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KDM6A expression loss is a common feature in low-grade non-invasive urothelial carcinomas of the urinary bladder

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Introduction and Objectives

KDM6A (syn. UTX) is an epigenetic regulator which is frequently mutated in urothelial carcinoma. Because of its location on the X-chromosome, the KDM6A gene is either single copy (males) or monoallelic due to imprinting (females). Truncating mutations should therefore lead to complete expression loss. Here we described the prevalence and clinical significance of KDM6A expression loss in the bladder urothelium.

Materials and Methods

A tissue microarray with more than 2,500 urinary bladder cancers, including 636 patients who underwent radical cystectomy for muscle-invasive disease (pT2-4), was analyzed by KDM6A immunohistochemistry (IHC). Selected tumors with and without KDM6A expression loss (n=78) were also sequenced.

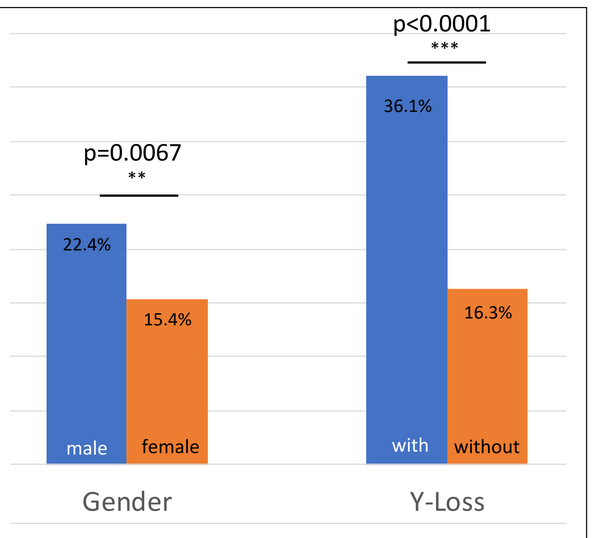
Immunostaining protocol

Antibody: ardoci GmbH, Hamburg, Germany , clone HMV311, Recombinant rabbit monoclonal IgG, Dilution: 1:150

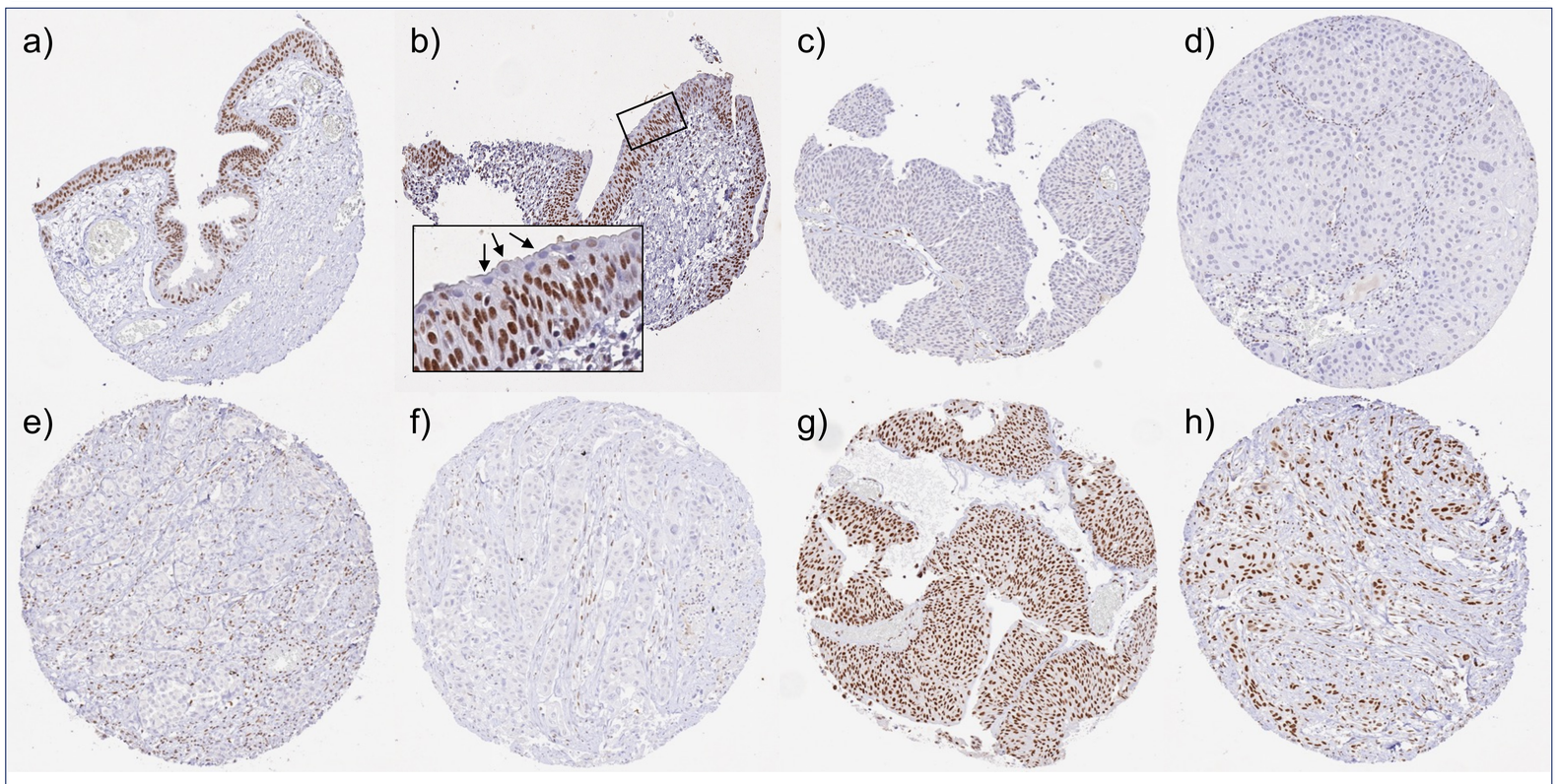
Antigen retrieval: 5 min at 121°C (autoclave) in pH 7.8 buffer

KDM6A, patient gender and Y-chromosome loss

KDM6A expression loss was generally more frequent in male than in female patients. Moreover, in males, KDM6A expression loss was linked to genomic deletion of the Y-chromosome (all analyses limited to the subset of cancers with muscle invasive disease).



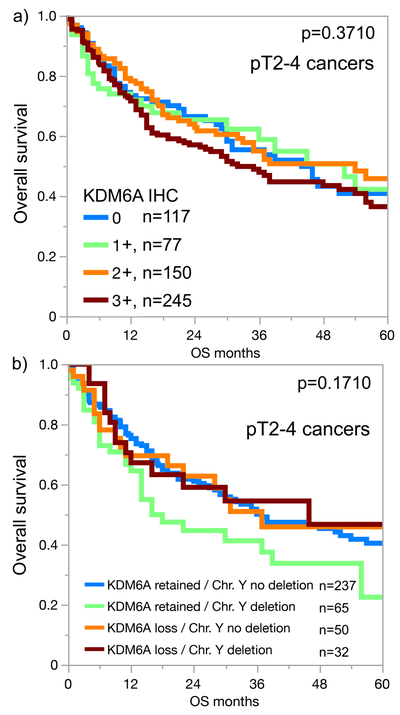
Results



The panels show strong nuclear staining of the normal urothelium of the urinary bladder a-b), b) often with less intense staining of the umbrella cells (indicated by arrows in the inset) as compared to the remaining epithelial cells, loss of KDM6A staining in c) a non-invasive pTa low grade, in d) a non-invasive pTa high grade carcinoma, and in e-f) two muscle invasive urothelial carcinomas, and retained KDM6A staining in g) a non-invasive pTa and h) a muscle invasive carcinoma.

KDM6A expression loss, tumor phenotype, patient prognosis

	n	KMD6A IHC result: loss (%)	retained (%)	P
All cancers	2125	22.8	77.2	
pTa G2 low	350	36.0	64.0	
pTa G2 high	152	23.0	77.0	0.0004
pTa G3	92	18.5	81.5	
pT2	378	17.2	82.8	
pT3	503	21.9	78.1	0.1894
pT4	247	18.2	81.8	
G2*	85	18.8	81.2	0.8466
G3*	1021	19.7	80.3	
pN0*	546	18.9	81.1	0.7870
pN+*	378	19.6	80.4	
R0*	463	21.2	78.8	0.4856
R1*	115	18.3	81.7	
L0*	209	17.7	82.3	0.4138
L1*	231	20.8	79.2	
V0*	355	18.3	81.7	0.1894
V1*	126	23.8	76.2	
Pn0*	55	20.0	80.0	0.4703
Pn1*	79	25.3	74.7	



KDM6A deficiency occurred most frequently in low-grade non-invasive bladder cancers.

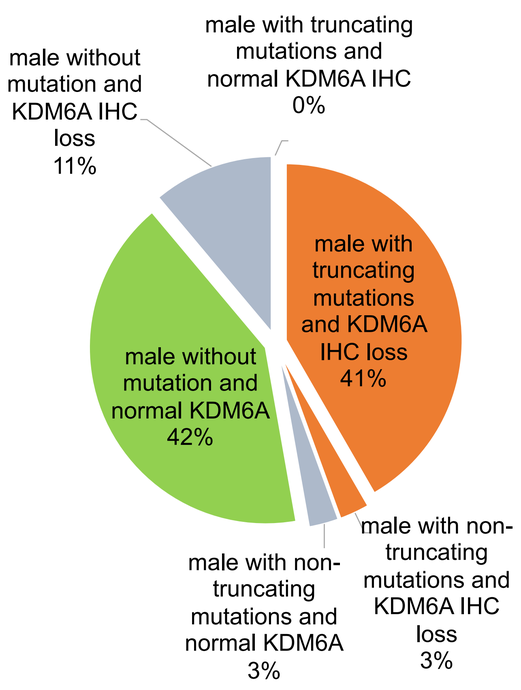
KDM6A expression levels were unrelated to patient prognosis in muscle invasive cancers.

The combined analysis of KDM6A deficiency and Y-chromosome loss did not provide prognostic information in muscle invasive cancers.

KDM6A mutation and expression loss in male and female patients

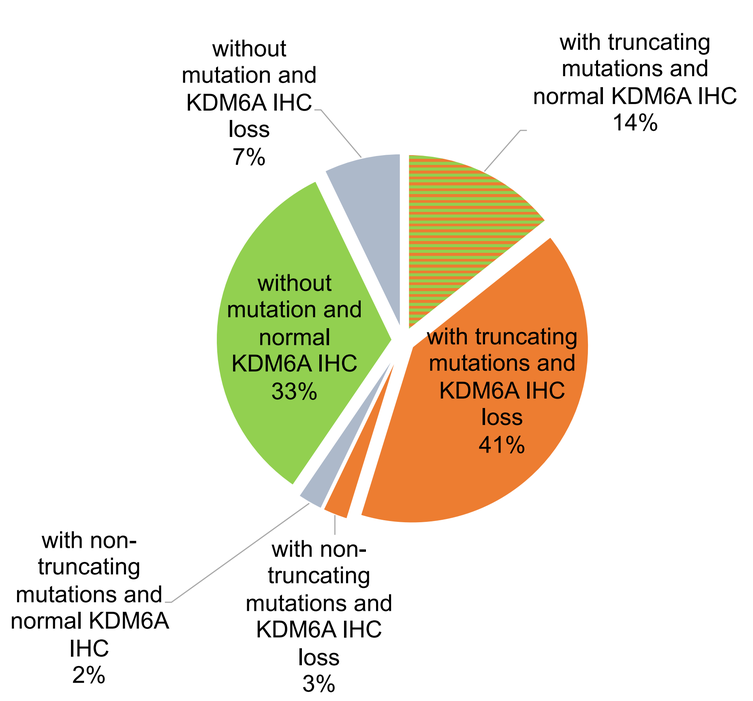
In male patients, all cancers with truncating KDM6A mutations had a complete loss of KDM6A expression.

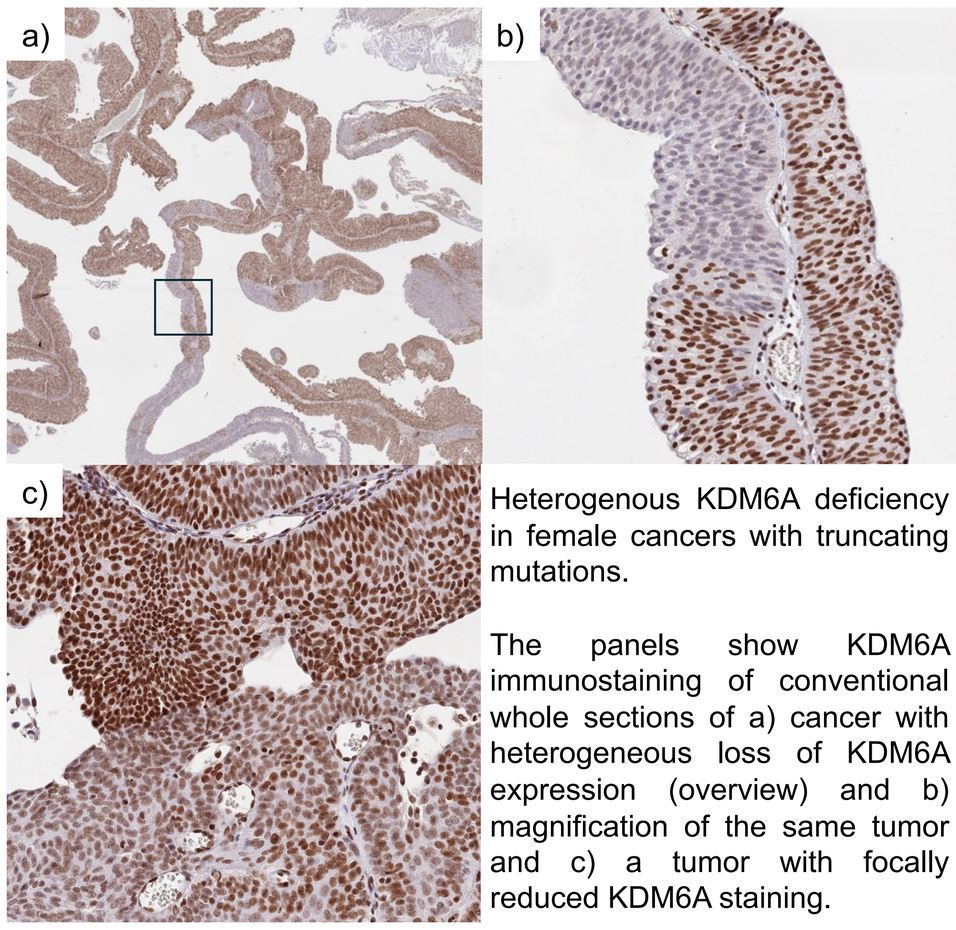
male patients (n=36)



In female patients, only 74% of cancers with truncating KDM6A mutations had a complete loss of KDM6A expression, providing striking evidence for incomplete imprinting of the KDM6A gene.

female patients (n=42)





Heterogenous KDM6A deficiency in female cancers with truncating mutations.

The panels show KDM6A immunostaining of conventional whole sections of a) cancer with heterogeneous loss of KDM6A expression (overview) and b) magnification of the same tumor and c) a tumor with focally reduced KDM6A staining.

Summary and Conclusions

- Our data demonstrate that truncating KDM6A mutations occur in a significant subset of urothelial carcinomas which is linked to low-grade non-invasive cancer, male gender and loss of the Y chromosome.
- The predominance of KDM6A loss in low-grade tumors makes KDM6A IHC a promising new tool for the identification of low-grade dysplasia in biopsies and early bladder cancer detection in cytology.

[1]Uhlen et al. A proposal for validation of antibodies. Nat Methods. 2016;13(10):823-7

Conflicts of interest: The KDM6A (HMV311) antibody clone was provided by ardoci GmbH (owned by a family member of GS)

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