Human Cancer Biology

Clinical Cancer Research

A High Frequency of Activating Extracellular Domain *ERBB2* (*HER2*) Mutation in Micropapillary Urothelial Carcinoma

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Abstract

Purpose: Micropapillary urothelial carcinoma (MPUC) is a rare and aggressive form of bladder cancer. We conducted genomic analyses [next-generation sequencing (NGS)] of MPUC and non-micropapillary urothelial bladder carcinomas (non-MPUC) to characterize the genomic landscape and identify targeted treatment options.

Experimental Design: DNA was extracted from 40 μm of formalin-fixed paraffin-embedded sections from 15 MPUC and 64 non-MPUC tumors. Sequencing (NGS) was performed on hybridization-captured, adaptor ligation–based libraries to high coverage for 3,230 exons of 182 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer. The results were evaluated for all classes of genomic alteration.

Results: Mutations in the extracellular domain of *ERBB2* were identified in 6 of 15 (40%) of MPUC: S310F (four cases), S310Y (one case), and R157W (one case). All six cases of MPUC with *ERBB2* mutation were negative for *ERBB2* amplification and Erbb2 overexpression. In contrast, 6 of 64 (9.4%) non-MPUC harbored an *ERBB2* alteration, including base substitution (three cases), amplification (two cases), and gene fusion (one case), which is higher than the 2 of 159 (1.3%) protein-changing *ERBB2* mutations reported for urinary tract cancer in COSMIC. The enrichment of *ERBB2* alterations in MPUC compared with non-MPUC is significant both between this series ($P < 0.0084$) and for all types of urinary tract cancer in COSMIC ($P < 0.001$).

Conclusions: NGS of MPUC revealed a high incidence of mutation in the extracellular domain of ERBB2, a gene for which there are five approved targeted therapies. NGS can identify genomic alteration, which inform treatment options for the majority of MPUC patients. *Clin Cancer Res; 20(1); 68–75. ©2013 AACR.*

Introduction

When cancer of the urinary bladder progresses to incurable metastatic disease, it is a major cause of morbidity and mortality around the world (1–3). Despite significant success of targeted anticancer therapy in other common solid tumors such as breast and lung cancer, patients with locoregionally advanced and metastatic urothelial carcinoma have limited therapy options, especially as chemoresistance develops to standard anticancer therapies (4, 5). The micropapillary variant of urothelial carcinoma (MPUC) was first described in 1994 (6). Encompassing approximately 5% of all bladder cancers, MPUC is a clinically important lesion characterized by a distinctive histology that features small micropapillae created by clusters of 4 to 5 cells across, peripherally situated nuclei, and cytoplasmic vacuoles with a strong tendency to develop intralymphatic permeation or simulate lymphovascular involvement because of the production of peritumoral stromal retraction artifacts (6–9). It is well-accepted that the diagnosis of MPUC indicates an adverse prognosis and pathologists have strongly recommended that even if the minority of a urinary bladder urothelial carcinoma features a MPUC pattern, the diagnosis of MPUC should either be made outright or the tumor should be classified as urothelial carcinoma with MPUC features (6–9). Among the noteworthy clinicopathologic features of MPUC is the association of metastatic disease at

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Translational Relevance

This study describes the application of a novel comprehensive next-generation sequencing-based diagnostic test on active clinical cases of relapsed urothelial carcinoma of the urinary bladder and how the results of the analysis can drive the selection of treatment for these patients by discovering unanticipated therapeutic targets. The micropapillary variant of urothelial carcinoma, a known clinically aggressive subtype of the disease, harbored an unprecedented high frequency of *ERBB2* mutations. Use of this approach could facilitate accrual to clinical trials of small molecules of antibodies in bladder cancer patients with *ERBB2* mutations.

the time of diagnosis for a tumor with either no invasion or limited invasion of the bladder wall. This finding is similar to that observed for micropapillary carcinomas occurring in other sites such as in the endometrium, breast, and lung (10–12).

Given the highly aggressive nature of MPUC, investigators have queried whether the disease may have characteristic driver mutations that could contribute to its propensity to develop early metastases and feature such a poor prognosis. In a previous study of 35 urothelial carcinomas, which included 1 MUPC case, a single mutation in the extracellular domain of *ERBB2* (S310F) was observed in the MUPC specimen (13). In this study we conducted a genomic analysis of 15 patients with MPUC and an expanded series of 64 patients with non-MPUC to characterize the genomic landscape of MUPC. Genomic analysis of this expanded series of patients allowed us to identify a subset of mutations in the extracellular regulatory domain of ERBB2 that are enriched in the MPUC subset of urothelial carcinomas.

Materials and Methods

Targeted next-generation sequencing (NGS) was performed on hybridization-captured, adaptor ligation–based libraries using DNA extracted from 4 formalin-fixed paraffin-embedded (FFPE) sections cut at 10 μm from 15 cases of MPUC and 64 cases of non-MUPC in a CLIA–certified lab (Foundation Medicine, Inc.). The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin-stained slides, and all samples forwarded for DNA extraction contained a minimum of 20% DNA derived from tumor cells. All MPUC samples were histologically confirmed by 3 pathologists (JSR, TN, and TAJ) using published criteria (7). DNA sequencing was performed for 3,230 exons of 182 cancer-related genes and 37 introns of 14 genes

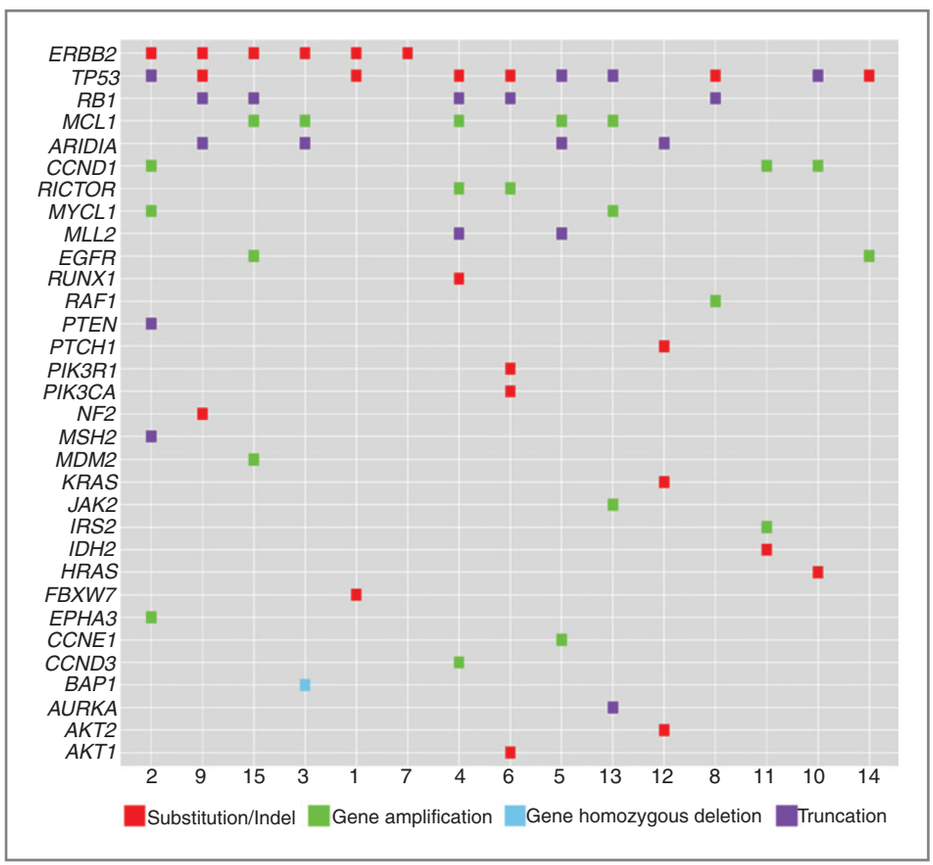

Figure 1. Tile plot of genomic alterations in 15 cases of micropapillary urothelial carcinoma.

Table 1. Clinicopathologic features and genomic alterations in 15 cases of micropapillary urothelial carcinoma of the urinary bladder

Study #	Gender	Age at time sample was obtained	Specimen sequenced	Tumor type	Tumor grade	Tumor stage	Coverage depth	Genomic alterations	Actionable alterations	AKT1	ATK2	ARID1A	AURKA	BAP1	CCND1	CCND3	CCNE1	EGFR	EPHA3	ERBB2 (allelic fraction)	FBXW7
1	F	71	Metastasis	MPUC	HG	IV	1,525	3	2											S310F (25%)	G423V
2	M	63	TURBT	MPUC	HG	IV	863	7	4						Amplification				Amplification	S310F (36%)	
3	M	67	Metastasis	MPUC	HG	IV	675	4	3			V661fs*15		Homozygous Loss						S310Y (19%)	
4	F	57	Cystectomy	MPUC	HG	IV	753	8	3							Amplification					
5	F	71	Metastasis	MPUC	HG	IV	757	5	3			S1356fs*101					Amplification				
6	M	66	TURBT	MPUC	HG	II	1,305	6	3	E17K											
7	M	55	TURBT	MPUC	HG	I	904	1	1											R157W (1%)	
8	F	61	Cystectomy	MPUC	HG	III	999	3	1												
9	M	55	Cystectomy	MPUC	HG	III	1,022	5	2			E1767*								S310F (25%)	
10	M	73	Cystectomy	MPUC	HG	II	1,100	3	1						Amplification						
11	M	86	Cystectomy	MPUC	HG	IV	870	3	1						Amplification						
12	M	68	TURBT	MPUC	HG	I	1,082	6	4		E17K	Y215*, Q1473*									
13	M	68	TURBT	MUPC	HG	II	999	6	3				S361*								
14	F	63	Metastasis	MUPC	HG	IV	1,022	2	1									Amplification			
15	M	67	TURBT	MUPC	HG	I	793	5	4									Amplification		S310F (43%)	

frequently rearranged in cancer (1.14 million total bp) on indexed, adaptor-ligated, hybridization-captured (Agilent SureSelect custom kit) DNA and fully sequenced using 49 bp paired reads on an Illumina HiSeq 2000. The MPUC cases were sequenced to at an average depth of 978X. The non-MPUC was sequenced to an average depth of 969X. As previously described (14), all samples were evaluated for genomic alterations including base substitutions, insertions, deletions, copy number alterations (amplifications and homozygous deletions), and select gene fusions/rearrangements. The bioinformatics processes used in this study included Bayesian algorithms to detect base substitutions, local assembly algorithms to detect short insertions and deletions, a comparison with process-matched normal control samples to detect gene copy number alterations and an analysis of chimeric read pairs to identify gene fusions. Actionable genomic alterations were defined as being linked to commercially available targeted therapies on the market or to targeted therapies being tested registered clinical trials. The 10 MPUC cases tested for HER2 (ERBB2) protein overexpression by immunohistochemistry (IHC) were analyzed using the Herceptest assay (Dako). Local site permissions and Albany Medical Center institutional review board approval were used for this study.

Results

The 15 MPUC samples were obtained from 10 male and 5 female patients with a mean age of 66 years (range 55–86 years). Sequencing was performed on the primary tumor in 11 (73%) cases (6 TURBT samples and 5 cystectomies) and on metastatic lesions in 4 (27%) cases. All tumors were high grade: 3 cases were stage I, 3 cases were stage II, 2 cases were stage III, and 7 cases were stage IV. A total of 67 genomic alterations (average 4.47 genomic alteration per tumor) were identified, including alterations in *TP53* (10 cases, 67%), *ERBB2* (6 cases, 40%), *MCL1* (5 cases, 33%), *RB1* (5 cases, 33%), and *ARID1A* (4 cases, 27%; Fig. 1 and Table 1). The 6 *ERBB2* mutations were all located within the extracellular domain of *ERBB2* and included S310F (4 cases), S310Y (1 case), and R157W (1 case; Fig. 2). No mutations were observed in the *ERBB2* tyrosine kinase domain. All 6 cases of MPUC with *ERBB2* mutation were negative for *ERBB2* amplification, and in the 3 cases where additional tissue was available for testing, the *ERBB2*-mutated MPUC were also negative for HER2 overexpression by IHC (Table 1 and Fig. 2).

In contrast, only 6 of 64 (9.4%) non-MPUC harbored *ERBB2* alterations, including S310F mutations (3 cases), amplification (2 cases, 40 and 15 copies), and an *ERBB2-GRB7* fusion (1 case; Supplementary Table S1 and Fig. S1). The *ERBB2* mutation frequency observed in both the MPUC and non-MPUC cohorts are higher than has been reported for urinary tract cancer in COSMIC (2/159 tumors, 1.3%; ref. 15). The enrichment of *ERBB2* alterations in MPUC compared with non-MPUC is significant between this series ($P < 0.0084$) and for all types of urinary tract cancer in

Table 1. Clinicopathologic features and genomic alterations in 15 cases of micropapillary urothelial carcinoma of the urinary bladder (cont'd)

HRAS	IDH2	IRS2	JAK2	KRAS	MCL1	MDM2	MLL2	MSH2	MYCL1	NF2	PTCH1	PIK3CA	PIK3R1	PTEN	RAF1	RB1	RICTOR	RUNX1	TP53
								S612*	Amplification					Q298*					R282W E294fs*52
					Amplification														
					Amplification Amplification		Truncation exon 48 M1417fs*15									G617*fs36	Amplification	R166Q	E258K, S215R S183*
												E542K	I290fs*4			S397*	Amplification		K132N
															Amplification	Y239*			R175H
G12D										E463K						Q762*			G244S Splice site 783-1G>A
	R140Q	Amplification																	
				G12V, G12C							V1081M								
			Amplification		Amplification				Amplification										R273C, Q52fs*71 G245S
					Amplification	Amplification										Q344*			

COSMIC ($P < 0.001$). All 9 MPUC cases with WT *ERBB2* harbored at least 1 actionable alteration, including mutations in *AKT1*, *AKT2*, *CCND1*, *EGFR*, *PIK3CA*, *PIK3R1*, and *RAF1*. The most frequent alterations in the non-MPUC group involved mutations in *TP53* (38 total; 58% of non-MPUC cases) and *CDKN2A/B* (25 total; 39% of non-MPUC cases). Alterations in chromatin remodeling genes, including truncating mutations in *KDM6A* (17 total; 27% of non-MPUC cases) and *ARID1A* (12 total; 19% of non-MPUC cases), were notable in the non-MPUC group (Supplementary Table S1 and Fig. S1).

Discussion

MPUC is a relatively rare subtype of urothelial carcinoma that comprises approximately 3,000 to 4,000 new cases diagnosed each year in the United States, an incidence just below that of the successfully targeted diseases including breast and lung cancers, melanoma, and hematologic malignancies such as chronic myelogenous leukemia (6–9). MUPC is widely considered to have an adverse prognosis, a reflection of the propensity to invade lymphovascular spaces and spread to distant sites early in the course of the disease (6–9). Any component of MPUC in a urothelial carcinoma of the bladder is considered to be significant, and studies have shown that as the proportion of the MPUC component increases, the prognosis worsens (16–18). Given that MPUC is well known to metastasize even when local invasion of the bladder muscle wall is absent, early radical surgery has been recommended for MPUC, as opposed to cases of conventional non-MPUC (8).

In an initial study of 35 urothelial carcinoma, which included a subset of cases used in this study, a single *ERBB2* mutation was identified in the only MPUC case profiled (13). In this expanded study, a significant enrichment of *ERBB2* mutation in MPUC (40%) versus non-MPUC (9.4%) was observed ($P < 0.0084$). When compared with the COSMIC database, which contains protein altering mutations in *ERBB2* in 2 of 158 (1.4%) carcinomas of the urinary bladder (15), the enrichment in MPUC is also highly significant ($P < 0.0001$). In the Bladder Urothelial Carcinoma TCGA dataset, although point mutations in *ERBB2* are not described, amplification of *ERBB2* was listed in 6% (9/150) of cases (The cBio Cancer Genomics Portal, April 2013). An enrichment of *ERBB2* mutation within a common cancer subtype has also been recently described in a series of *CDH1*-mutated invasive lobular carcinomas of the breast with a frequency of 23% compared with a frequency of 2% in all breast cancers (19). Separate studies have reported *ERBB2* amplification predominantly based on FISH analysis in 8% to 9% of primary urothelial carcinomas, and at a higher frequency in lymph node metastases (20). In addition, in a study of non–muscle invasive bladder cancers, *ERBB2* amplification has been observed in high-grade urothelial carcinomas (HG-UC) at a similar incidence of 9%, but not in any of the papillary urothelial neoplasms of low malignant potential or low-grade urothelial carcinomas studied, and has been associated with recurrence and progression in high-grade urothelial carcinoma (21). Her2 overexpression has been identified in 19% (22/116) of bladder cancers, with significant enrichment in grade III and

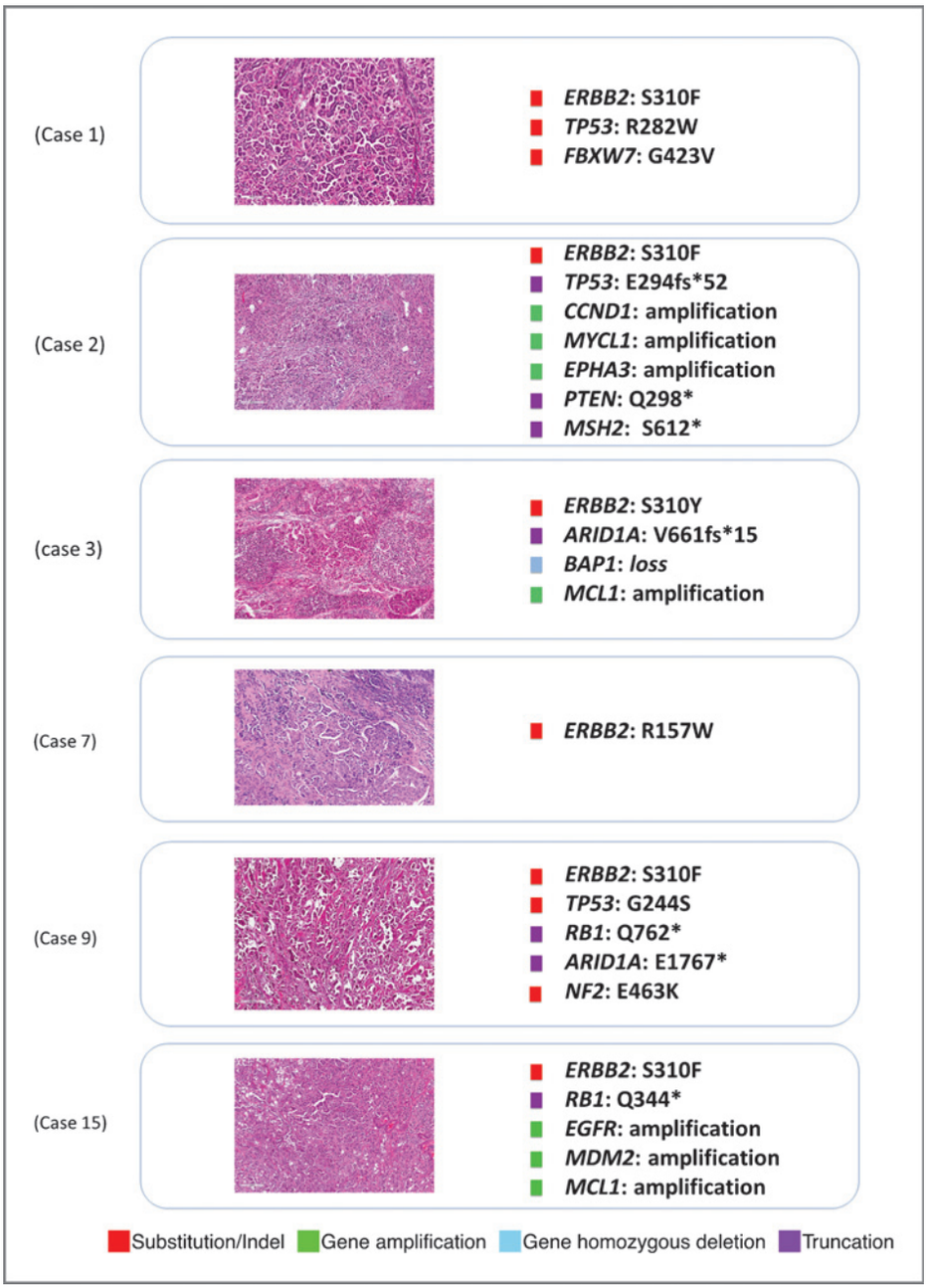

Figure 2. Histology and list of genomic alterations in 6 cases of micropapillary urothelial carcinoma featuring mutations in the *ERBB2* gene.

muscle invasive tumors (22). However, studies have reported inconsistent results about the prognostic value of HER2 expression detected by IHC (23). In this study, 3 (100%) of *ERBB2* mutated MPUC were negative for HER2 expression by IHC.

All 6 *ERBB2* mutations identified in MPUC in this study were localized to the extracellular domain, with 5 of the 6 mutations at S310, and no mutations were found within the tyrosine kinase domain. This contrasts with other tumor types where the majority of *ERBB2* mutations are located within the kinase domain. In all tissues described in COSMIC, lung adenocarcinoma, and breast cancer, 78%, 97%, and 81% of *ERBB2* alterations are located within the kinase domain, respectively (Fig. 3). In addition, *ERBB2* alterations in lung cancer are predominantly in-frame insertion mutations in the kinase domain and breast cancer, although similar to lung cancer in the localization of the large majority of *ERBB2* alterations to the kinase domain are point mutation, with in-frame insertions relatively uncommon (15). Although the S310Y/F mutations have been characterized as oncogenic (24), the biological underpinnings of the extracellular domain location of the MUPC *ERBB2* point mutations are unclear and warrant further investigation.

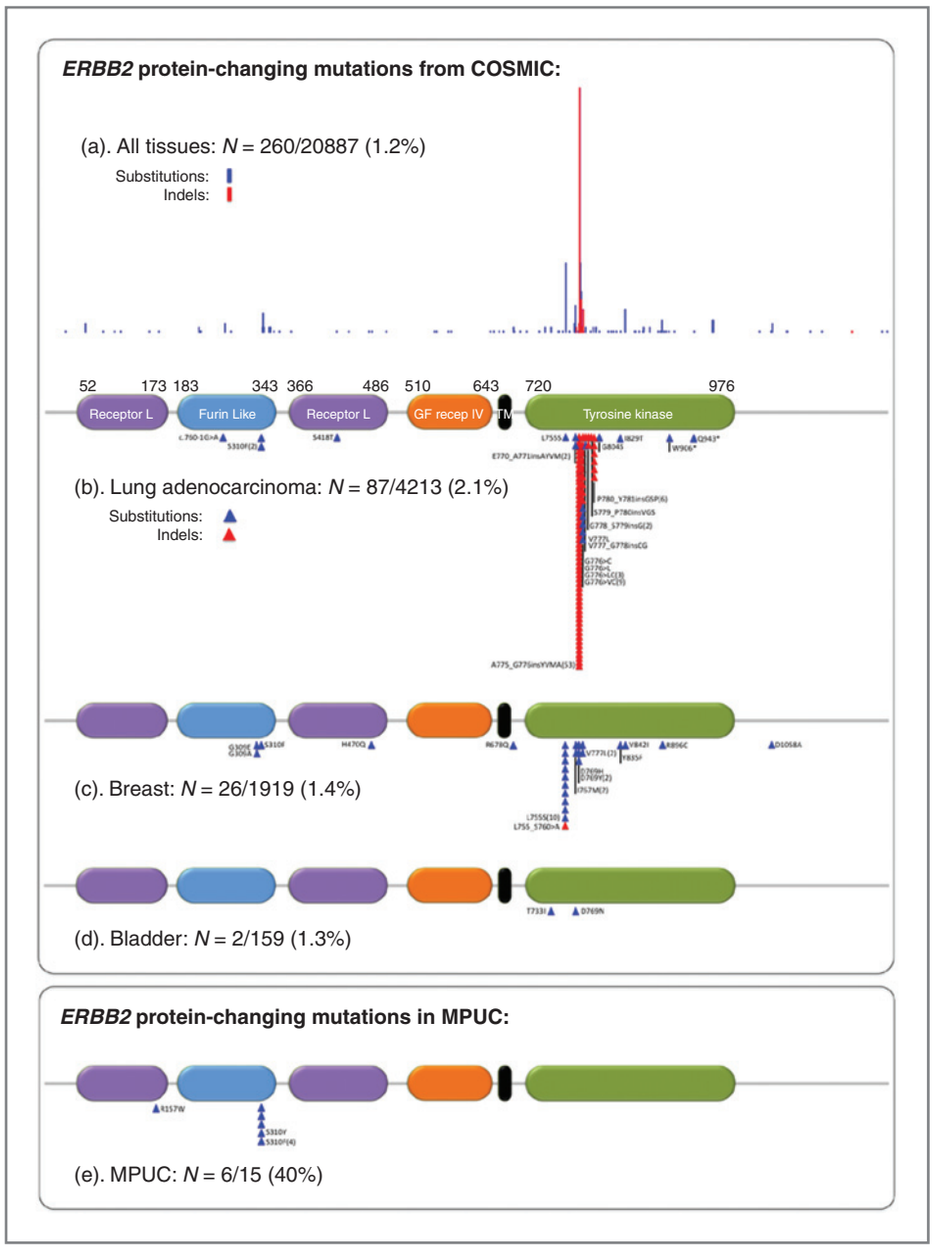

Figure 3. Relative incidence of ERBB2 mutations in lung cancer, breast cancer, urinary bladder cancer (all urothelial carcinomas) in the COSMIC database, and micropapillary urothelial carcinoma in this study.

It is now established that, in addition to *ERBB2* amplification, activating *ERBB2* mutations may also predict sensitivity to anti-HER2–targeted therapies (23–27). Irreversible HER2 (ERBB2) inhibitors are emerging and seem to show greater potency and durability than on the market reversible inhibitors in both clinical and preclinical settings (25, 26). The S310F/Y ERBB2 extracellular domain mutations seen in 5 cases of MPUC and 3 cases of urothelial carcinoma are considered to be an activating mutation and sensitive to irreversible dual Egfr/Erbb2 inhibitors (24, 28–30). Although kinase domain alterations in *ERBB2* are considered to be homologous to those encountered in the *EGFR* gene (24), the effect of the S310F/Y extracellular domain ERBB2 mutations found in the MPUC cases cannot be as easily extrapolated from EGFR extracellular domain mutations characterized to date. Although the mechanism of receptor activation has not yet been characterized for these EGFR extracellular domain mutations, it is tempting to speculate that the underlying tumorigenic mechanism is caused by a less tethered conformation of the extracellular domain as most amino acid substitutions localize to interdomain interfaces (28).

Currently available therapies targeted to Her2, such as trastuzumab and lapatinib, are under investigation for treatment of *ERBB2*-amplified urothelial carcinomas; however, phase III trial data has yet to emerge (31). For

nonamplified, point-mutated bladder cancers such as the 6 *ERBB2*-mutated MPUC analyzed in this study, the standard tests for *HER2* (*ERBB2*) amplification/overexpression status (IHC and FISH) were uniformly negative, and thus these aggressive tumors would not have been detected as being driven by *ERBB2* activation. Recent clinical trials for breast and lung cancers bring promise of targeting *ERBB2*-mutated (HER2 IHC/FISH negative) tumors, and results from this study argues that this approach should be extended to urinary bladder cancer, especially when the tumor features an MPUC pattern. The micropapillary architecture and well-documented aggressive clinical course attributed to MPUC has also been linked to micropapillary carcinomas of the endometrium, breast, and lung (10–12); however, no association with *ERBB2* mutations in these other aggressive types of micropapillary carcinomas has been reported. With the ability to identify functionally significant alterations that may not be observed with standard IHC analysis, this study illustrates the impact of histologic subtyping based on the genomic landscape and the resulting potential to elucidate targeted therapies that may be applicable.

Disclosure of Potential Conflicts of Interest

J.S. Ross is employed (other than primary affiliation; e.g., consulting) as a medical director in Foundation Medicine, Inc. J.S. Ross has commercial research grant from Foundation Medicine, Inc. and has ownership interest (including patents) in Foundation Medicine, Inc. G.A. Palmer is employed (other than primary affiliation; e.g., consulting) as a VP in Foundation Medicine, Inc. G.A. Palmer has ownership interest (including patents) in Foundation Medicine, Inc. S. Ali is employed (other than primary affiliation; e.g., consulting) as an associate director in Foundation Medicine, Inc. S. Ali has ownership interest (including patents) in Foundation Medicine, Inc. G. M. Frampton is employed (other than primary affiliation; e.g., consulting) as a scientist in Foundation Medicine, Inc. G.M. Frampton has ownership interest (including patents) in Foundation Medicine, Inc. J. Curran is employed (other than primary affiliation; e.g., consulting) as a medical director in Foundation Medicine, Inc. J. Curran has commercial research grant from Foundation Medicine, Inc. J. Curran has ownership interest (including patents) in Foundation Medicine, Inc. S.R. Downing has ownership interest (including patents) in Foundation Medicine, Inc. R. Yelensky has ownership interest (including patents) in Foundation Medicine, Inc. D. Lipson is employed (other than primary affiliation; e.g., consulting) as a Director in Foundation Medicine, Inc. D. Lipson has ownership interest (including patents) in Foundation Medicine, Inc. M. Hawryluk has ownership interest (including patents) in Foundation Medicine, Inc. P.J. Stephens is employed (other than primary affiliation; e.g., consulting) as a VP Cancer Genomics in Foundation Medicine, Inc. P.J. Stephens has ownership interest (including patents) in Foundation Medicine, Inc. No potential conflicts of interest were disclosed by the other authors.

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