999	M	34	3	3b	1	N/A	N/A	0
212	MIX	Münster	1999	F	68	3	3b	N/A	N/A	N/A	0
214	MIX	Münster	1999	M	59	3	3a	0	N/A	N/A	0
217	MIX	Münster	1999	F	74	3	4a	0	N/A	N/A	N/A
220	SqCC	Münster	2000	F	61	2	3b	N/A	N/A	N/A	0
222	MIX	Münster	2002	M	74	3	3b	N/A	N/A	N/A	0
224	SqCC	Münster	2002	F	62	3	3a	0	N/A	N/A	0
226	SqCC	Münster	2002	M	75	2	3a	2	1	1	0
229	SqCC	Münster	2002	M	63	2	2b	0	N/A	N/A	0
231	SqCC	München	2005	M	70	3	3b	0	N/A	N/A	N/A
233	MIX	München	2005	F	88	3	3a	0	N/A	N/A	N/A
236	SqCC	München	2006	F	64	2	3a	N/A	1	N/A	N/A
238	SqCC	München	2006	M	62	3	2b	0	N/A	N/A	0
240	SqCC	München	2007	M	55	2	3a	0	N/A	N/A	0
242	SqCC	München	2007	F	58	2	2b	0	0	0	0
247	MIX	Aachen	2008	F	79	3	4	0	0	1	0
252	MIX	Aachen	2009	F	76	3	3	N/A	1	1	1

256	MIX	Aachen	2005	M	72	3	3	0	1	1	0
259	SqCC	Aachen	2008	F	54	3	4	0	N/A	0	N/A
J60	UC	Jena	1996	M	68	3	2	N/A	N/A	N/A	N/A
J66	UC	Jena	1997	M	56	3	3	N/A	N/A	N/A	N/A
J101	UC	Jena	2001	M	67	3	3	2	N/A	N/A	N/A
J108	UC	Jena	1997	M	58	3	3	N/A	N/A	N/A	N/A
J135	UC	Jena	1996	W	82	3	2	N/A	N/A	N/A	N/A
J139	UC	Jena	1996	M	57	2	3	N/A	N/A	N/A	N/A
J143	UC	Jena	1996	M	69	3	3	N/A	N/A	N/A	N/A
J178	UC	Jena	1997	M	72	3	2	N/A	N/A	N/A	N/A
J223	UC	Jena	1996	W	69	3	2	N/A	N/A	N/A	N/A
J224	UC	Jena	1996	M	71	3	2	N/A	N/A	N/A	N/A
J228	UC	Jena	1996	M	66	3	2	N/A	N/A	N/A	N/A
J311	UC	Jena	1999	M	58	3	2	N/A	N/A	N/A	N/A
J381	UC	Jena	1997	M	66	3	3	N/A	N/A	N/A	N/A
J578	UC	Jena	1997	M	66	3	2	2	N/A	N/A	N/A

ID = identification, SqCC = pure squamous cell carcinoma of the bladder, MIX = urothelial carcinoma with squamous differentiation, UC = urothelial carcinoma, F = female, M = male, N/A = not available.

al but are indifferent from conventional high grade urothelial carcinoma on hematoxylin eosin-based routine histological evaluation. In this context, there is a need to better understand mere squamous differentiation and partial squamous differentiation especially in non-schistosoma-associated bladder cancers, to draw further conclusions and consequences of a "basal/squamous-like" subtype. However, previous studies on genetic aberrations in SqCC either focused on schistosomiasis-associated tumors [11, 12], hardly separated schistosomiasis-associated and non-schistosomiasis-associated SqCC or compared SqCC and squamous parts of mixed urothelial/squamous cancers in single cases only [13]. The most comprehensive study of structural genetic data on schistosoma- and non-schistosoma-associated SqCC and UC by El-Rifai et al. suggested different genetic pathways for these tumors due to varying gains and losses of chromosomes [14], but a detailed analysis of non-schistosoma-associated SqCC and the squamous part of mixed urothelial/squamous cancers (MIX) is also lacking. In a previous study our workgroup has evaluated squamous differentiation in bladder cancers [15], and precisely immunophenotyped a cohort of patients with non-schistosomiasis-associated pure SqCC of the urinary bladder and mixed urothelial and squamous cancers. Given the paraffin material and histopathological data of this cohort, the aim of the study presented here was to evaluate the structural genetic changes of the three bladder cancer subtypes non-schistosomiasis-associated squamous carcinoma of the bladder (SqCC), mixed urothelial carcinoma with partial squamous differentiation (MIX) and pure urothelial carcinoma (UC) by fluorescence in situ hybridization and comparative genomic hybridization.

Materials and methods

Specimens and preparations

Retrospectively diagnostic formalin-fixed paraffin tissues (FFPE) from six Institutes of Pathology in Germany were collected. Cases were recruited between 1993 and 2009. In total we collected n = 35 pure squamous cell carcinoma (SqCC) and n = 40 mixed UC/SqCC (MIX). In a prior study of our workgroup tissue microarrays (TMAs) of these samples have been constructed and immunohistochemically evaluated [15]. For analysis of MIX tumors we used the squamous cell tumor part (completely positive for KRT5/6 and KRT5/14, but negative for KRT20) [15]. Additionally, n = 36 urothelial carcinomas (UC) from Aachen and Jena served as a control group. The age of the tissue blocks as well as the formalin concentrations, fixation times and paraffin composition among the samples were highly variable. An overview of cases is shown in **Table 1**.

Approval of the local ethics committee for retrospective use of diagnostic FFPE tissue was

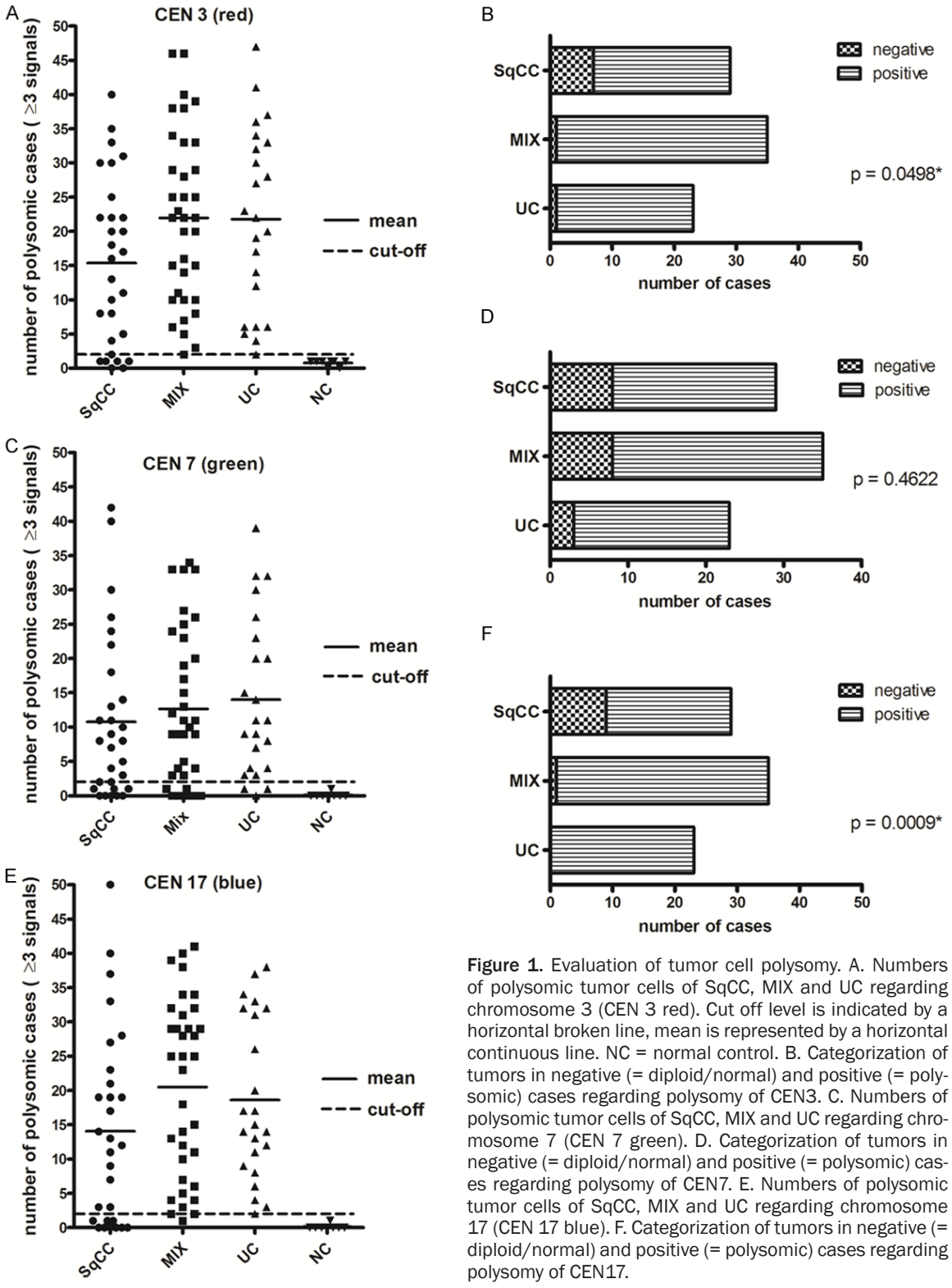

Figure 1. Evaluation of tumor cell polysomy. A. Numbers of polysomic tumor cells of SqCC, MIX and UC regarding chromosome 3 (CEN 3 red). Cut off level is indicated by a horizontal broken line, mean is represented by a horizontal continuous line. NC = normal control. B. Categorization of tumors in negative (= diploid/normal) and positive (= polysomic) cases regarding polysomy of CEN3. C. Numbers of polysomic tumor cells of SqCC, MIX and UC regarding chromosome 7 (CEN 7 green). D. Categorization of tumors in negative (= diploid/normal) and positive (= polysomic) cases regarding polysomy of CEN7. E. Numbers of polysomic tumor cells of SqCC, MIX and UC regarding chromosome 17 (CEN 17 blue). F. Categorization of tumors in negative (= diploid/normal) and positive (= polysomic) cases regarding polysomy of CEN17.

obtained (RWTH Aachen EK 9/12). Paraffin slides of TMAs were used for FISH, and DNA was extracted from paraffin slides of the residual tissue blocks after careful manual microdissection of tumor areas under a stereomicroscope using standard QiAamp™ DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's orders.

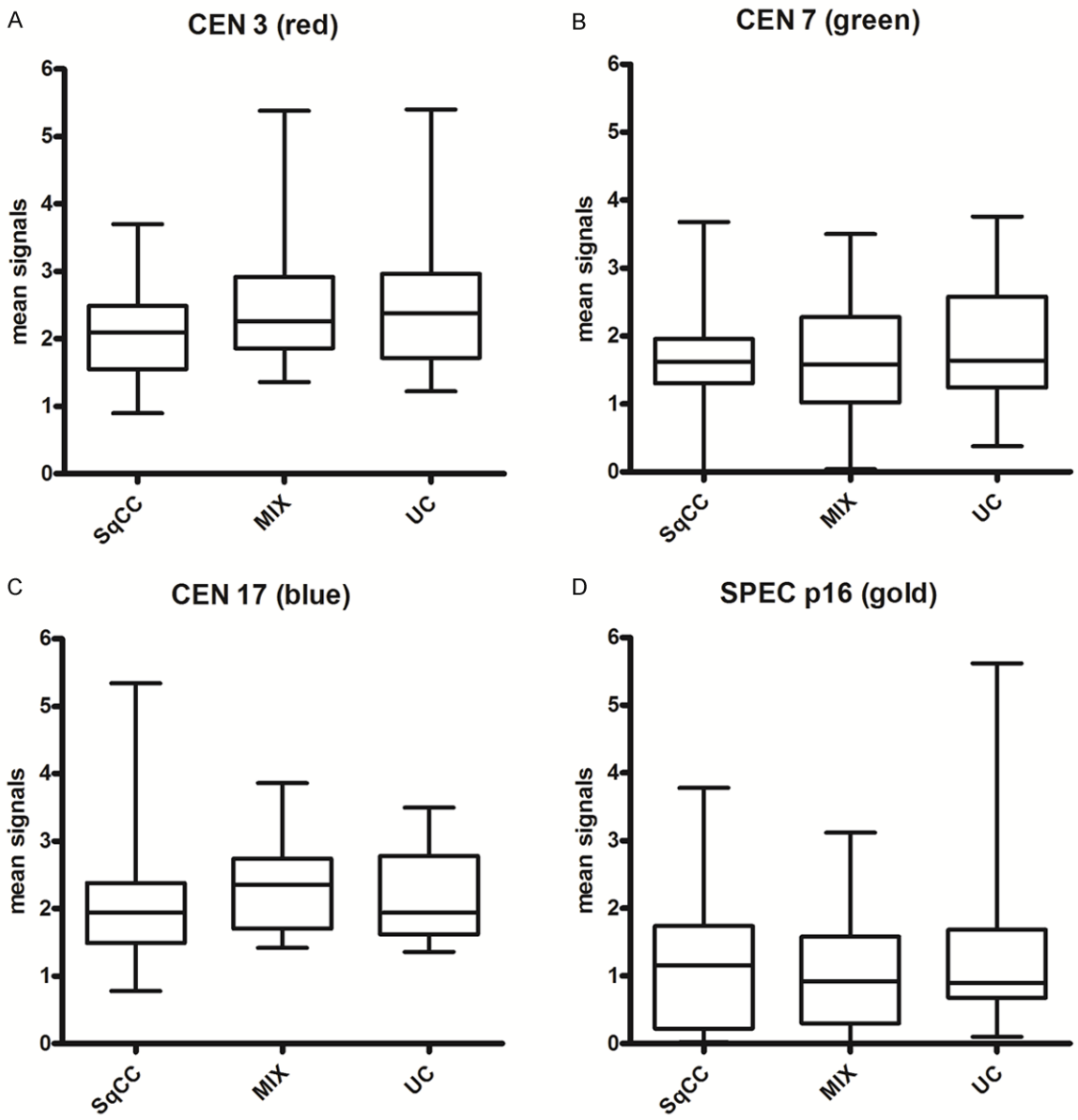

Figure 2. Differences of means of chromosome/locus copies of UC, MIX and SqCC. A. Box plot of CEN3 signals of SqCC, MIX and UC. Mean is indicated by a horizontal line. B. Box plot of CEN7 signals of SqCC, MIX and UC. C. Box plot of CEN17 signals of SqCC, MIX and UC. D. Box plot of SPEC p16 signals of SqCC, MIX and UC.

Fluorescence in situ hybridization (FISH)

FISH was performed on 5 µm paraffin-slides of TMAs carrying both tumor and reference cores. ZytoLight SPEC p16/CEN3/7/17 Quadruple Color Probes (Zytovision, Bremerhaven, Germany) was used according to the manufacturer's protocol and standard in-house modifications for pretreatment of tissue sections. Signals were detected with an Axiovert S100 Fluorescence Microscope (Carl Zeiss, Oberkochen, Germany), suitable filter sets and DISKUS software (Hilgers, Technisches Buero, Koenigswinter, Germany).

For each TMA hybridization efficacy was evaluated on normal urothelium reference cores and for each patient signals of 50 tumor cell nuclei were counted. Polysomy of tumour cells was determined by a cut-off value in normal tissue cells according to the literature: mean number of polysomic cells + (standard deviation x 3). All cells with ≥3 signals were assumed to be polysomic [16].

Comparative genomic hybridization (CGH)

Due to highly variable DNA quality and subsequent problems in probe preparation for Array-

Table 2. All structural chromosomal aberrations detected with CGH

ID	total changes	losses	gains	ampli-fications	
SqCC (n = 35)					
3	2	2	0	0	dim (3p, 10q)?
39	4	2	2	0	dim (3p14pter, 18q13qter) enh (1q, 12p)
107	0	0	0	0	no changes
167	4	2	2	0	dim (6q, 12q21q24.1) enh (10p, 12p)
170	3	2	1	0	dim (8p, 18q) enh (5p)
182	8	3	5	0	dim (6q16.1q22.33, 17p, 20p) enh (3q21.1qter, 5q11.2q21.3, 9p, 10p12.33pter, 17q)
189	3	1	2	0	dim (3p) enh (3q22.1qter, 9q12q21.31)
197	3	3	0	0	dim (3p?, 8p?, 10q22.2qter)
220	20	16	4	0	dim (2q21.2q23.2, 2q36.1qter, 3p, 4p, 4q31.3qter, 5q34qter, 6q22.31q26, 8p?, 9, 10q26.11qter, 11q23.2qter, 13q22.3qter, 15q, 18q, 20, 21q) enh (3q, 8q, 11q12.1q14.2, 18p)
226	9	9	0	0	dim (2p15pter, 3p, 4p, 8p?, 9, 10q22.1qter, 11p, 13q21.1qter, 21)
229	0	0	0	0	no changes
236	0	0	0	0	no changes
240	1	1	0	0	dim (20q)
242	0	0	0	0	no changes
5	6	5	1	0	dim (3p14pter, 4p, 9q, 10q22qter, 11q14qter) enh (9p)
10	5	1	4	0	dim (10q) enh (1q, 3p21qter, 8, 17q23qter)
11	9	4	4	1	dim (3p14pter, 5q32qter, 7q33qter, 8p) enh (3p14qter?, 7p, 8q11.2q23, 18) amp (11q14q23)
29	5	3	2	0	dim (3p, 11q14.1qter, 18q?) enh (3q, 18p?)
41	0	0	0	0	no changes
91	6	4	2	0	dim (3p, 6q16qter, 10q22qter, 18q) enh (3q, 18p)
98	0	0	0	0	no changes
102	9	6	3	0	dim (2q35qter, 3p, 4q28.3qter, 5q11.2q31.1, 8p, 18q) enh (3q25.1qter, 11q12.2q13.4, 13q22.2pter)
117	3	3	1	0	dim (8p, 14q31.1qter, 16?) enh (1p21.1p33)
121	11	5	6	0	dim (2q22.1qter, 3p, 5q, 8qter?, 10q23.1qter) enh (1q21.1q25.3, 2p11.1p16.1, 7, 11, 18p, 20)
132	1	1	0	0	dim (3p)
135	4	4	0	0	dim (11q13, 15q22qter, 17, 20qter)
136	4	2	2	0	dim (3p14p21, 16) enh (4q, 9p)
199	4	1	3	0	dim (18q) enh (11q12q13.4, 16?, 17)
206	12	7	5	0	dim (1p, 3p, 4p, 4q26qter, 8p, 14, 21q) enh (2q31.2q35, 5p, 7q, 9q, 12p)
208	13	5	8	0	dim (3p, 5q, 6q15q22.33, 10q26.11qter,18) enh (6p, 8q22.1qter, 9q, 12p12.3q21.3, 13q14.11qter, 17q, 20, 21)
210	12	3	9	0	dim (3p, 11q21qter, 15q) enh (1p34.1p35.1, 5p, 6p, 7p13q21.11, 8q22.3q23.3, 9, 11q13.2q14.3, 17p, 20)
224	8	6	2	0	dim (2q, 6q, 9q, 11q14.1qter, 13q, 20p) enh (18p, 20q)
231	1	0	1	0	enh (1q31.1q32.2)
238	7	4	3	0	dim (3p11.2p21.33, 8p, 10q25.1qter, 18q) enh (6p21.33pter, 8q?,18p?)
259	11	5	6	0	dim (5q, 7q31.13qter?, 11q21qter, 13q, 18q) enh (3q25.1qter, 5p, 7q31.1pter, 8q21.1qter, 11q12.2p13.3, 14)
MIX (n = 40)					
80	3	1	2	0	dim (10q23qter) enh (5p, 6q12q23)

150	4	4	0	0	dim (9, 10q24.2qter, 11, 17p)
158	4	4	0	0	dim (6p?, 15q22qter, 17p, 20)
180	21	13	8	0	dim (1p, 2p, 2q34qter, 3p, 4p, 4q26qter, 5q, 10q23.31q25.3, 11p, 11q23.1qter, 17p, 18q, 20p) enh (5p, 6p, 6q21q24, 7p, 8p, 10p, 11q12.1q14.1, 20q)
56	5	3	2	0	dim (3p12p14, 7q, 10q21qter?) enh (3q, 7p)
113	0	0	0	0	no changes
1	8	5	3	0	dim (2p?, 2q33qter, 8p, 11q23qter, 16q21qter) enh (1p12p31, 8q11.22q23.1, 12q14q21)
6	0	0	0	0	no changes
12	13	6	7	0	dim (3p, 8p, 9p13pter, 10q22.2q23, 11p, 11q23qter) enh (1q21qXq31.3, 5p13.2p14.3, 6q22.3qter, 7q?, 8q21.1qter, 9q?, 10p11.2p13)
62	0	0	0	0	no changes
88	5	1	4	0	dim (11q14qter) enh (1q, 3q26qter, 7p12.2q32, 8q23.1qter)
90	8	6	2	0	dim (3p?, 6p21p22?, 9q22.3qter, 10q22qter, 15q22qter, 18q?) enh (5p, 8q)
110	16	5	11	0	dim (3p14.1p24, 6p, 11p, 16q, 17q24.2pter) enh (1q, 2p11.2p21, 3q, 4q13.2q24, 5q11.2q14.2, 6q, 8q23.1qter, 10p, 12p, 16p, 20q)
120	7	1	6	0	dim (3p12.2p23) enh (1q12q32.2, 3q, 5p?, 7, 8q, 9)
125	6	6	0	0	dim (3p14.3p21.3, 10q21.1qter, 11q12.3q13.4?, 16, 17p?, 20q)
148	9	6	3	0	dim (3p14.1pter, 10q22.1qter, 15q22.1qter, 16, 17p, 20) enh (3p14.1qter, 5p, 9q21.3qter)
152	10	9	1	0	dim (2q36.1qter, 3p12.1p21.32, 4q, 5q, 7q32.3qter, 11q22.3qter, 15q22.1qter, 16p, 20q13.12qter) enh (5p)
154	4	2	2	0	dim (8p, 16?) enh (1? oder 1p33q31.3, 12q21.1q23.2)
156	0	0	0	0	no changes
160	7	3	3	1	dim (9q22.3qter, 16p, 17q25.1pter) enh (1p32q32, 3q, 5p) amp (12p)
162	10	7	3	0	dim (1q31.3qter, 2q34qter, 3p14.1p22.1, 9q31.1qter, 1q24.2qter, 15q22.1qter, 16q) enh (11q14.1q24.1, 13q21.1qter, 18p)
173	11	8	3	0	dim (1?, 2?, 4q28qter, 10q, 11p, 11q21qter, 15q, 18q) enh (5p, 8q21.1qter, 18p)
195	8	4	4	0	dim (3p, 3q12.3q21.2, 4, 5q) enh (2q23.2q33.2, 6p21.31pter, 1p14.1q12.1, 12p)
214	5	1	4	0	dim (18q) enh (5p, 7q12.1q21.12, 17p, 20)
233	8	3	5	0	dim (5q32qter, 6p, 9q21.13qter, 18q) enh (3q26.2qter, 8q, 10p, 12p, 20?)
252	6	0	6	0	enh (1q, 7?, 12, 16?, 17?, 20)
256	5	3	2	0	dim (6q22.1qter, 10q25.1qter, 13q31.1) enh (3q24qter?, 10p)
186	8	8	0	0	dim (2q34qter, 3p11.1p21.3, 4q21.22qter, 5q, 7q22.2qter, 11q21qter, 13q12.11q21.1, 18q21.31qter)
201	10	7	3	0	dim (4p, 5q32qter, 8p, 9, 10q25.1qter, 11p, 21q) enh (5p, 8q, 17p)
212	4	2	2	0	dim (8p, 10q25.1qter) enh (8q, 11q)
222	5	5	0	0	dim (1p, 9q, 10q, 14q, 18q)
247	14	5	9	0	dim (5q, 6q, 10q?, 11p?, 18q) enh (6p, 8q21.3qter, 9p13.3q22.2, 11q12.1q13.5, 12p11.22q12.3, 14q23.2q31.3, 17q, 18p, 20)
36	1	0	1	0	enh (5p?)
77	3	2	1	0	dim (2q35qter, 3p) enh (5p)
89	0	0	0	0	no changes
129	8	3	4	1	dim (3p12p24, 5q31qter, 18q) enh (5p, 8, 12, 13q14.3q31.3) amp (11q21q23.1)
138	14	9	5	0	dim (3q24pter, 4q23qter, 5q32qter, 6q, 7p11.2q21.3, 10q25.1qter, 11q14.1qter, 17p, 18q) enh (1p31.1q24.3, 3q26.1qter, 5p, 7p14.1pter, 17q)
141	5	3	2	0	dim (8p, 16, 18q) enh (3q?, 8q)
146	11	7	4	0	dim (3p, 5q23.1qter, 9q22.33qter, 10q23.2qter, 11p?, 15q22.1qter, 16p) enh (3q?, 5p, 6q, 12p)
217	4	2	2	0	dim (4q28qter, 11q22.1qter) enh (17q, 18p)

UC (n = 36)					
143	0	0	0	0	no changes
J60	10	4	6	0	dim (2q32qter, 5q, 11p12pter,12p,Y) enh (3, 5p, 8q21.3q22.3,
J135	17	9	7	1	dim (2q14.2, 4p, 6q, 8p12pter, 11p, 13q13q31.3, 14q21qter, 16p, 18q) enh (1p31p1q32.2, 3q, 4q?, 5p, 7, 16q22.1qter, 18p, 20q) amp (8q23)
J178	16	7	9	0	dim(1p, 2q36qter, 5q, 6q, 8p, 10q, 18q) enh(1q, 5p, 6p22p24, 9p, 10p12pter, 13q31qter, 17q23.2qter, 18p, 20q)
J223	9	3	6	0	dim (8p, 11q23qter, 18q21qter?) enh(3q24qter, 6p22pter, 8q, 9, 11p?, 18q, 14q)
J224	4	3	1	0	dim (2q36qter, 10q, 11q22qter) enh(6p23pter)
J228	0	0	0	0	no changes
J578	0	0	0	0	no changes
J311	0	0	0	0	no changes
J66	3	1	2	0	dim (6q) enh(8q21.1qter, 11q14.3qter)
J101	0	0	0	0	no changes
J108	7	2	5	0	dim(4q32.1qter,12q21qter), enh(1q21q31,2q,3p25pter,5p,18p)
J143	5	2	3	0	dim (6p22pter, 17p) enh (3q25q26, 8q, 9p23pter)
J139	6	4	1	1	dim (4q31qter, 9, 15q22qter, 17p, Y) enh(7) amp(10q22q23)
J381	8	4	4	0	dim (4p, 5q11.2q23, 8p, 18q) enh(5p, 8q, 10q25qter, 20)
43	4	1	3	0	dim (10q22.2qter) enh (6q12q33, 8q, 17q)
66	3	1	2	0	dim (8p) enh (5p, 10q23.1q24.1)
45	5	3	2	0	dim (9?, 10q21.3qter, 18q) enh (8, 13q)
179	15	12	3	0	dim (2q32.1qter, 3p12p24, 4p, 5q, 8p21pter, 10q, 11p, 11q23.1qter, 13q, 17p, 18q, 20p) enh (5p, 6q24pter, 10q)
22	16	9	7	0	dim (3p?, 4p, 5q14.3q22.2, 6q14.1qter, 7p, 8p, 9q, 11p, 18q) enh (3q, 11q12.3q14.3, 12q12q21.33, 16, 17q, 18p, 20q)
24	20	12	8	0	dim (2p22.3pter, 2q22.3q32.3, 4p, 4q32.3qter, 5q, 6q, 9p?, 9q, 10q24.2qter, 11p, 16p, 17p) enh (3q, 5p, 8q21.3q23.3, 11q12.1q14.1, 16q, 17q11.1q23.2, 20)
29	17	10	7	0	dim (2q14.1q24.3, 4p, 5q12.1q21.1, 5q33.2qter, 6q, 9q, 10q, 11p, 13q, 17p) enh (8q, 10p, 11q11q13.3, 12q11q21.33, 17q, 20)
2	21	11	10	0	dim (1q31.1qter, 4p, 5q, 6q12q23.2, 8p, 9p, 9q, 10q24.2qter, 11p, 16q, 18q) enh 2q11.2q22.3, 3p24.1pter, 3q13.33q26.33, 4q31.1q34.2, 5p, 6q23.2qter, 7p12.1q31.33, 10p, 16p, 18p)
30	10	6	4	0	dim (3p13p24.1, 8p, 16p, 17p, 18q, 20p) enh (2q32.1q35, 8q21.13qter, 10p, 12p11.1p13.31)
78	6	3	3	0	dim (8q23.1qter, 9, 14q) enh (1q, 2p16, 17q)
77	7	4	3	0	dim (10q13.1qter, 15q21.1qter, 16p, 17p) enh (5p, 18p, 20p)
71	4	1	3	0	dim (6q) enh (1q23.2q31.3, 5p, 18q)
70	6	2	3	1	dim (11pX, 17p) enh (3q, 8q21.1qter, 10p) amp(12q14.1q23.1)
47	11	5	6	0	dim (4q21.1qter, 6q, 7q21.3qter, 8p, 16q) enh (1q, 7p-q21.3, 8q22.2qter,9p, 10p, 17q)
46	3	1	2	0	dim (3p?) enh (3q26.2qter, 17q24.3qter)
44	7	4	3	0	dim (1p, 3p24.3, 6q, 8p, 11p) enh (8q, 17q, 18p)
45	3	3	0	0	dim (10q, 11q22.3, 15q?)
35	5	3	2	0	dim (4q31.3qter, 8p, 9q) enh (8q, 20p)
32	9	7	2	0	dim (4q24qter, 5q32qter, 7p, 9p, 113q12.11q21.33, 17p, 18q) enh (10p, 18p)
33	13	2	11	0	dim (2q34qter, 18q21.2qter) enh (1q42.3qter, 3q13.11q13.33, 7?, 8q11.22q23.3, 10q, 17, 18p, 20)
34	5	5	0	0	dim (4?, 5q, 6q, 10q, 12q14.1q23.1)

n = number, p = short arm of chromosome, q = long arm of chromosome, dim = diminished, enh = enhanced, ? = inconclusive, ter = terminal.

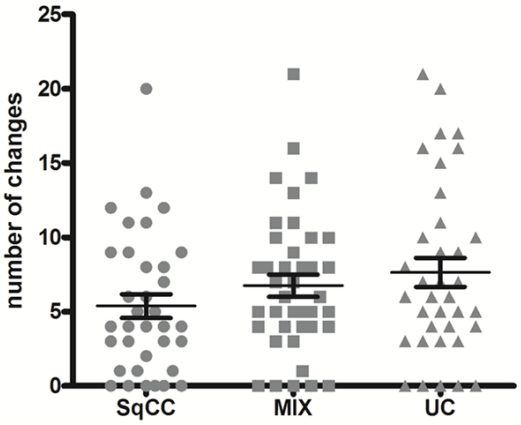

Figure 3. Mean of chromosomal aberrations for each tumor group in CGH. Each sample is represented by a symbol, mean of each tumor group is indicated by a horizontal line, standard error of the mean (SEM) is shown by a linked double horizontal line.

CGH we performed traditional CGH in order to investigate as much cases as possible.

Amplification of DNA via degenerate oligonucleotide-primed-polymerase chain reaction (PCR) and Taq-polymerase primed-PCR, labeling with biotin-16-dUTP for tumor-DNA and digoxigenin-11-dUTP for reference-DNA as well as hybridization and detection were performed as described earlier [17]. Analysis included ten to fifteen metaphase-chromosomes per case.

Statistical analysis

Statistical analysis using Fisher's exact test, 1-way ANOVA-test and unbound t-Test was performed with the GraphPad software (GraphPad Software, Inc., La Jolla, California, USA). Numbers are presented as frequencies and percentages. A *P*-value of less than 0.05 was considered significant.

Results

Polysomy of tumor cells evaluated by FISH

For FISH-analysis n = 29 pure SqCC, n = 35 MIX and n = 23 UC could be successfully analyzed. Due to a low hybridization efficiency of tissue sections (<0.9) it was not possible to analyze deletions (p16-probe (gold)) with reasonable accuracy. Therefore, we only focused on polysomy of CEN3 (red), CEN7 (green) and CEN17 (blue). Cells with ≥3 signals were assumed to be polysomic, and according to the formula: cut off = mean number of polysomic cells + (standard deviation × 3), the calculated cut-offs in normal cells for CEN3 (red) were 2.13, and for CEN7 (green) and CEN17 (blue) were 1.175 each. All three tumor groups showed similar variations regarding the number of polysomic cells in all probes (**Figure 1**). Constantly, the mean number of polysomic cells in SqCC was lower, indicating less aneuploid tumor cells in SqCC compared to MIX and UC. Significant differences of polysomic and non-polysomic cases within the three groups were only found for the CEN3-probe (red) (*P* = 0.0498) and the CEN17-probe (aqua) (*P* = 0.0009).

Differences of chromosome/locus copies among UC, MIX and SqCC

Of all tumors 50 tumor cell nuclei were evaluated for each probe; the mean results are shown in **Figure 2**. Means of the three tumor groups of the CEN3-probe (red) and the CEN17-probe (blue) varied around 2, the CEN7-probe (green) was slightly below 2 and the means of the SPEC p16-probe (gold) was around 1. There was a slight tendency of lower copy numbers of chromosomes 3, 7 and 17 and higher numbers of the p16-locus in SqCC compared to UC and MIX, but there was no significant statistical difference among the three tumor groups (*P* = 0.45).

CGH of UC, MIX and SqCC

CGH was successfully performed on n = 35 SqCC, n = 40 MIX and n = 36 UC samples. Structural genomic variations were found in n = 30 SqCC (85.7%), n = 35 MIX (87.5%) and n = 32 UC samples (88.9%). **Table 2** depicts all variations (**Table 2**). SqCC showed the lowest number of variations/tumor (mean = 5.37, standard error of the mean (SEM) 0.80, 95% confidence interval of the mean (CI) 3.75-6.99); MIX tumors had a mean of 6.75 changes/tumor (SEM 0.74, CI 5.25-8.25) and UC exhibited most changes with a mean of 7.64 changes/tumor (SEM 0.98, CI 5.65-9.63; *P* = 0.1754, **Figure 3**).

Chromosomal variations of organ confined tumor stages (pT2) versus advanced tumor stages (pT3-4)

In SqCCs mean number of changes increased with extravesical tumor stage (pT2 tumors: mean 3, SEM 1.48, CI-0.62-6.62; pT3-4 tumors: mean 5.964, SEM 0.90, CI 4.12-7.81). However, the case numbers for stages pT2 were low, and the difference was not statistically significant (*P* = 0.1386). In MIX and UC no increase of

Table 3. Gains and losses of short and long arms of chromosomes for each tumor group

Chr. arm	SqCC (n = 35)				MIX (n = 40)				UC (n = 36)			
	losses		gains		losses		gains		losses		gains	
	n	%	n	%	n	%	n	%	n	%	n	%
1p	1	*2.9*	2	*5.7*	3	*7.5*	4	*10*	2	*5.5*	1	*2.8*
1q	0	*0*	4	*11.4*	2	*5*	7	*17.5*	1	*2.8*	6	*16.7*
2p	1	*2.9*	1	*2.9*	3	*7.5*	1	*2.5*	1	*2.8*	1	*2.8*
2q	4	*11.4*	1	*2.9*	7	*17.5*	1	*2.5*	8	*22.2*	3	*8.3*
3p	18	*51.4*	2	*5.7*	15	*37.5*	1	*2.5*	5	*13.9*	3	*8.3*
3q	0	*0*	7	*20*	2	*5*	10	*25*	0	*0*	10	*27.8*
4p	4	*11.4*	0	*0*	3	*7.5*	0	*0*	8	*22.2*	0	*0*
4q	3	*8.6*	1	*2.9*	7	*17.5*	1	*2.5*	7	*19.4*	2	*5.5*
5p	1	*2.9*	4	*11.4*	0	*0*	16	*40*	0	*0*	11	*30.6*
5q	5	*14.3*	1	*2.9*	10	*25*	1	*2.5*	10	*27.8*	0	*0*
6p	0	*0*	3	*8.6*	4	*10*	3	*7.5*	1	*2.8*	3	*8.3*
6q	6	*17.1*	0	*0*	3	*7.5*	5	*12.5*	11	*30.6*	1	*2.8*
7p	0	*0*	3	*8.6*	1	*2.5*	5	*12.5*	2	*5.5*	5	*13.9*
7q	2	*5.7*	3	*8.6*	3	*7.5*	4	*10*	1	*2.8*	3	*8.3*
8p	9	*25.7*	1	*2.9*	6	*15*	2	*5*	12	*33.3*	1	*2.8*
8q	1	*2.9*	7	*20*	0	*0*	13	*32.5*	1	*2.8*	15	*41.7*
9p	2	*5.7*	4	*11.4*	4	*10*	2	*5*	6	*16.7*	4	*11.1*
9q	2	*5.7*	4	*11.4*	7	*17.5*	3	*7.5*	8	*22.2*	1	*2.8*
10p	1	*2.9*	2	*5.7*	0	*0*	5	*12.5*	0	*0*	8	*22.2*
10q	9	*25.7*	0	*0*	17	*42.5*	0	*0*	11	*30.6*	3	*8.3*
11p	1	*2.9*	1	*2.9*	9	*22.5*	1	*2.5*	9	*25*	1	*2.8*
11q	7	*20*	6	*17.1*	12	*30*	4	*10*	3	*8.3*	4	*11.1*
12p	0	*0*	4	*11.4*	0	*0*	7	*17.5*	1	*2.8*	1	*2.8*
12q	1	*2.9*	0	*0*	0	*0*	3	*7.5*	2	*5.5*	2	*5.5*
13p	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*
13q	4	*11.4*	2	*5.7*	2	*5*	2	*5*	4	*11.1*	3	*8.3*
14p	1	*2.9*	1	*2.9*	0	*0*	0	*0*	0	*0*	0	*0*
14q	2	*5.7*	1	*2.9*	1	*2.5*	1	*2.5*	2	*5.5*	2	*5.5*
15p	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*
15q	3	*8.6*	0	*0*	7	*17.5*	0	*0*	3	*8.3*	0	*0*
16p	2	*5.7*	1	*2,9*	7	*17.5*	2	*5*	4	*11.1*	2	*5.5*
16q	2	*5.7*	1	*2.9*	6	*15*	1	*2.5*	2	*5.5*	3	*8.3*
17p	2	*5.7*	2	*5.7*	7	*17.5*	3	*7.5*	9	*25*	1	*2.8*
17q	1	*2.9*	4	*11.4*	2	*5*	4	*10*	0	*0*	10	*27.8*
18p	1	*2.9*	7	*20*	0	*0*	4	*10*	0	*0*	9	*25*
18q	10	*28.6*	1	*2.9*	11	*27.5*	0	*0*	11	*30.6*	1	*2.8*
20p	3	*8.6*	3	*8.6*	3	*7.5*	4	*10*	2	*5.5*	6	*16.7*
20q	3	*8.6*	4	*11.4*	4	*10*	6	*15*	0	*0*	7	*19.4*
21p	1	*2.9*	1	*2.9*	0	*0*	0	*0*	0	*0*	0	*0*
21q	4	*11.4*	1	*2.9*	1	*2.5*	0	*0*	0	*0*	0	*0*

Chr. arm = chromosome arm, n = number, p = short arm of chromosome, q = long arm of chromosome, SqCC = pure squamous cell carcinoma of the bladder, MIX = urothelial carcinoma with squamous differentiation, UC = urothelial carcinoma.

changes with tumor stage could be found (MIX pT2: mean 8.9, SEM 2.72, CI 2.20-15.51; MIX pT3-4: mean 6.3, SEM 0.70, CI 4.88-7.72; P = 0.1953; UC pT2: mean 9.2, SEM 1.60, CI 5.79-12.56; UC pT3-4: 6.3, SEM 1.14, CI 3.88-8.65; P = 0.14).

Chromosomal variations and tumor grading

Correlations with tumor grade showed in all tumor groups the same trend of increasing genetic changes in less differentiated G3 tumors compared to G2 tumors. In SqCCs G2 tumours harboured a mean of 4.1 changes (SEM 1.44, CI 0.96-7.18), whereas G3 tumours showed a mean of 6.2 changes (SEM 0.90, CI 4.37-8.11; P = 0.186). In mixed tumors there were 6.2 changes in G2 tumors (SEM 3.05, CI -1.67-14) and 6.9 changes in G3 tumors (SEM 0.72, CI 5.39-8.32), and in pure UCs there was the same trend but there were only 2 cases of G2 tumours (G2: mean 15.5 changes, SEM 0.5, CI 9.12-21.85; G3: mean 7.17 changes, SEM 0.98, CI 5.18-9.17). Due to low numbers no statistical analysis was performed.

Most frequent chromosomal changes

Table 3 shows an overview of the gains and losses on short (p) and long (q) arms of the chromosomes compared in each tumor group (**Table 3**). Chromosomes 19, 22, X and Y were excluded due to high variability.

Most frequent changes in SqCC were losses of 3p (51.4%)*, 8p (25.7%), 10q (25.7%), 18q (28.6%), and gains of 3q (20%), 8q (20%), 18p (20%). In MIX tumors losses were found on 3p (37.5%), 5q (25%), 10q (42.5%), 11p (22.5%)*, 11q (30%) and 18q (27.5%), and gains on 3q (25%), 5p (40%) and 8q (32.5%). UCs presented with the most frequent losses on 5q (27.8%), 6q (30.6%)*, 8p (33.3%), 9q (22.2%), 10q (30.%), 11p (25%)*, 17p (25%) and 18q (30.6%), as well as gains on 3q (27.8%), 5p (30.6%), 8q (41.7%), 10p (22.2%), 17q (27.8%) und 18p (25%). In statistical analysis significant differences between groups were found for losses on 3p (P = 0.0041), 6q (P = 0.0202) and 11p (P = 0.0237), as well as for gains on 5p (P = 0.0202).

Discussion

This is the first larger study comparing immunohistochemically proven non-schistosoma associated pure SqCC, mixed urothelial carcinoma with partial squamous differentiation and pure UC. In previous studies either not exactly characterized/specified tumors or only small numbers of cases have been analyzed. Our data support the concept that SqCC of the bladder is less aneuploid and therefore genetically more stable than UC. This is in line with the largest non-specified FISH-study of cell suspensions of n = 94 SCCs and n = 96 UCs from Pycha et al., who also found less polysomic tumour cells in SqCCs than in UCs (CEN17 30.8% versus 85.4% UC, CEN 7 81.9% vs. 97.9%) [18]. For chromosome 7 our study could not show statistically significant differences. However, this might be due to the technical difference of cell suspensions and artifact-rich tissue sections (partial nuclei or overlay of nuclei) [19]. Further, Urovysion® FISH was developed for the detection of aneuploidy in UC, and Reid Nicholson et al. found only two of 15 SqCCs on tissue sections FISH-positive (Abbott criteria), strongly questioning its diagnostic value in non-urothelial tumors. However, analysis of their published data also showed polysomies of chromosome 3 and 17 in one case, as well as polysomy of chromosomes 3, 7 and 17 in the other case [20]. In contrast, we found higher numbers of positive SqCCs (22/29, 76%) and polysomic tumor cells: 76% of cases showed polysomy of chromosome 3 and 68% polysomy of chromosome 17. Kipp et al. analyzed tissue sections of n = 7 SqCC by Urovysion® FISH and detected homozygous deletions of 9p21 in SqCC significantly more frequent than in other tumor types [21]. They also reported polysomies of the other chromosomes in 31% of 9p21-deleted cells.

We substantiated our study with further analysis of our cohort by CGH. Similar to UC [22] we expected an increase of genetic alterations by tumor stage and grade in squamous tumors as a consequence of accumulation of genetic hits. However, in our invasive cancers we found no significant correlations of genetic alterations within any tumor group regarding increasing tumor stage (T2 vs. T3-4) or grade (G2 vs. G3). Overall we detected less genetic alterations in SqCC (mean 5.37) than in any other tumor type (MIX mean 6.75, UC mean 7.64), but the results showed no statistical significance. The findings are similar to the differences in tumor cell polysomy found with FISH.

An important question is, if there is a difference in the genetic profile of schistosoma- and non-schistosoma-associated-SqCC. Comparing our non-schistosoma associated samples to the reported schistosoma-associated data [3], we found losses of 3p, 8p, 8q and 18q in both settings. El Rifai et al. also compared schistosoma- and non-schistosoma-associated samples

and detected in non-schistosoma-associated samples most frequently losses of 13q, 3p, 9p and gains of 1q, 8q and 20q [3, 14]. The results for the losses of 3p and gains of 8q are in line with ours, however, we found alterations of 3p much more frequently (51% vs. El-Rifai et al. 18%). Alterations of chromosomes 9p and 13q as well as 1q and 20q were not among the most frequent alterations in our study.

Another point to address is, whether there is a specific difference in genetic aberrations in SqCC and MIX or UC. The earlier study of Rifai et al. dealing with not-exactly characterized samples reported gains and high-level amplifications of 5p and losses of 3p as specific for SqCC, with gains of 5p only in schistosoma-associated SqCC [14]. Our precisely defined cohort showed three characteristics: a) the loss of 3p was more frequent in SqCC than in MIX or UC, b) the loss of 11p was less frequent in SqCC than in others, and c) there were less gains of 5p in SqCC compared to MIX and UC ($P = 0.0795$). However, we found gains of 5p in 30% of UC. Loss of chromosome arm 11p was rare in all groups in the study of El-Rifai et al. [14]. Fadl-Elmula et al. analyzed two cases of urothelial carcinoma with secondary squamous differentiation. They showed in both cases UC-typical changes and an isochromosome 5p in one case and the loss of 11p in the other case, which allowed no discrimination between MIX and UC [13]. The loss of 3p was also reported in squamous cell tumors of the head and neck [23] and esophagus [24, 25], with a reported loss of 57% (21/37) in SqCC of the esophagus [25]. In a meta-analysis of CGH data of 5918 cancers Baudis et al. described frequent losses of 3p and gains of 3q, 8q as well as variable gains of 1q and 5p in cancer subsites with predominantly squamous cell tumours (i.e., head and neck, non-small cell lung cancer, cervix carcinoma, vulva carcinoma, esophagus carcinoma) [23]. Interestingly, in our analysis we also found frequent loss of 3p and gains of 3q, 8q and 5p, but more often in UC than in SqCC, which does not indicate a specific role in squamous cell cancers but a general involvement in the malignant transformation of the urothelium.

Comparing the exact regions of losses in 3p, we found more often a complete loss of 3p or from 3p14 until the p-terminal end in SqCC, whereas the losses in MIX and UC are more focal. There are various genes located in region 3p, e.g., FHIT (fragile histidine triad-3p14.2), CTNNB1 catenin (cadherin associated protein-3p21), VHL (von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase-3p25.3) [25], or MLH1 (mutL homolog 1, colon cancer nonpolyposis type 2-3p21.3) [23]. So far, only FHIT was reported to be involved in the tumorigenesis of bladder tumors [26]. It encodes the FHIT-protein, a diadenosintriphosphate-hydrolase of the "histidine triad (HIT)"-super family of nucleotid-binding proteins [26, 27], which acts as a tumour suppressor. The tumour suppressive mechanism is not fully understood, yet [26, 28, 29], but it seems to induce apoptosis [29], and interacts with several pathways regulating oxidative stress and cell cycle control [28, 29]. Furthermore, region 3p14.2 includes a very active "common fragile site (CFS)" FRA3B, susceptible for strand breaks or rearrangements (genetic instability), and triggering tumorigenesis [28, 29]. Therefore, loss of 3p14.2 as part of FRA3 is reported quite often in tumours of the lung, esophagus, cervix, breast and head and neck [26, 28]. In bladder tumors Han et al. reported a negative correlation of FHIT expression with tumour grade, but not with tumour stage or recurrence [26]. Zhang et al. suggested FHIT expression as a prognostic marker, as patients with positive FHIT-expression showed significantly longer survival times in their study [30]. FHIT-expression has not yet been investigated in pure, non-schistosoma associated SqCCs of the bladder, but Gutierrez et al. found a methylation of FHIT (40% of cases) and other loci in schistosoma-associated SqCCs [31].

To conclude, SqCCs show less polysomy and genetic alterations than MIX and UC. However, the structural genetic profile for SqCC, MIX and UC is similar, with no absolutely specific alterations for each group. Loss of 3p was more frequent in SqCC, but so far we do not know the driver-event for squamous carcinogenesis. Our results build bridges to the recently proposed subtypes of muscle invasive bladder cancer, as the described "basal/squamous-like" subtype perfectly overlaps with our MIX tumors, especially the "transdifferentiated" subtype of Gaisa et al. [15]. Our cytogenetic data strengthen the concept of different squamous phenotypes in bladder cancer: mixed tumors show similar alterations than UC, thus seem to fit into the "squamous-like" subtype and should be con-

sidered a further more aggressive development of urothelial carcinoma, while mere SqCC appear to be a separate tumor group. Gathering detailed knowledge on exact subtypes of bladder cancer is extremely important for the development of biology-based individualized therapies and further molecular studies on squamous differentiated bladder tumours are needed.

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Disclosure of conflict of interest

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