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Micropapillary urothelial carcinoma: evaluation of HER2 status and immunohistochemical characterization of the molecular subtype

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Abstract

Comprehensive molecular analyses of urothelial bladder cancer (UBC) have defined distinct subtypes with potential therapeutic implications. In this study, we focused on micropapillary urothelial carcinoma (MPUC), an aggressive, histomorphologically defined rare variant. Apart from genetic alterations shared with conventional UBC alterations of the *HER2* gene have been reported in higher frequencies. However, only small cohorts of MPUCs have been analyzed and the real impact is still unclear. We collected a cohort of 94 MPUCs and immunohistochemically tested HER2, basal (CD44, CK5, EGFR, p63) and luminal (CD24, FOXA1, GATA3, CK20) markers to allocate MPUC to a molecular subtype. Additionally, HER2 amplification status was assigned by chromogenic in-situ-hybridization. Sanger sequencing of Exon 4 and 8 was used to test for *HER2* mutations. Kruskal-Wallis test was calculated to compare marker distribution between proportions of the MPUC component. 2+/3+ HER2 staining scores were identified in 39.6% of 91 analyzed MPUCs and were not differentially distributed among the proportion of the MPUC component (p=0.89). Additionally, CISH analysis revealed 30% of *HER2* amplified tumors independently of the MPUC fraction. In 6/90 evaluable MPUCs a p.S310F *HER2* mutation was detected. Overexpression of luminal markers was observed in the majority of MPUC. Our investigations of the largest cohort of analyzed MPUC demonstrate that HER2 overexpression and amplifications are common genetic alterations and identification of overexpressed luminal markers allows sub-classification to the luminal subtype. These findings highlight the need of histomorphological recognition of MPUC and analysis of *HER2* status and the luminal molecular subtype for potential targeted therapeutic strategies.

Keywords: urothelial bladder cancer; micropapillary variant; *HER2*; molecular subtype; targeted therapy.

1. Introduction

Urothelial bladder cancer (UBC) is the fifth most common malignancy worldwide in men and one of the most cost-consuming cancers as a result of financial expenses not only for therapy but also for life time follow-up examination. In the recent years and due to the lack of therapeutic advances and targeted therapies, comprehensive molecular characterization studies put a lot of effort and insight into the genomic landscape of UBC and provided several potential targets. Moreover, whole-genome mRNA expression profiling studies proposed distinct molecular categories of UBC similar to the breast cancer classification and defined different prognostic groups and appropriate therapeutic options [1].

In this study, we focus on micropapillary urothelial carcinomas (MPUC), a rare subtype first reported by Amin et al. in 1994, accounting for 0.7-6% of all UBC [2]. According to micropapillary carcinomas in other organs such as lung or breast, MPUC is histomorphologically characterized by several small-sized nests of tumor cells within lacunar spaces which may or may not have a central vascular core located [3]. Clinical and prognostic comparison between MPUC and conventional UBC showed a poorer prognosis for this distinct variant, probably due to a more aggressive behavior with frequent lymphatic invasion resulting in a higher rate of metastases [4, 5].

Besides these differences in the clinical behavior, very limited data exist about the molecular background of this specific subtype. Interestingly, by testing the Human epidermal growth factor receptor 2 (*HER2*) gene amplification among UBC, mutated tumors presented more often with specific features such as micropapillary morphology [6]. *HER2* is a receptor tyrosine-protein kinase and a member of the epidermal growth factor receptor family. Activation of the receptor through dimerization and autophosphorylation initiates several signaling pathways including cell proliferation. In breast cancer, amplification or overexpression of *HER2* are known to be responsible for the development of aggressive

subtypes and the use of anti-HER2 antibodies combined with standard chemotherapy is well established and a prime example for an applied targeted therapy [7]. Concerning the possible association of MPUC and *HER2* status, several studies identified a higher rate of *HER2* amplification, mutations and/ or overexpression compared to the detected numbers in conventional urothelial tumors [8-11]. However, due to the low incidence rates of MPUC the available reports are conducted in low sample sized cohorts (below 60 cases). Therefore, the real impact of a potential target among this histomorphological subgroup remains unclear.

The aim of our study was to collect the largest cohort of MPUC to date and to validate the expression, mutation and amplification status of the *HER2* gene as a possible therapeutic strategy among MPUC tumors. Moreover, with regard to the recently identified molecular categories of UBC we used basal and luminal immunohistochemical markers to assign MPUC to a specific subgroup.

2. Materials and methods

2.1 Study cohort

In total, 94 archival formalin-fixed, paraffin-embedded MPUC of the urinary bladder were collected from several cooperation partners and from the files of the Institute of Pathology of the Friedrich-Alexander-University Erlangen-Nürnberg. Complete histomorphological reevaluation of at least one hematoxylin and eosin (H&E)-stained tumor slide according to the current WHO classification system was performed by two experienced uropathologists (A. H., S. B.) Reevaluation included assessment of the percentage of the micropapillary component in tumors with mixed morphology [3]. All cases presented the classical appearance of invasive micropapillary carcinomas and were included if at least 15% of the tumor showed clear micropapillary differentiation. Table 1 represents the clinico-pathological characteristics of the analyzed MPUC cohort. Prior institutional review board (University Hospital Erlangen) approval was obtained for molecular analysis on archival material.

Representative pictures of the micropapillary histomorphological features are shown in Figure 1.

2.2 Immunohistochemistry analysis

A tissue microarray (TMA) was constructed containing cores specifically punched from marked micropapillary regions of 94 MPUCs. Immunohistochemistry was performed on TMA slides including only the variant component using a Ventana Bench Mark Ultra automatic stainer. All antibodies used by this method are listed in Table 2. Single markers were chosen to classify the MPUC cohort: CK5, CD44, p63 und EGFR for defining the basal and CD24, CK20, GATA3 and FOXA1 for the luminal subtype. Manual staining was performed for FOXA1 (polyclonal, 1:1000; Abcam, ab23738). CD24 immunohistochemistry was performed automatically (Dilution 1:5; SWA-11; *Ventana Medical Systems, Inc.*; Tucson, USA) by our cooperation partners at the Institute of Pathology, University Hospital Bonn, Germany. HER2 immunohistochemistry was evaluated according to the recommendations of the American Society of Clinical Oncology and the College of American Pathologists [12]. All other immunohistochemical markers were analyzed according to the immunoreactive score (IRS) by Remmele and Stegner resulting from multiplication of percentage score (0=0, 1=<10%, 2=10-50%, 3=51-80%, 4=>80%) and intensity score (assessment of the staining intensity of positive cells: 0=negative, 1=weak, 2=intermediate, 3=strong). IRS=0 was considered negative, IRS 1-5 weakly positive and IRS 6-12 strongly positive [13].

2.3 HER2-Chromogenic in situ hybridization (CISH)

CISH analysis was performed on TMA slides using the SPEC HER2/CEN 17 Probe Kit (Zytovision) following the manufacturer's instructions. Scoring was performed according to the revised ASCO/CAP guidelines for HER2 amplification in breast cancer [12].

2.4 Microdissection and DNA isolation

According to marked H&E-stained sections of the evaluated tumors manual microdissection of the micropapillary component and DNA isolation was performed as described previously [14]. For DNA isolation the Maxwell 16 LEV Blood DNA Kit (Promega, Mannheim, Germany) was used according to the manufacturer´s instructions and included only the variant component.

2.5 HER2 exon 4 and exon 8 mutation analysis

Exon 4 and 8 of the *HER2* gene were amplified by PCR (used primers: for Exon 4: sense: 5´-CAG AAG GTG ACA GAA GGG-3´; antisense: 5´-CTG TCT GAG AGA AGA GGG-3´ and for Exon 8: sense: 5´-TCA TGG TGG TGC ACG AAG, antisense: 5´-AAC TGC AGC TGG CCT CGG-3´) (Metabion, Martinsried, Germany) in a total volume of 25µl containing approximately 100ng DNA, 0.2mM dNTP, 0.18µM primers and 0.025U/µl GoTaq (Promega, Mannheim, Germany). Thermal cycling conditions: initial denaturation for 3 min at 94°C, 45 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 1 min, final primer extension at 72°C for 10 min. Gradient PCR was used for optimization of cycling conditions. PCR-products (size: Exon 4: 226bp, Exon 8: 211bp) were purified using the Qiagen Dye Ex 2.0 TM Spin Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Sanger Sequencing analysis was performed with PCR primers using Applied Biosystems Big Dye Terminator v1.1 Cycle Sequencing Kit and an Applied Biosystems ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA).

2.6 Statistical analysis

Kruskal-Wallis test was used to assess differences between the proportion of the MPUC components and were calculated using R, version 3.2.3. The heatmap.2 function of the gplots package within the R 3.2.3 statistical environment was used for non-hierarchical clustering based on immunoreactive scores of the evaluated markers.

3. Results

3.1 *HER2 protein expression and amplification*

In total, 91/94 (96.8%) MPUC cases were evaluable for HER2 immunohistochemistry. Figure 2 shows representative images of the scoring. 36/91 (39.6%) tested MPUC demonstrated a HER2 score of 2+ and 3+ (Table 3). Comparing tumors with different proportions of the MPUC component we observed no differences in the distribution of HER2 protein overexpression rate (p=0.89). CISH analysis was evaluable in 70 out of 94 tumors. Representative images are displayed in Figure 2. *HER2* amplification was found in 21/70 (30.0%) MPUC. *HER2* amplified tumors were independently distributed among MPUC, regardless of the proportion of the MPUC component (p=0.61, Table 3). Correlation of amplification status with a high protein expression showed 16/21 (76.2%) *HER2* amplified MPUCs with a HER2 protein expression score of 2+ or 3+, in 5 amplified cases protein expression was weak (score 1+) or absent (Table 4).

3.2 *HER2 mutation analysis*

The *HER2* mutation status of exon 4 and 8 was analyzed in 90 available MPUC samples. Sequencing data of both exons were available for 89/90 (98.7%) tumors (Table 5). The only alteration identified was a p.S310F mutation in 6/90 (6.7%) MPUCs located within exon 8 (Figure 3A). In one case results were only available for exon 8 and presented wildtype *HER2* sequence. Furthermore, we detected four MPUCs showing the minor T allele of the single nucleotide polymorphism (SNP) rs56114611 located within exon 4 (Figure 3B). Moreover, the combination of sequencing and CISH data revealed two *HER2* p.S310F mutated MPUCs with concomitant *HER2* amplification and three cases with a 2+/3+ HER2 scoring.

3.3 *Immunohistochemical analysis of basal and luminal markers*

90/94 MPUC with immunohistochemical results were included in the heat map analysis. Non-hierarchical clustering determined similarity of the used luminal and basal markers. Figure 4

illustrates the combination and expression levels of the different markers. Strong expression of luminal markers was found in the vast majority of cases, with a weak and strong expression of CD24 in 93.1%, CK20 in 76.9%, GATA3 86.8% and FOXA1 in 85.2%. In contrast, basal markers were predominantly not expressed. With regard to the distinct percentages of the MPUC component, we did not identify a differential distribution of the luminal or basal markers (Table 6).

4. Discussion

MPUC is associated with aggressive behavior and poor survival most probably due to frequent lymphatic invasion and metastases [5, 15]. In comparison with MPUC of the upper urinary tract both malignancies present with similar aggressive characteristics [16]. Adverse outcomes despite aggressive platinum-based chemotherapy and limited response to Bacillus-Calmette-Guerin instillation therapy have been reported in MPUC, but little is known about alternative treatment strategies and most authors recommend early aggressive surgical treatment [5]. Recently, our own studies on MPUC showed worse outcome compared to conventional UBC after transurethral resection and radiochemotherapy [17]. In another study it has been shown that any amount of the MPUC variant is significant and should be reported [18]. Moreover, the biological scenario of the variant is largely unknown: a recent study reported that TERT mutations identified in UBC were also frequently observed among MPUC [19]. Improvement of our knowledge about this morphological variant and search for new therapy strategies are urgently needed.

Among breast cancer patients HER2 IHC analysis is used to identify patients suitable for anti-HER2 targeted therapies [7]. In conventional UBC, HER2 overexpression frequencies have been reported ranging from 9.2 to 71% [20-22]. In the largest series of 1005 invasive UBC only 9.2% presented a positive (Score 2+ to 3+) HER2 staining [22]. Recently, due to the need of a guided exploration Moktefi et al. reported, that HER2 evaluation in UBC should

include immunohistochemistry as screening step adding FISH testing if needed. Additionally, MPUC in this study presented with a higher proportion of HER2 positive tumors [23]. In concordance with this the few available small sample sized MPUC cohorts reported higher numbers of HER2 positive tumors: Schneider and colleagues found 30/61 (50%) positive (2+ and 3+) MPUC cases [10]. Another study detected an increased HER2 protein expression in 13/19 (68%) MPUC [11]. Moreover, an overexpression rate of 74% of 27 investigated MPUC tumors has been shown. However, more than half of these positive cases were scored 2+ according to the ASCO scoring system [24]. Recently, Behzatoglu et al. showed a 3+ staining in 34 of 60 MPUC [9]. In our own study, a 2+/3+ HER2 staining was found in 38.3% of evaluable MPUC. The variability of IHC results among conventional or MPUC studies may be due to the cohort size as well as methodical differences. Despite standardized evaluation recommendations, assessment of IHC suffers from high interobserver variability depending on training of the pathologist [25]. In our department, HER2 immunohistochemical assay is validated in annual round-robin tests and protocols are certified according to ISO 17020. Furthermore, evaluation of this study was performed by two experienced pathologists. Due to this high standardization and the large number of evaluated MPUC we summarize, that HER2 overexpression is particularly seen among MPUC.

Tschui et al. aimed to better characterize *HER2* amplified UBC and observed micropapillary morphology as a hallmark of these mutated tumors [6]. During the last years, several studies reported *HER2* amplifications in MPUC cohorts with variable frequencies of 15 to 42% [10, 26]. We found *HER2* amplifications in 21 of 70 investigated MPUCs via CISH analysis. The high variability of reported frequencies may be due to the relatively small cohorts investigated in most studies. Additionally, the diversity may result from differences in methodology, i.e. CISH or FISH, and analysis of TMA spots or whole tumor sections. Due to the high number of MPUC investigated in our study, the frequency of 30% *HER2* amplifications, which lies in between the reported values, seems to be a realistic estimation of the actual percentage. In comparison to reported amplifications rates of 5.1-10% in unselected cohorts of UBC, *HER2*

amplified tumors seem to be remarkably more frequent among the micropapillary variant [22, 27, 28]. Since our CISH analyses were performed on TMA slides, cases with heterogeneous distribution of *HER2* amplifications may have been missed [22]. Summarized, almost one third of MPUC showed an *HER2* amplification opening a promising therapy option for this rare subtype for example as a complementary therapeutic element in a neoadjuvant setting [29].

Another point of interest is the absence of HER2 protein expression (score 0 or 1+) in 24% of *HER2* amplified MPUCs. This finding is in line with different studies showing a considerable discrepancy between results of immunoreactive score and amplification status. By using FISH analysis Caner et al. observed in 3 out of 16 overexpressed tumors a *HER2* amplification among UBC [27]. In addition, heterogeneous pattern between amplified cases and mRNA/protein expression were identified among the TCGA cohort. The analysis of different “omics” levels in the TCGA cohort revealed, that amplified tumors with low expression levels tended to have increased gene methylation pointing to an important impact for epigenetic regulation and a complex landscape [29].

Using next generation sequencing (NGS) analysis Ross et al. found *HER2* mutations (p.S310F/Y, p.R157W) in 6/15 tested MPUC, whereas HER2 protein overexpression and amplification was not detected [8]. In our study 6.7% of evaluable MPUCs revealed p.S310F mutation and none of the other reported ones. P.S310Y/F alterations are probably activating mutations which have also been detected in other cancer entities and are considered to be sensitive to HER2 inhibitors [30]. The high discordance may be due to the very few samples used in the NGS study as well as threshold values set for evaluation of NGS results. Notably, there is no information whether these findings were validated by a second sequencing method [8]. Since we did not perform NGS based analysis we may have missed some mutations. However, a similar rate of 7% *HER2* mutations was identified among the TCGA

cohort [31]. Our results suggest that somatic *HER2* mutations do not seem to play a dominant role among MPUC patients.

Several comprehensive characterization studies based on mRNA expression profiling proposed molecular classification systems of UBC into prognostic significant distinct subtypes, whereby transfer of mRNA-based subtyping to immunohistochemical marker profiles is currently being discussed [1, 32]. A consensus paper summarized high CK5/14 and low levels of FOXA1/GATA3 as the characterizing markers of the basal type [33]. CD44, p63 and EGFR were previously identified and used as basal markers [1]. CK20 and CD24 are predominately represented among luminal tumors [1, 34]. In our study, by using a limited and selected immunohistochemical panel we detected a strong expression of the luminal markers CD24, FOXA1, GATA3 and CK20, whereas basal markers (CD44, CK5, EGFR, p63) were predominantly not expressed, assuming that MPUC represent the luminal subtype of UBC. Our results are in line with previous findings of the study by Guo et al. identifying the luminal character based on whole mRNA and immunohistochemical analyses of CD44, CK14, GATA3 and UPK2 in a cohort of 43 MPUC samples [35]. Additionally, *HER2* amplification and overexpression were significantly more often identified in tumors with luminal characteristics and therefore operate in the context of the luminal pathway [29]. This co-occurrence of HER2 amplified and overexpressed samples among luminal tumors is in line with our findings among MPUC cases evolving through the luminal molecular pathway.

5. Conclusions

To the best of our knowledge, this is the largest cohort of MPUC to date being comprehensively evaluated for *HER2* status by using immunohistochemistry, CISH and mutational analysis. Additionally, we were able to assign MPUC to the luminal molecular subtype using TMA based immunohistochemical analysis. Our findings and the size of our

cohort may better reflect the actual frequency of overexpressed and/or amplified tumors compared to available low size studies. Given the relatively high frequency of HER2 overexpression and/or amplification in almost one third of MPUC, our study demonstrates the importance of recognition of this histomorphological variant and its potential therapeutic impact. However, *HER2* mutations do not seem to play a major role in MPUC due to their low frequency. Summarized, routine implementation of HER2 immunohistochemistry at least and/or assessment of *HER2* amplification status to improve therapeutic strategies of this aggressive subtype should be considered.

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Figure legend

Figure 1: A-D Histomorphological spectrum of MPUC (H&E-stained, 200x magnification). **A, B:** Typical slender papillary proliferations with peritumoral clefts mimicking lymphovascular invasion. **C:** Several micropapillae may be seen within a single lacuna. **D:** Cytoplasmic vacuoles forming ring-like structures are also characteristic features of MPUC.

Figure 2: A, B: Representative pictures of HER2 immunohistochemistry (400x magnification). **A:** HER2 immunoscore 2+. **B:** HER2 immunoscore 3+; **C, D:** Sample images of *HER2* amplification (630x magnification). **C:** Tumor with a high-level *HER2* amplification. **D:** Aneuploid tumor with 6 or more HER2 signals in the majority of tumor cells.

Figure 3: A: Sanger sequencing of exon 8 shows a p.S310F mutation. **B:** Sanger sequencing of exon 4 shows the SNP rs56114611.

Figure 4: Heatmap presenting immunohistochemistry results for luminal and basal markers. Each column represents 1 TMA spot; cases with <3 basal and luminal markers were excluded, white fields represent not available TMA-spots; red fields represent high immunoreactive scores (IRS), green fields represent low IRS.

Table 1. Study characteristics of the analyzed cohort

	MPUC cohort n (%)
Total number of cases	94
Age distribution, yr	
Minimum/ maximum	41-99
Median age	68
Not available	6
Gender	
Male	61 (81.3)
Female	14 (18.7)
Not available	19
Stage distribution	
pT1	7 (8.6)
pT2	24 (29.6)
pT3	29 (35.8)
pT4	21 (25.9)
Unknown	13
Grading WHO 2016	
Low-grade	0
High-grade	94 (100.0)
Grading WHO 1973	
G1	0
G2	9 (9.6)
G3	85 (90.4)
Proportion of the MPUC component	
15-70%	24 (25.5)
80-90%	17 (18.1)
95-100%	53 (56.4)

MPUC: Micropapillary urothelial carcinoma, WHO: Word Health Organization

Table 2. Antibodies used for immunohistochemistry on a Bench Mark Ultra automatic stainer (Ventana).

Antibody	Company	Clone	Dilution, 1:
CD44	Dako	DF1485	40
CK20	Dako	Ks20.8	50
CK5	Zytomed	XM26	50
EGFR	Novocastra	EGFR25	50
GATA3	DCS	L50-823	ready to use
HER2	Dako	Poly	1000
p63	DCS	SFI-6	100

Table 3. HER2 protein overexpression and amplification rates among the MPUC cohort.

	MPUC cohort n (%)	Proportion of the MPUC component n (%)			Comparison
		15-70%	80-95%	100%	*p*-value
Total number of cases	94	24	17	53	
HER2 protein expression					
0	23 (25.3)	6 (26.1)	3 (18.8)	14 (26.9)	*p*=0.89
1+	32 (35.2)	7 (30.4)	6 (37.5)	19 (36.5)	
2+	23 (25.3)	9 (39.1)	5 (31.2)	9 (17.3)	
3+	13 (14.3)	1 (4.3)	2 (12.5)	10 (19.2)	
Not available	3	1	1	1	
***HER2* amplification status**					
Non-amplified	49 (70.0)	13 (68.4)	9 (81.8)	27 (67.5)	*p*=0.61
Amplified	21 (30.0)	6 (31.6)	2 (18.2)	13 (32.5)	
Not available	24	5	6	13	

Table 4. HER2 immunohistochemistry in combination with *HER2* CISH results.

	MPUC cohort n (%)	HER2 protein expression n (%)				
		0	1+	2+	3+	Not available
Total number of cases	94	23	32	23	13	3
***HER2* amplification status**						
Non-amplified	49 (70.0)	13 (92.9)	22 (84.6)	10 (52.6)	4 (36.4)	0
Amplified	21 (30.0)	1 (7.1)	4 (15.4)	9 (47.4)	7 (63.6)	0
Not available	24	9	6	4	2	3

Table 5. Molecular analysis of exon 4 and 8 of the *HER2* gene.

	MPUC cohort	Proportion of the MPUC component n (%)		
	n (%)	15-70%	80-95%	100%
Total number of cases	94	24	17	53
***HER2* exon 4+8 analyzed samples**				
Wildtype	84 (93.3)	23 (100.0)	14 (82.4)	47 (94.0)
Partly evaluable*	1	0	1	0
Mutated	6 (6.7)	0	3 (17.6)	3 (6.0)
Not available	4	1	0	3

*Only exon 8 was analyzed and presented with wildtype sequence.

Table 6. Basal and luminal markers among the MPUC cohort

	MPUC cohort n (%)	Proportion of the MPUC component n (%)			Comparison
		15-70%	80-95%	100%	*p*-value
Total number of cases	94	24	17	53	
Basal markers					
CD44					
Negative	80 (92.0)	20 (90.9)	14 (93.3)	46 (92.0)	*p*=0.81
Weak	7 (8.0)	2 (9.1)	1 (6.7)	4 (8.0)	
Strong	0	0	0	0	
Not available	7	2	2	3	
CK5					
Negative	78 (87.6)	17 (81.0)	14 (87.5)	47 (90.4)	*p*=0.90
Weak	9 (10.1)	3 (14.2)	2 (12.5)	4 (7.7)	
Strong	2 (2.2)	1 (4.8)	0	1 (1.9)	
Not available	5	3	1	1	
p63					
Negative	69 (82.1)	17 (77.3)	13 (86.7)	39 (83.0)	*p*=0.35
Weak	8 (9.5)	3 (13.6)	1 (6.7)	4 (8.5)	
Strong	7 (8.3)	2 (9.1)	1 (6.7)	4 (8.5)	
Not available	10	2	2	6	
EGFR					
Negative	53 (58.9)	12 (52.2)	10 (66.7)	31 (59.6)	*p*=0.55
Weak	31 (34.4)	10 (43.5)	4 (26.7)	17 (32.7)	
Strong	6 (6.7)	1 (4.3)	1 (6.7)	4 (7.7)	
Not available	4	1	2	1	
Luminal markers					
CK20					
Negative	21 (23.1)	2 (8.3)	5 (31.2)	14 (27.5)	*p*=0.60
Weak	9 (9.9)	1 (4.2)	2 (12.5)	6 (11.7)	
Strong	61 (67.0)	21 (87.5)	9 (56.3)	31 (60.8)	
Not available	3	0	1	2	
GATA3					
Negative	12 (13.2)	1 (4.4)	2 (12.5)	9 (17.3)	*p*=0.59
Weak	31 (34.1)	7 (30.4)	6 (37.5)	18 (34.6)	
Strong	48 (52.7)	15 (65.2)	8 (50.0)	25 (48.1)	
Not available	3	1	1	1	
FOXA1					
Negative	13 (14.8)	3 (13.0)	3 (21.4)	7 (13.7)	*p*=0.97
Weak	17 (19.3)	2 (8.7)	2 (14.3)	13 (25.5)	
Strong	58 (65.9)	18 (78.3)	9 (64.3)	31 (60.8)	
Not available	6	1	3	2	
CD24					
Negative	6 (6.9)	0	0	6 (11.8)	*p*=0.96
Weak	21 (24.1)	6 (28.6)	4 (26.7)	11 (21.5)	
Strong	60 (69.0)	15 (71.4)	11 (73.3)	34 (66.7)	
Not available	7	3	2	2	

Micropapillary urothelial carcinoma: evaluation of HER2 status and immunohistochemical characterization of the molecular subtype

Highlights

- Evaluation of 94 invasive, rare micropapillary urothelial carcinomas (MPUC)
- Comprehensive characterization of *HER2* by using IHC, CISH and mutation analysis
- Almost one third of amplified and/or overexpressed HER2 tumors
- Molecular classification of MPUC into the luminal subtype by using IHC markers
- Need of histomorphological recognition to test for *HER2* and to improve therapy

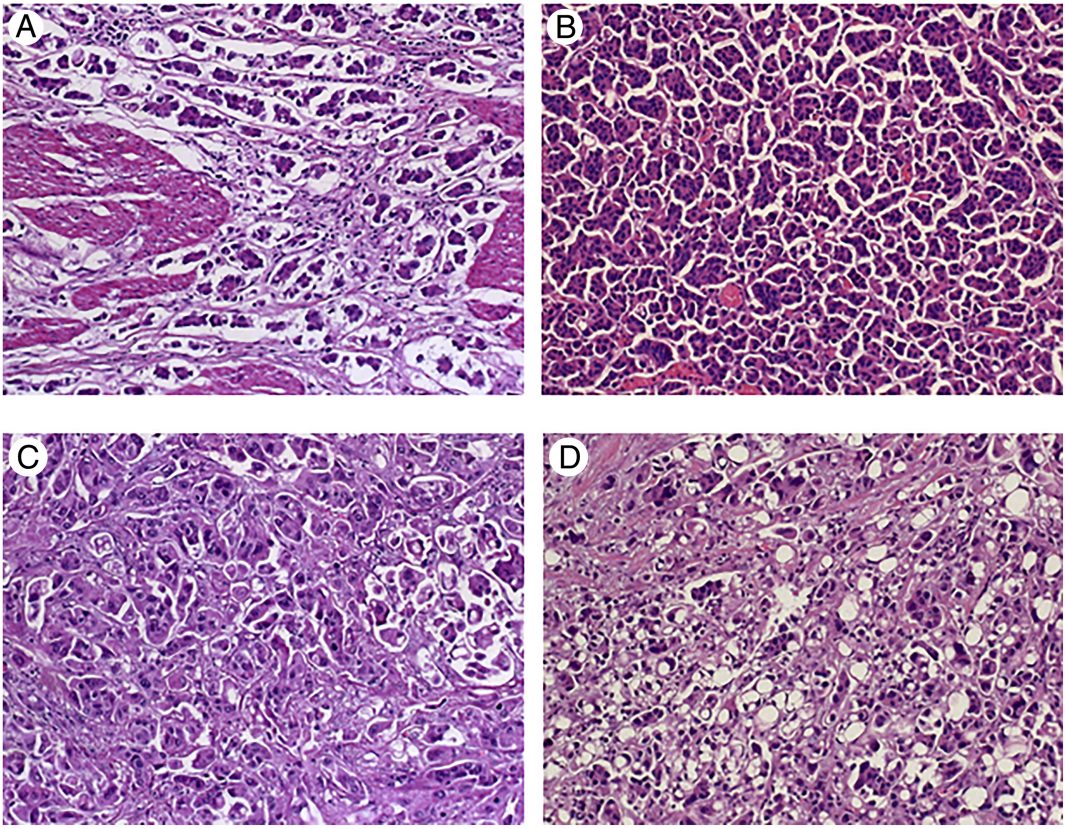

Figure 1

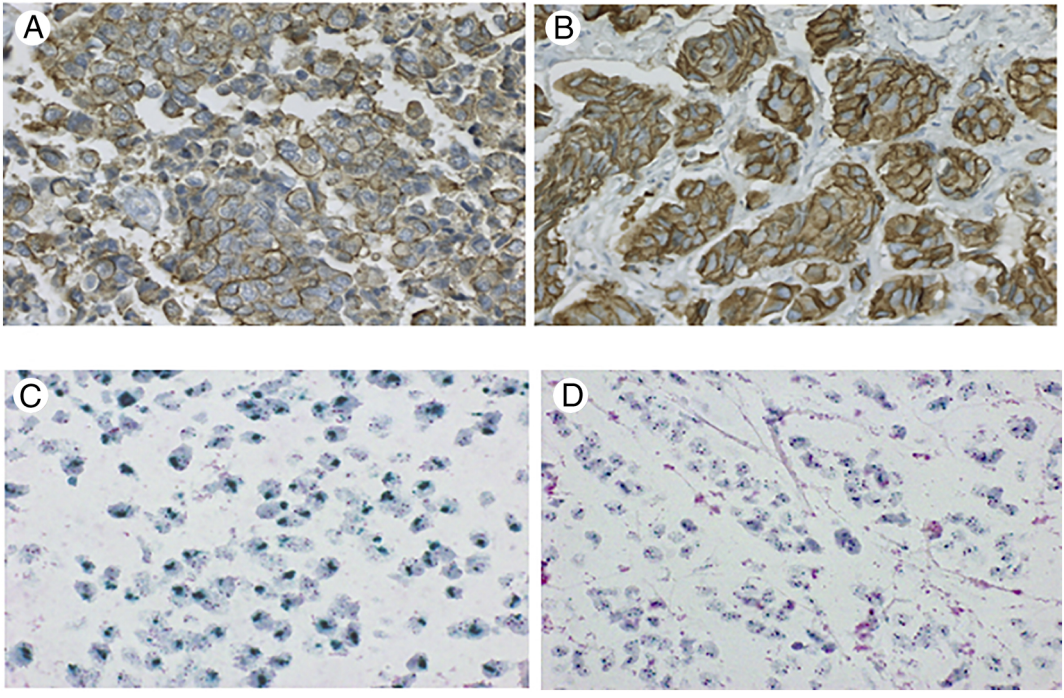

Figure 2

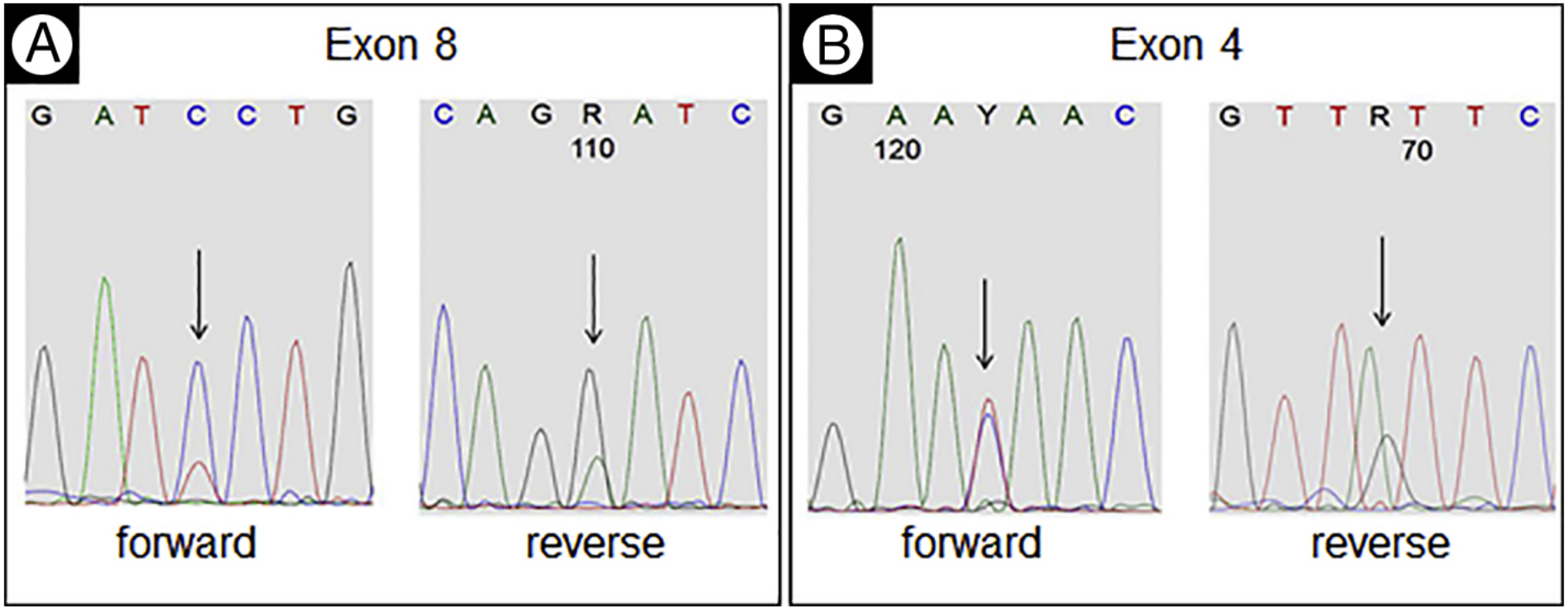
A
Exon 8
G A T C C T G
C A G R A T C
110
forward
reverse
B
Exon 4
G A A Y A A C
120
G T T R T T C
70
forward
reverse

Figure 3

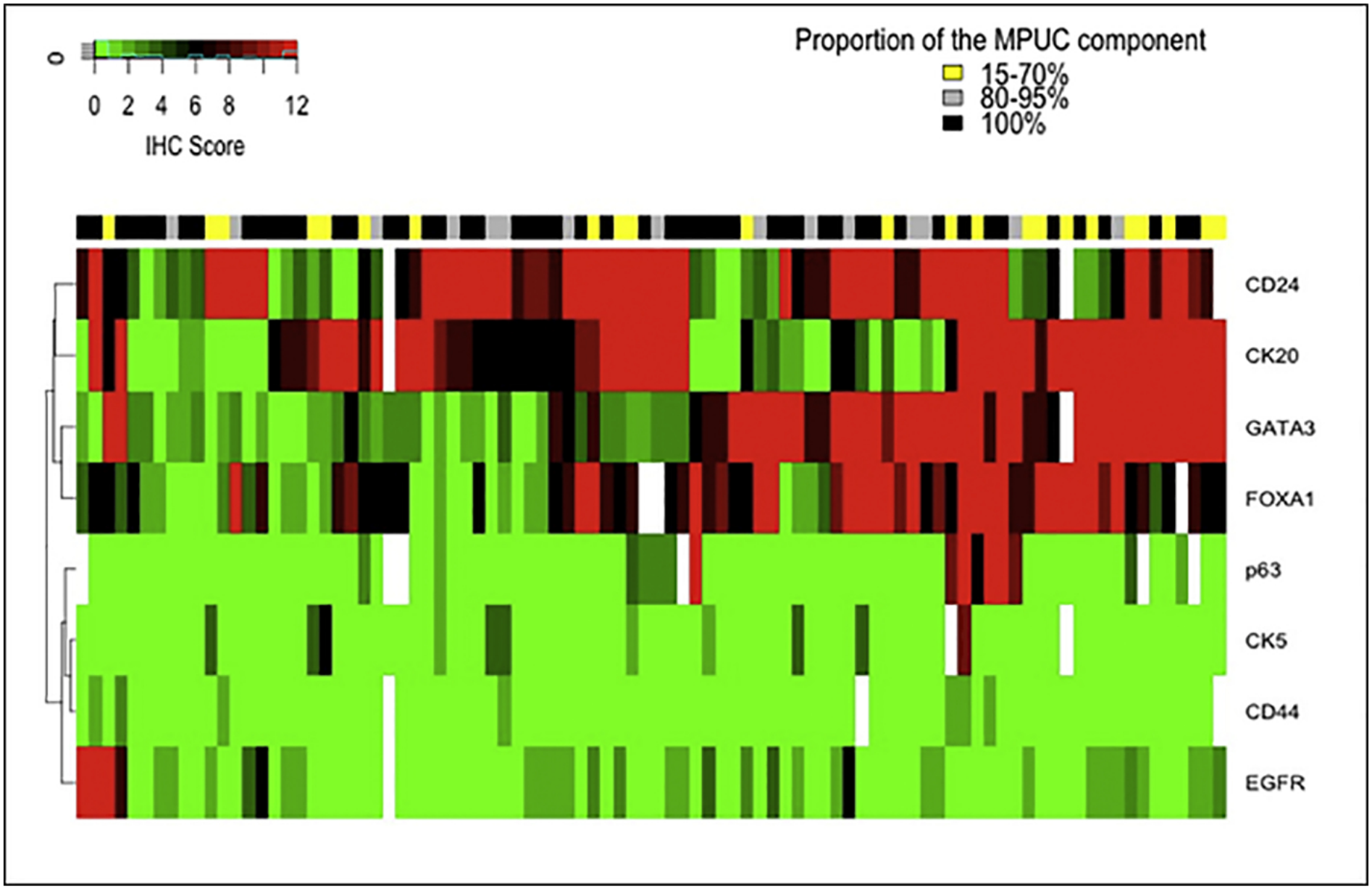
0
0 2 4 6 8 12
IHC Score
Proportion of the MPUC component
15-70%
80-95%
100%
CD24
CK20
GATA3
FOXA1
p63
CK5
CD44
EGFR

Figure 4