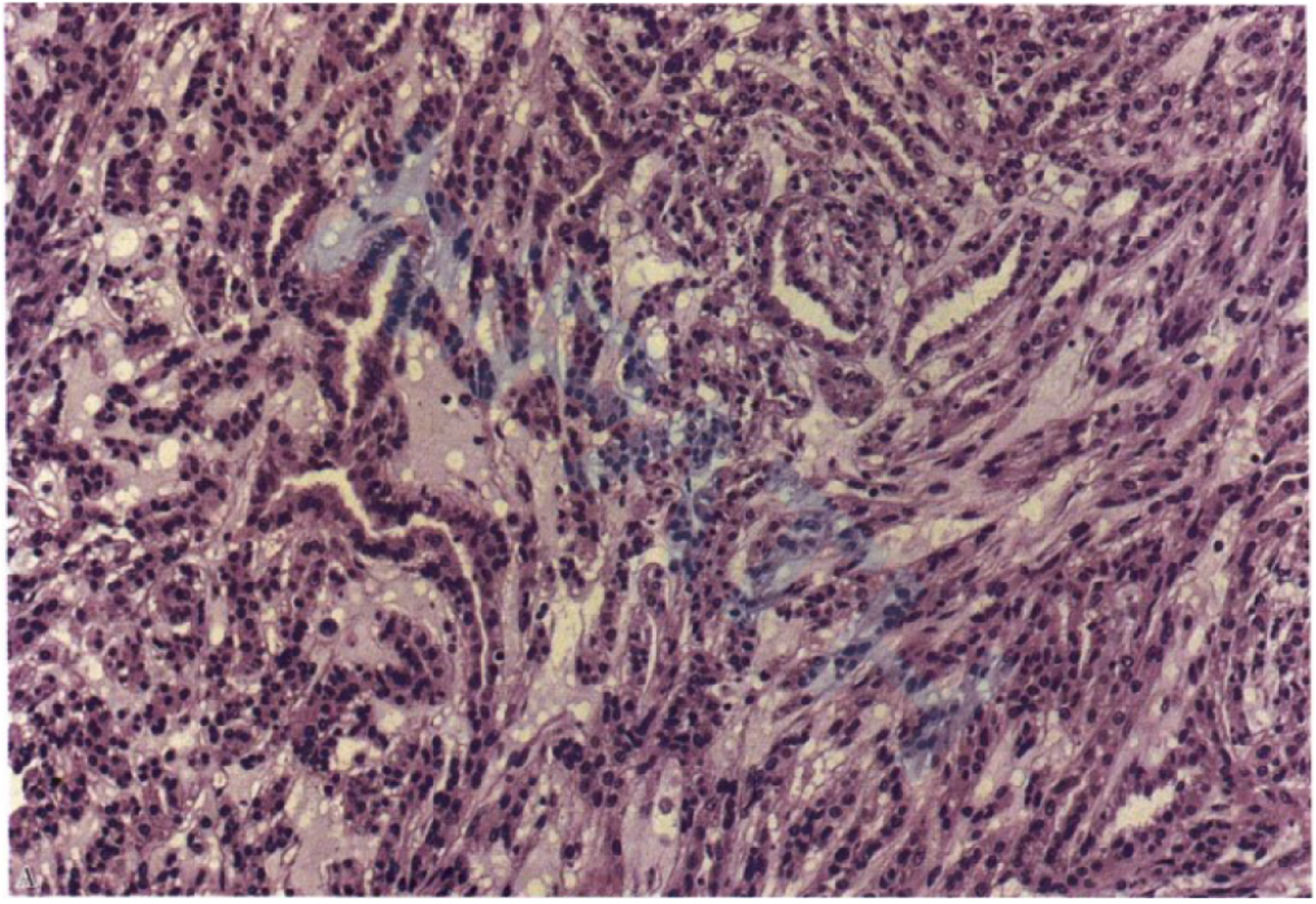
Am J Surg Pathol Volume 42, Number 12, December 2018

VSTM2A Overexpression Is a Sensitive and Specific Biomarker for Mucinous Tubular and Spindle Cell Carcinoma (MTSCC) of the Kidney

汇报人：颜临丽

黏液小管状和梭形细胞癌mucinous tubular and spindle cell carcinoma (MTSCC)

- 定义：MTSCC是一种肾脏上皮性肿瘤，特征性的由管状结构组成，合并有温和的梭形细胞和黏液样间质。
- 发病率：<1%肾脏肿瘤，年龄13-81岁（平均：58岁），女：男=3：1
- 部位：主要位于肾皮质，也可以发生在髓质
- 大体：界限清楚的实性包块，切面灰黄-棕褐色
- 组织学：肿瘤由紧密排列的、小而狭长的小管组成，特征性的移行为梭形细胞。小管细胞立方，局部胞浆透明、嗜酸性或空泡状。核级为低级别。间质为嗜碱性黏液。
- 有报道一些MTSCC病例，肿瘤含有高级别核和肉瘤样变。



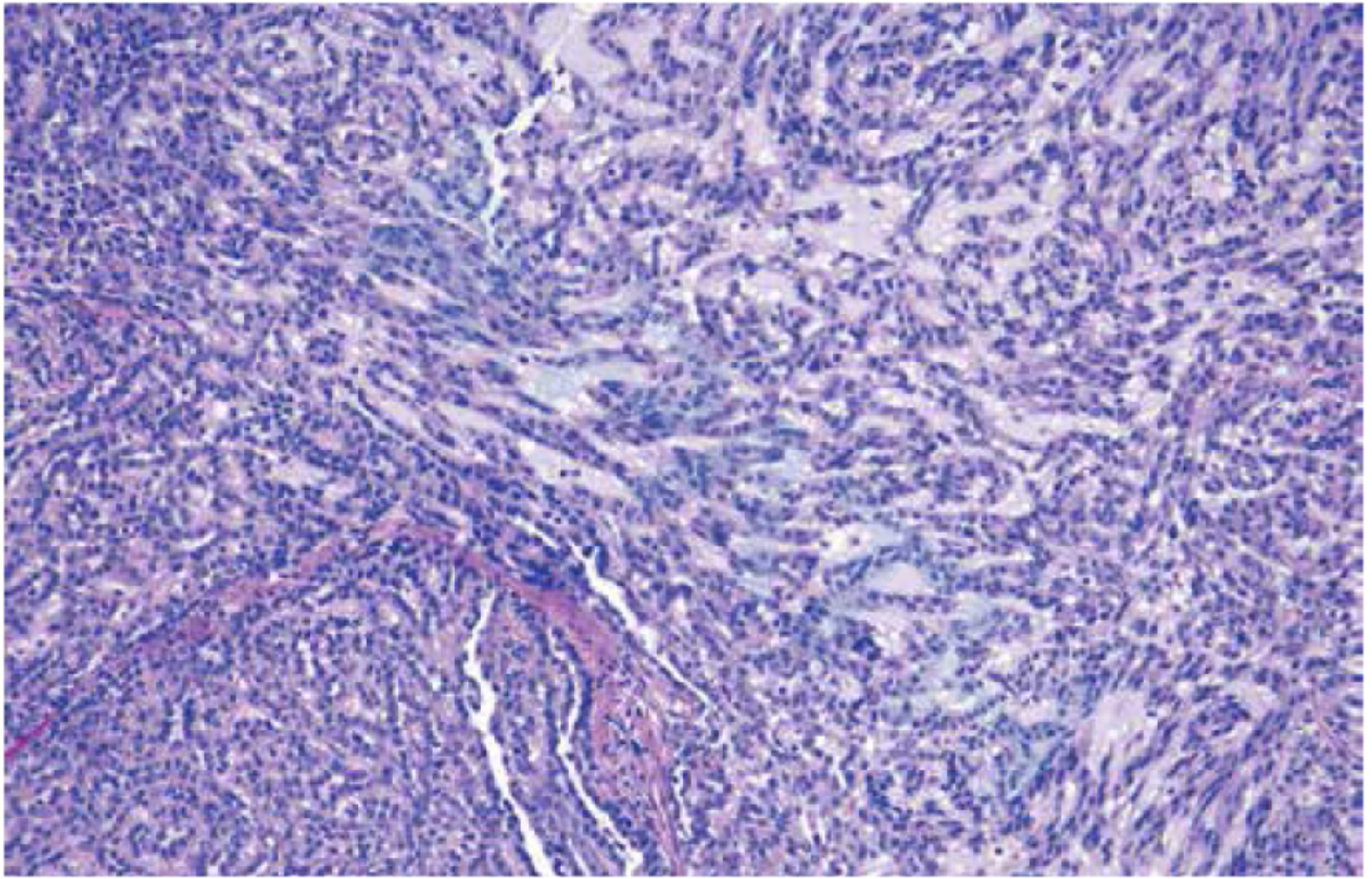


Fig. 1.35 Mucinous tubular and spindle cell carcinoma. The tumour is composed of tubular cells, which are commonly elongated or cord-like, and spindle cells with bland nuclei; abundant mucin is observed in the stroma.

黏液小管状和梭形细胞癌mucinous tubular and spindle cell carcinoma (MTSCC)

- 免疫组化：CK7、PAX2、P504S阳性
- 遗传学：比较基因组杂交技术显示低级别肿瘤有多种染色体的缺失，包括1，4，6，8,9，13，14，15，和22 。细胞遗传学分析显示1例高级别肿瘤伴有肉瘤样转化的由染色体14和15的缺失，及染色体2，5，7，9，10，12，17，19,20，22，and X的获得
- 预后：大多数肿惰性进程，罕见复发，伴有高级别转化的肿瘤可能远处转移及致命

BACKGOUND

- Recent large next-generation sequencing (NGS) data sets of renal cell carcinoma (RCC) provide opportunities to discover and characterize biomarkers, disease mechanisms, tumor phenotypes, and therapeutic targets.
- To better understand the etiology and molecular subtypes of RCC, we sought to identify cancer-specific and lineage-specific biomarkers by performing an integrative analysis of RNA sequencing (RNA-seq) data from TCGA (The Cancer Genome Atlas) index samples, MCTP (Michigan Center for Translational Pathology) cohorts, and the Knepper data set of microdissected rat nephrons

- MTSCC cases occasionally show extensive morphologic and immunohistochemical overlap with PRCC, especially PRCC with low-grade spindle cell foci

- VSTM2A (V-set transmembrane domain containing 2A) and IRX5 (Iroquois homeobox gene 5) were identified as cancer-specific and lineage-specific biomarkers in MTSCC, respectively

MATERIALS AND METHODS

- RCC Cohorts
- RNA-seq Analysis
- RNA In Situ Hybridization
- Fluorescence In Situ Hybridization
- Statistical Analysis

RNA In Situ Hybridization

- The RNA ISH signal was identified as brown, punctate dots, and the expression level was scored as follows:

0= no staining or<1 dot per 10 cells,

1 = 1 to 3 dots per cell,

2=4 to 9 dots per cell (few or no dot clusters),

3= 10 to 15 dots per cell(<10% in dot clusters),

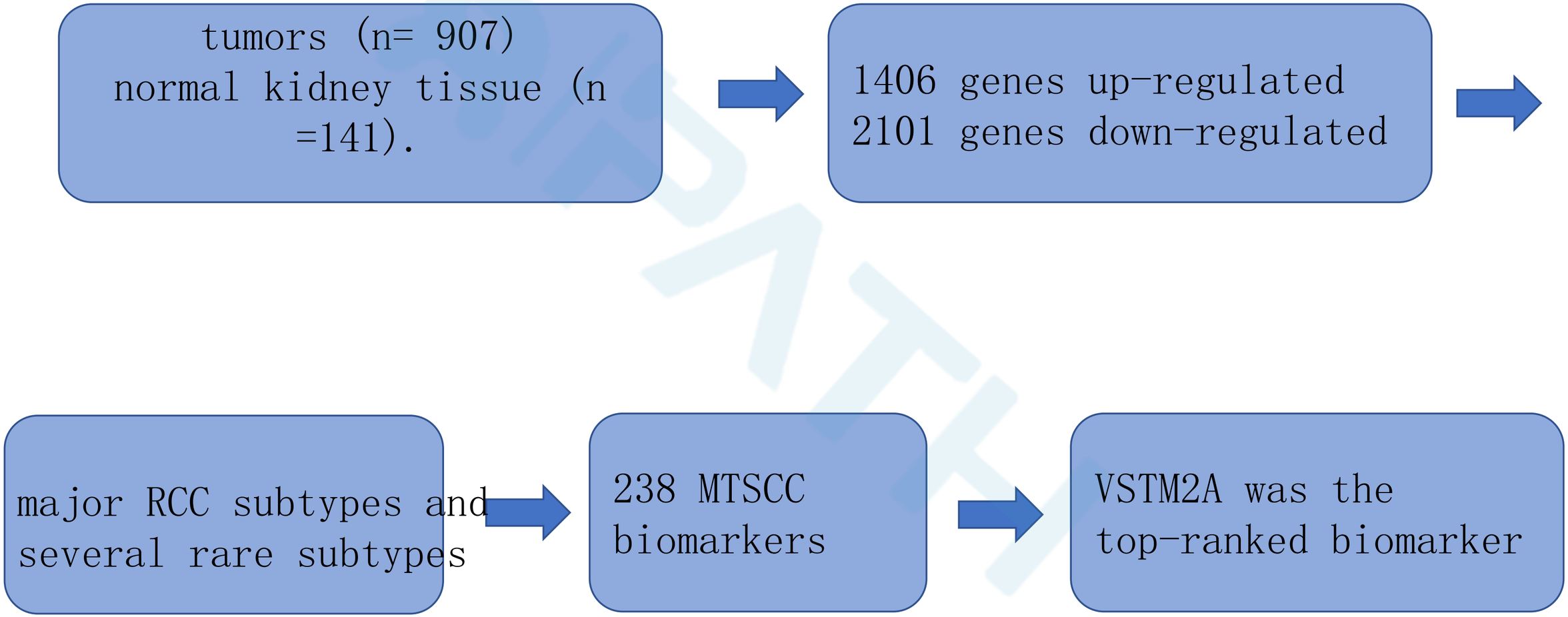
4= >15 dots per cell (>10% in dot clusters).

- As previously described, a cumulative RNA ISH product score was calculated for each evaluable tissue core as the sum of the individual products of the expression level (0 to 4) and percentage of cells [0 to 100; ie, (A%×0)+(B%×1)+(C%×2)+(D%×3)+(E%×4); total range= 0 to 400]

RESULTS

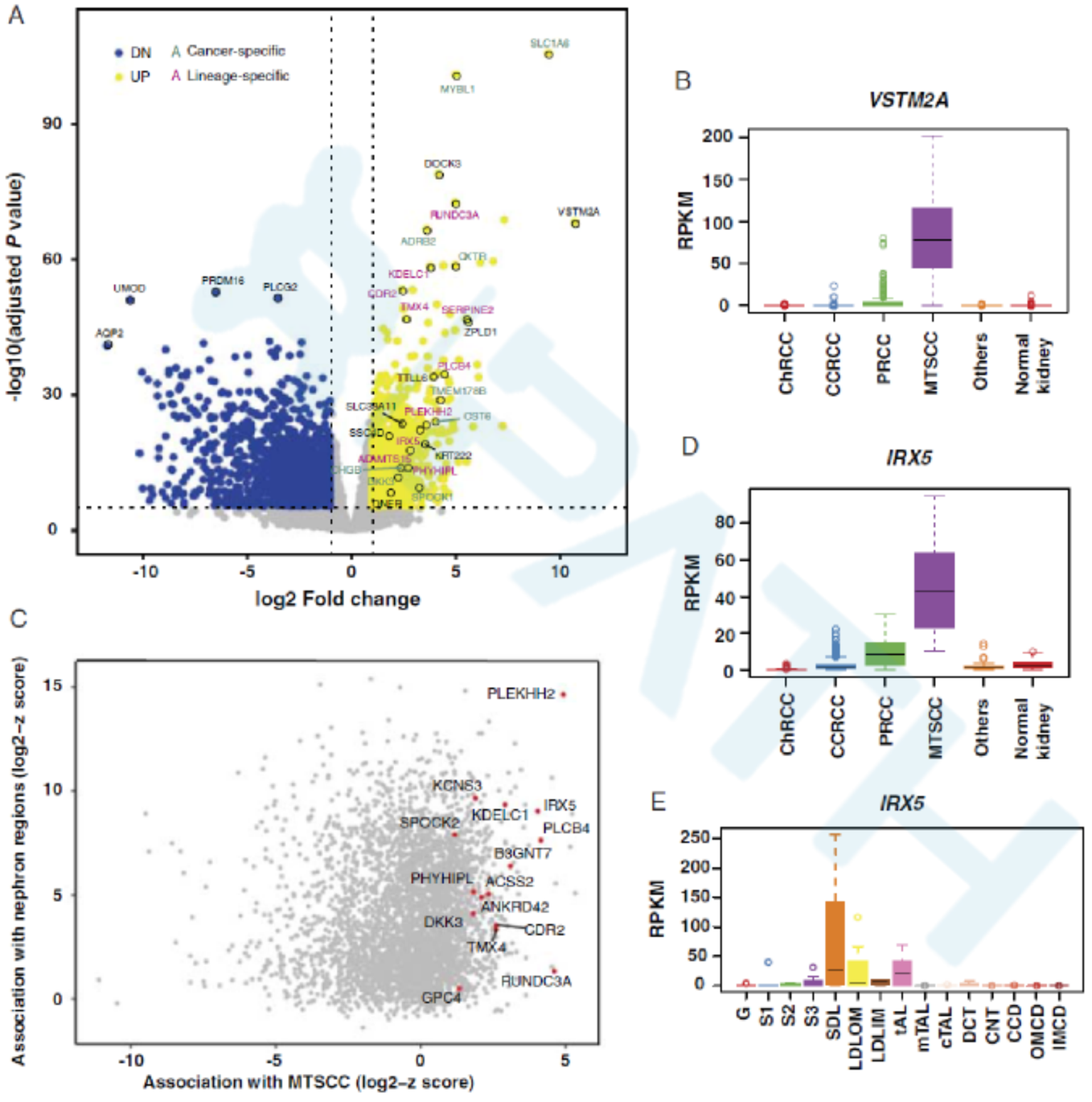
- Nomination of Cancer-specific and Lineage-specific Biomarkers in MTSCC
- Clinical Characteristics of the Cohort
- VSTM2A is a Cancer-specific Marker for MTSCC
- IRX5 is a Lineage-specific Biomarker for MTSCC
- Diagnostic Performance of VSTM2A and IRX5 for MTSCC

cancer-specific biomarkers



lineage-specific biomarkers

We noted that in addition to overexpression of IRX5 in MTSCC tumors (4-fold change and ranked No. 15），certain nephron segments, that is in the loop of Henle, also expressed this gene。


A
DN
UP
A Cancer-specific
A Lineage-specific
-log10(adjusted P value)
log2 Fold change
SLC1A6
MYBL1
DOCK3
RUNDC3A
VSTM2A
ADRB2
OXTR
KDELC1
CDR2
TMX4
SERPINE2
ZPLD1
PLCB4
TTLL6
TMEM178B
SLC38A11
PLEKHH2
GSTT2
SSC5D
IRX5
KRT222
ADAMTS15
CHGB
PHYHIPL
DKK3
SPOCK1
TMEM
UMOD
PRDM16
PLCG2
AQP2
B
VSTM2A
RPKM
ChRCC
CCRCC
PRCC
MTSCC
Others
Normal kidney
C
Association with nephron regions (log2−z score)
Association with MTSCC (log2−z score)
PLEKHH2
KCNS3
IRX5
KDELC1
SPOCK2
PLCB4
B3GNT7
PHYHIPL
ACSS2
ANKRD42
DKK3
CDR2
TMX4
RUNDC3A
GPC4
D
IRX5
RPKM
ChRCC
CCRCC
PRCC
MTSCC
Others
Normal kidney
E
IRX5
RPKM
G
S1
S2
S3
SDL
LDLOM
LDLIM
tAL
mTAL
cTAL
DCT
CNT
CCD
OMCD
IMCD

RCC Cohorts

TABLE 1. Clinical Characteristics of the Study Cases

Tumor Type	Total Patients (n)	Age (y)*	Male/ Female (Ratio)	Tumor Size (cm)*	WHO/ ISUP Grade
MTSCC	33	62 (21-78) 69 (53-82)	0.53:1 0:1	4.2 (1.3-16.5) 4.7 (3-6.6)	Low grade (29) High grade (4)
Type 1 PRCC	40	60 (41-87)	4:1	3.6 (1.3-16.9)	Grade 2 (28) Grade 3 (11) Grade 4 (1)
Type 2 PRCC	8	72 (58-80)	7:1	3.6 (1.3-7.9)	Grade 3 (7) Grade 4 (1)
Unclassified RCC†	2	62	1	10.2	Grade 3 (2)
CCRCC	15	63 (30-82)	2.75:1	6.0 (2.2-12.5)	Grade 2 (3) Grade 3 (6) Grade 4 (6)
ChRCC	15	61 (37-72)	2:1	4.3 (2.3-11)	NA

*Median (range).
†One patient has missing data.
NA indicates not available.

VSTM2A RNA ISH

- almost no dots were detected in renal cortical or medullary cells
- All classic MTSCC tumors (n=29) demonstrated homogeneous moderate to high expression of VSTM2A (mean ISH score=265; range=150 to 350) ranged from 80% to 100%.
- Four MTSCC tumors with high-grade features demonstrated moderate expression of VSTM2A (mean ISH score=225; range=180 to 255) ranged from 80% to 90%.

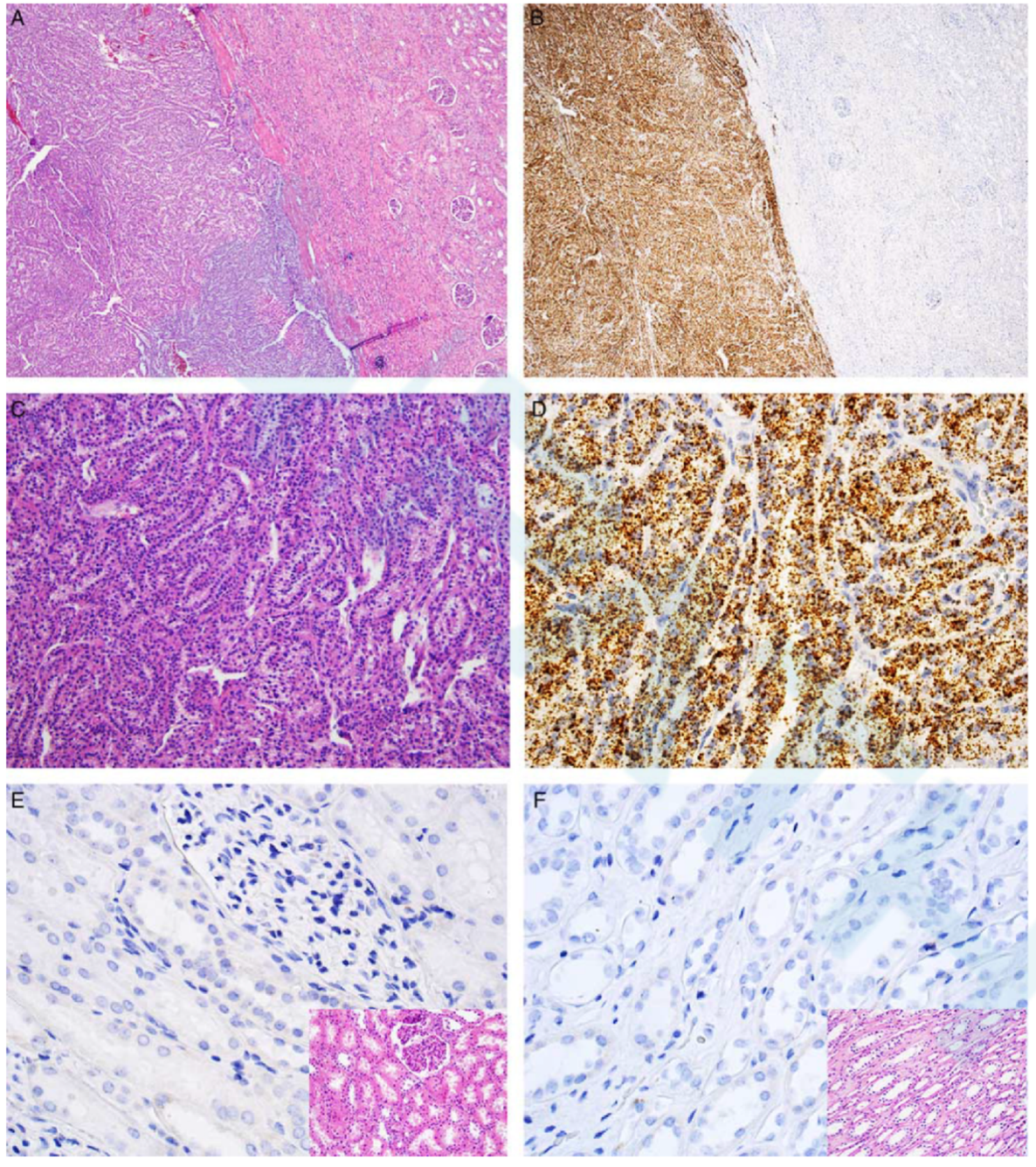
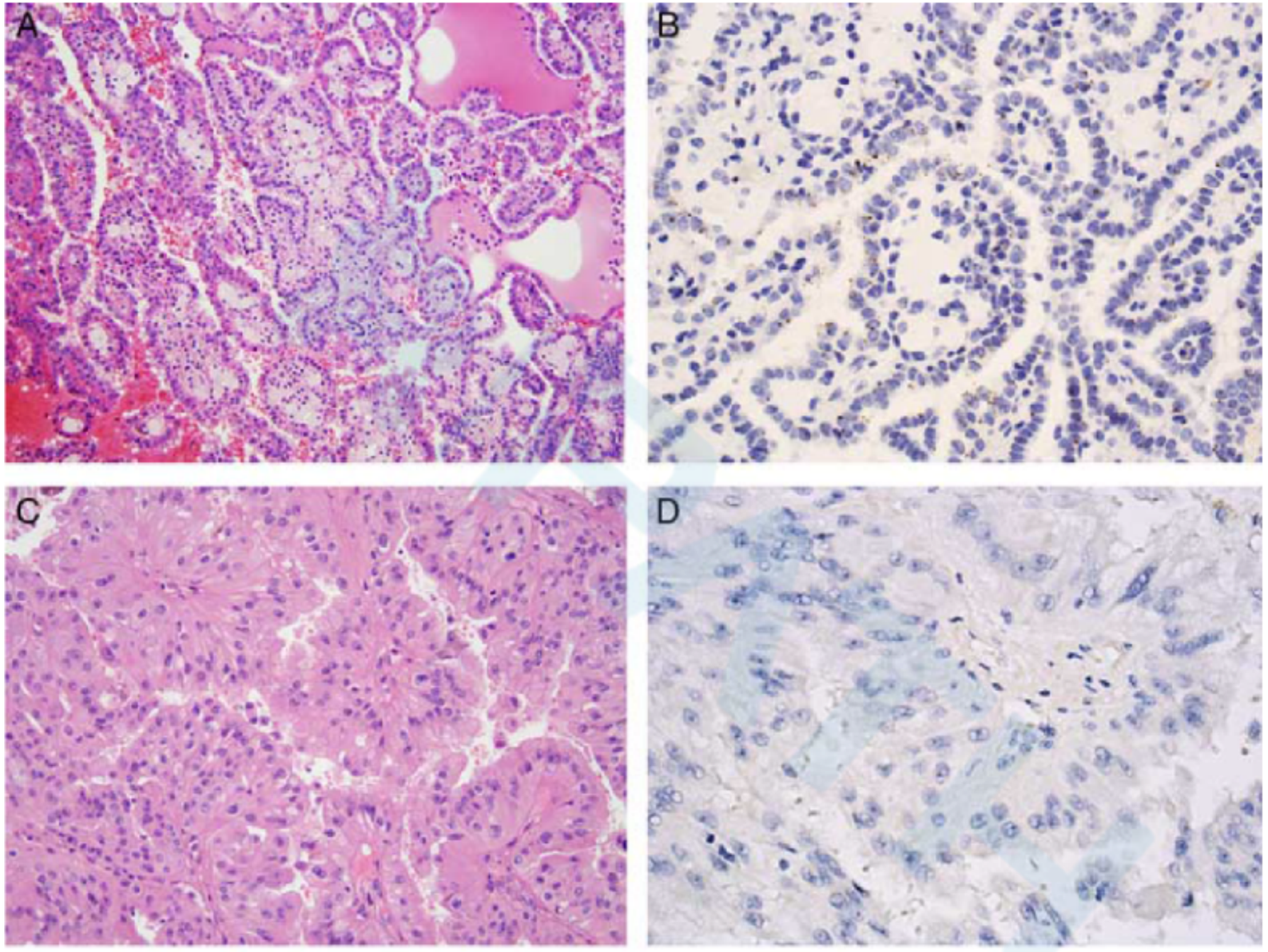
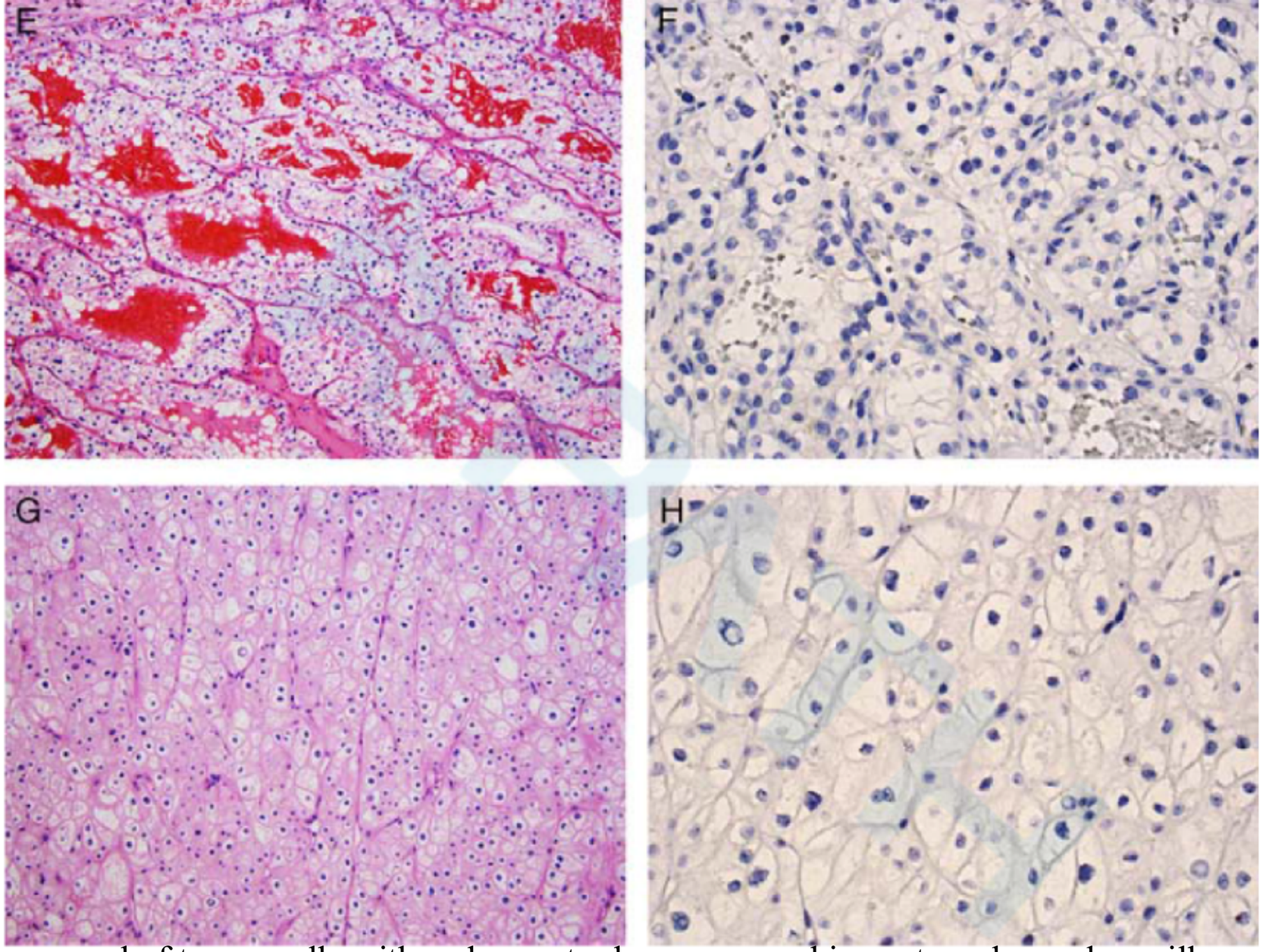


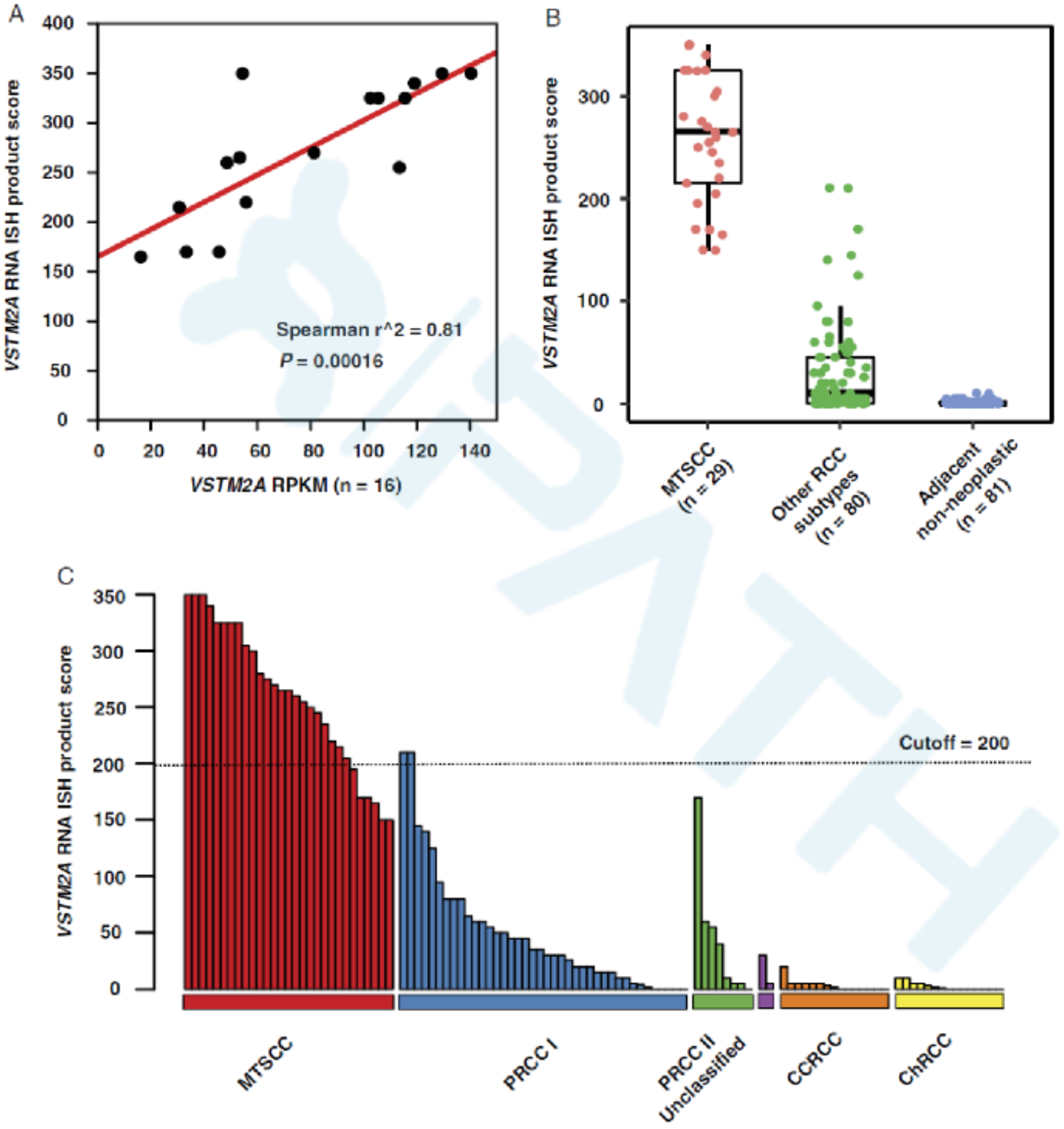
FIGURE 2. *VSTM2A* expression in MTSCC by RNA ISH. A and C, Classic MTSCC with elongated tubules in a myxoid matrix (H&E
3 and D, *VSTM2A* expression in MTSCC by RNA ISH, with brown, punctate dots and no background staining. E and F, Negativ
expression of *VSTM2A* in renal cortex and medulla, respectively; inset: H&E.



VSTM2A expression in other RCC subtypes. A, Type 1 PRCC, is characterized by small cuboidal cells covering thin papillae with a single line of uniform nuclei and small nucleoli (H&E). B, Type 1 PRCC exhibited low expression of VSTM2A by RNAISH. C, Type 2 PRCC, characterized by pseudostratified columnar epithelium on papillary cores, with abundant and eosinophil cytoplasm, large nuclei and prominent nucleoli (H&E). D, Type 2 PRCC was negative for VSTM2A expression by RNA ISH.

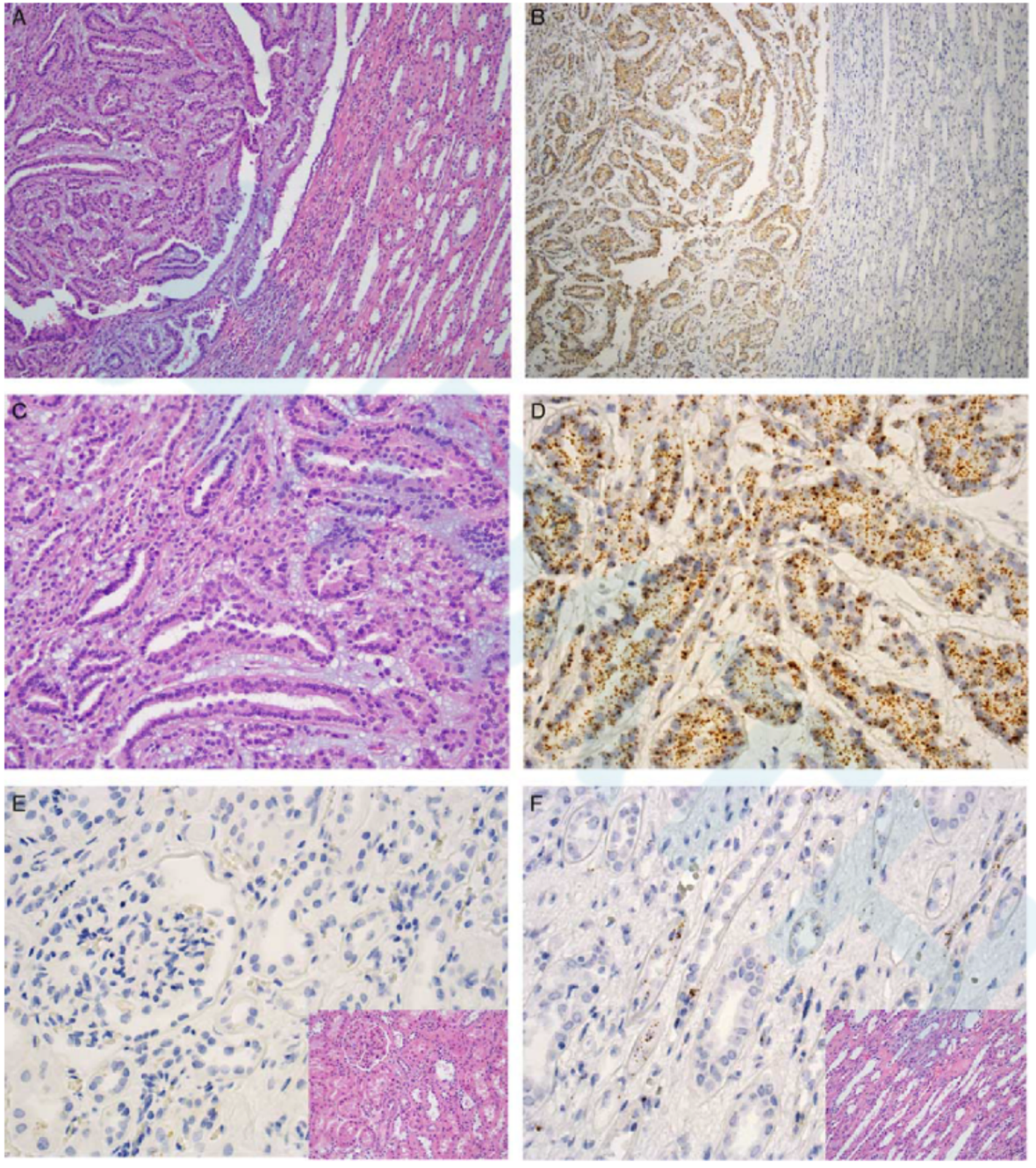


E, CCRCC is composed of tumor cells with a clear cytoplasm arranged in nests and pseudopapillary structures with a delicate vascular networkH&E). F, CCRCC was negative for VSTM2A expression by RNA ISH. G, ChRCC is composed of large cells with defined cell membranes (H&E). H, ChRCC was negative for VSTM2A expression by RNA ISH.


A
VSTM2A RNA ISH product score
VSTM2A RPKM (n = 16)
Spearman r^2 = 0.81
P = 0.00016
B
VSTM2A RNA ISH product score
MTSCC (n = 29)
Other RCC subtypes (n = 80)
Adjacent non-neoplastic (n = 81)
C
VSTM2A RNA ISH product score
Cutoff = 200
MTSCC
PRCC I
PRCC II Unclassified
CCRCC
ChRCC

IRX5 RNA ISH

- IRX5 was absent in the non-neoplastic renal cortex, but was expressed in certain medullary tubules,presumably the loop of Henle
- Classic MTSCC tumors (n=29) showed moderate to high expression of IRX5 (mean ISH score=150; range=0 to 300) ranged from 0% to 100%
- Four high grade MTSCC tumors demonstrated low expression of IRX5 (mean ISH score=70; range=50 to 120) ranged from 40% to 60%.



GURE 5. *IRX5* expression in MTSCC by RNA ISH. A and C, Classic MTSCC (H&E). B and D, *IRX5* expression in MTSCC by RNA H. E, Negative expression of *IRX5* in renal cortex by RNA ISH; inset: H&E. F, Certain tubules stained positive for *IRX5* in renal edulla by RNA ISH; inset: H&E.

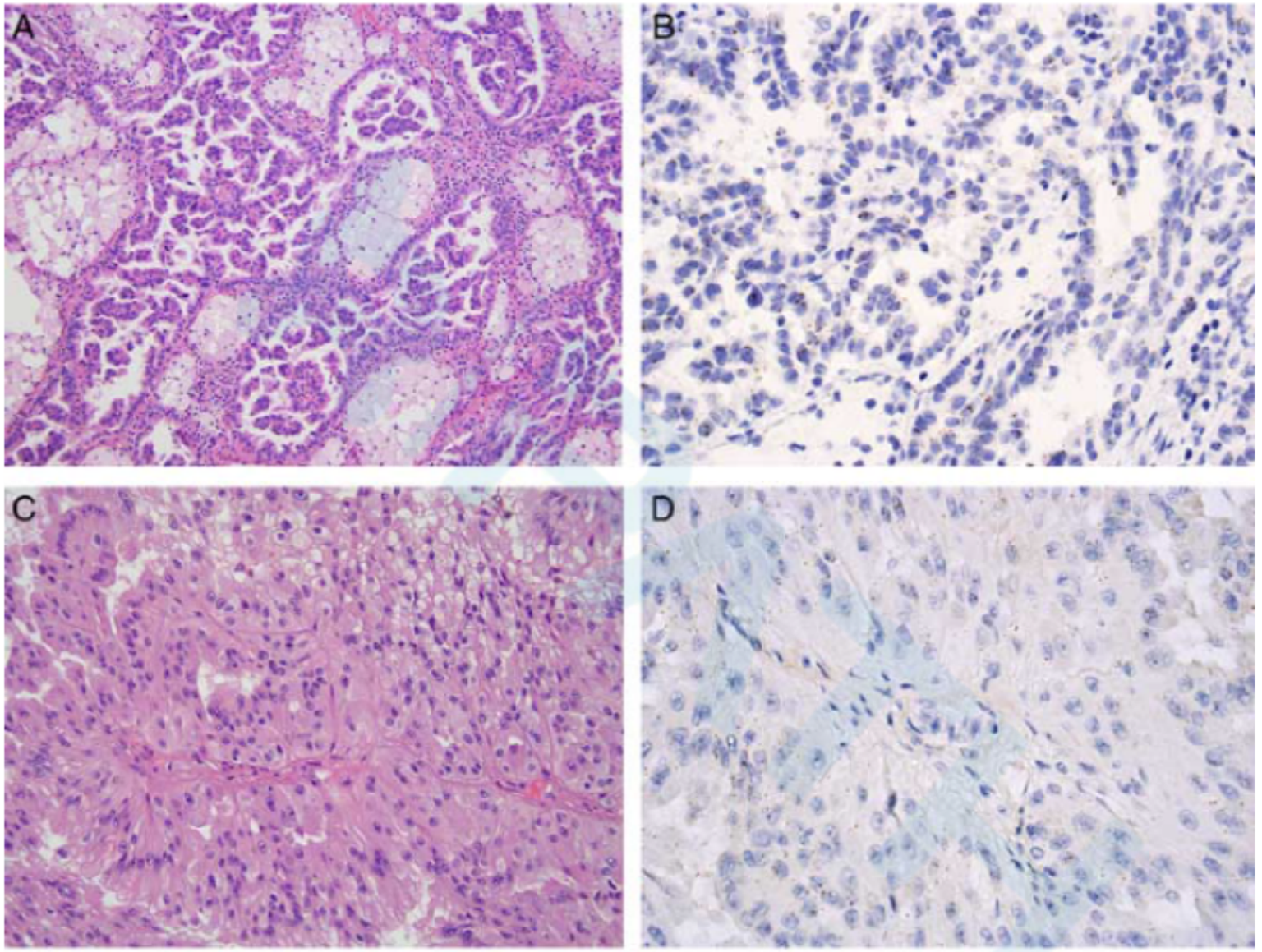
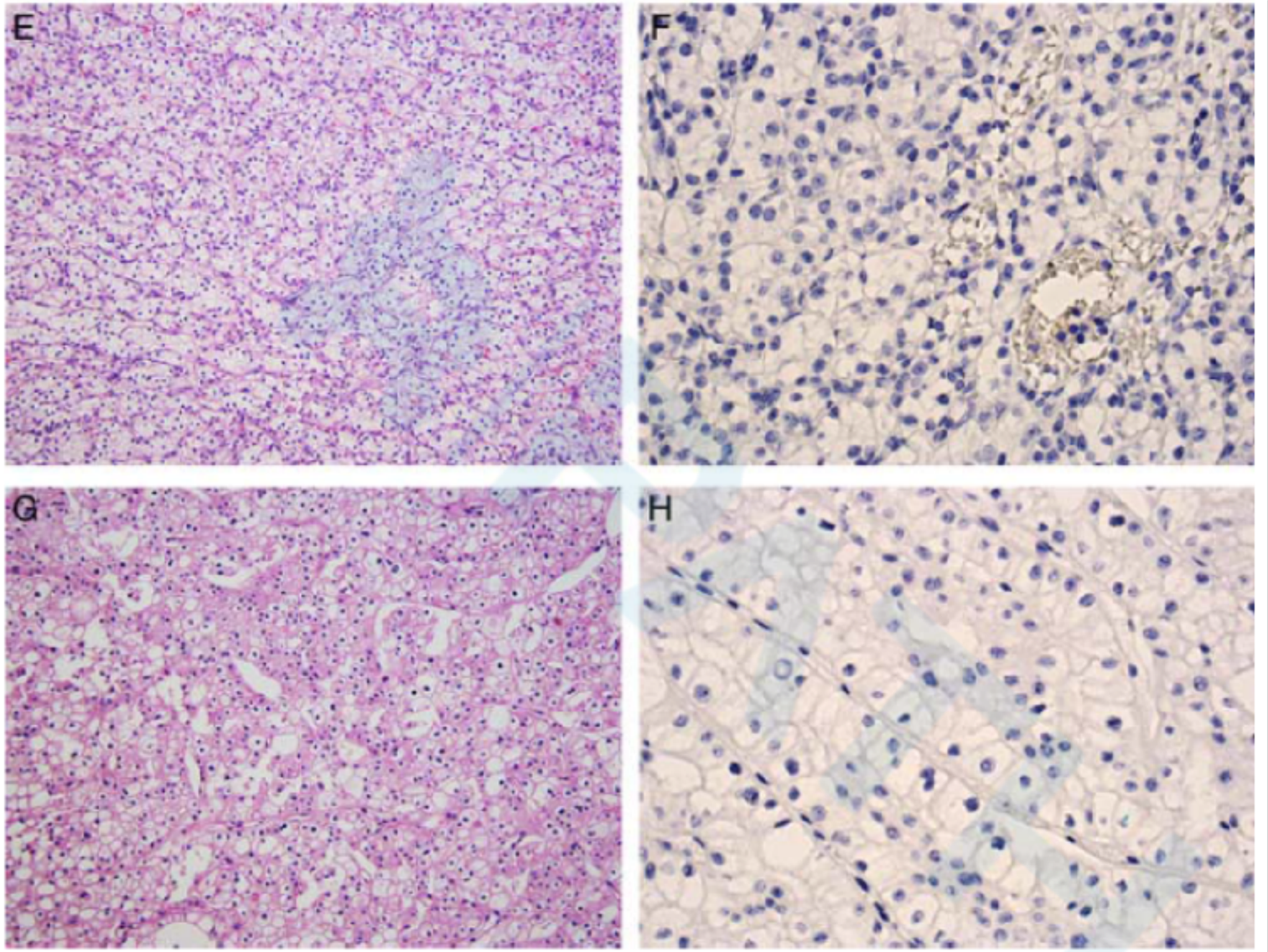
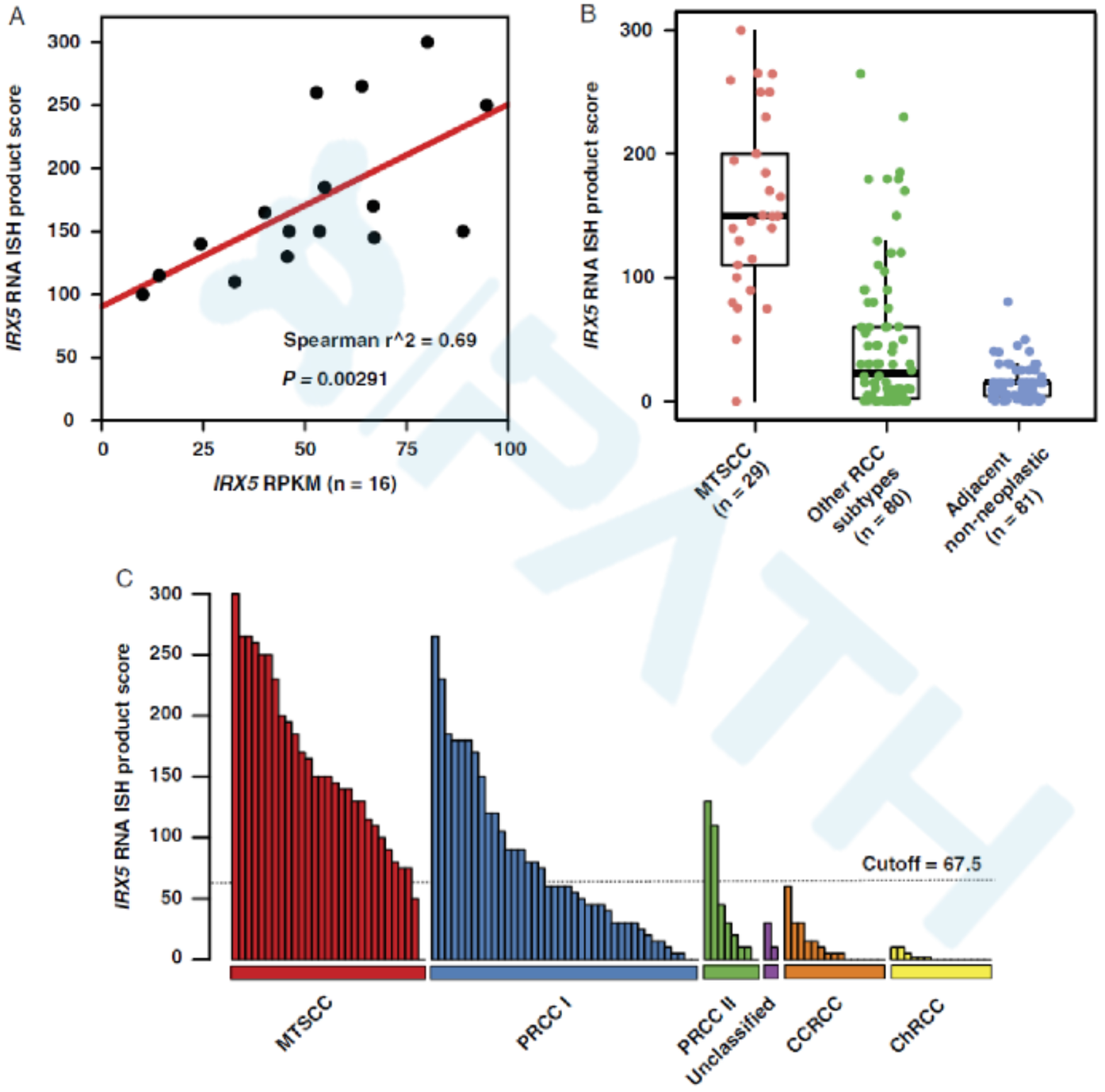


FIGURE 6. IRX5 expression in other RCC subtypes. A, Type 1 PRCC. B, Type 1 PRCC demonstrated low expression of IRX5 by RNAISH. C, Type 2 PRCC (H&E). D, Type 2 PRCC demonstrated low expression of IRX5 by RNA ISH.



E, CCRCC (H&E). F, CCRCC was negative for IRX5 expression by RNA ISH. G, ChRCC (H&E). H, ChRCC was negative for IRX5 expression by RNA ISH.


A
IRX5 RNA ISH product score
IRX5 RPKM (n = 16)
Spearman r^2 = 0.69
P = 0.00291
B
IRX5 RNA ISH product score
MTSCC (n = 29)
Other RCC subtypes (n = 80)
Adjacent non-neoplastic (n = 81)
C
IRX5 RNA ISH product score
Cutoff = 67.5
MTSCC
PRCC I
PRCC II
Unclassified
CCRCC
ChRCC

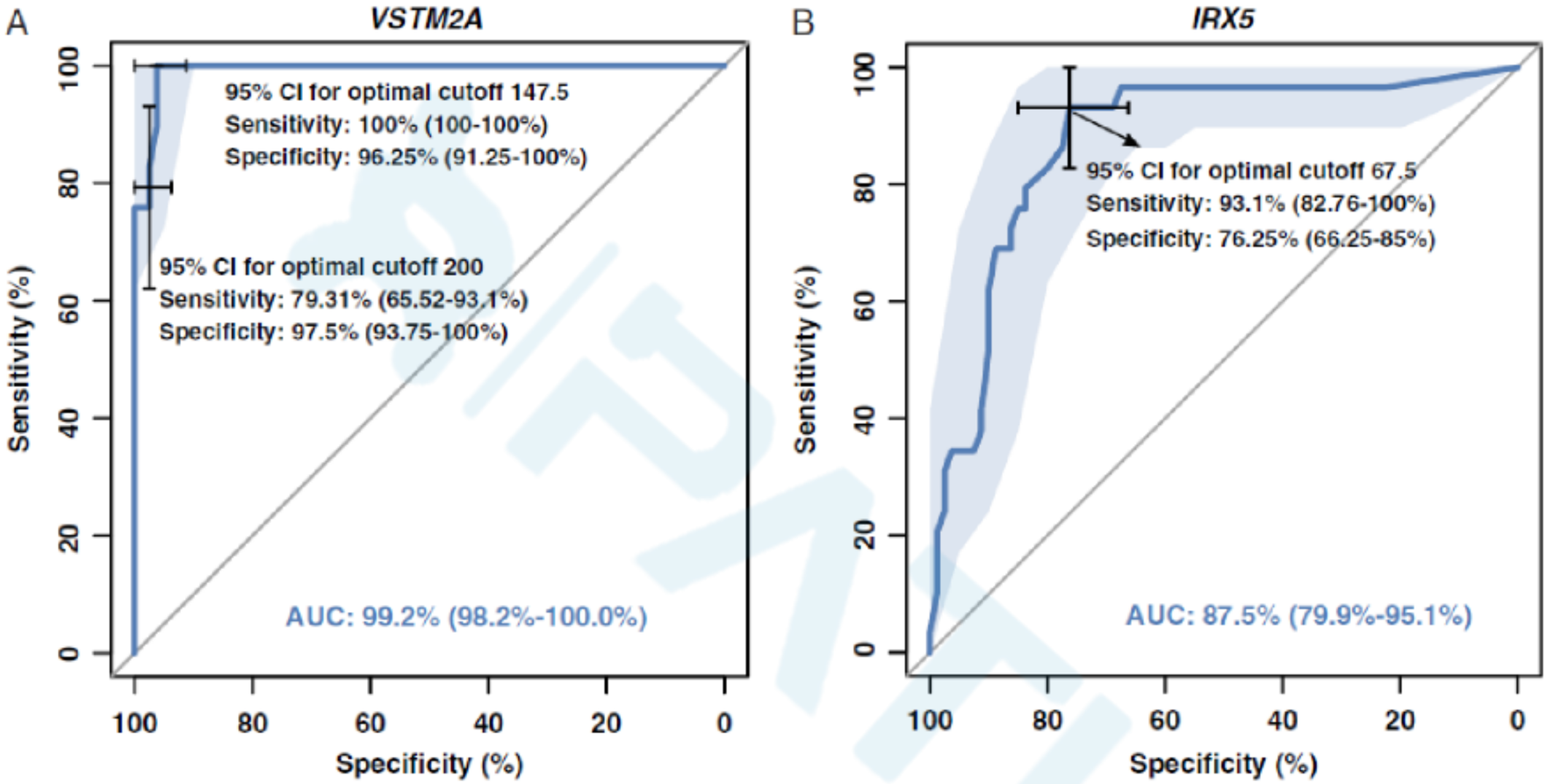


FIGURE 8. Diagnostic values of *VSTM2A* (A) and *IRX5* (B) for MTSCC. CI indicates confidence interval.

In summary,VSTM2A expression (AUC: 99.2%) demonstrated better diagnostic efficacy than IRX5 (AUC: 87.5%).

DISCUSSION

expression patterns of VSTM2A

- Our analysis of the GTEX data noted highly restricted VSTM2A expression in human brain samples
- VSTM2A is nearly absent in non-neoplastic renal parenchyma and is specifically and homogeneously expressed only in MTSCC tumor cells.

cutoff points of VSTM2A

ISH score cutoff	sensitivity	specificity
147.5	100%	96.25%
200	79.31%	97.5%

Hence, our results indicate that for an individual tumor with morphologic features that can be observed in either MTSCC or PRCC, high VSTM2A expression above an ISH score cutoff of 200 will support an interpretation of MTSCC.

MTSCC higher nuclear grade

- A prior study ---all 6 high-grade MTSCC cases showed monosomy of chromosomes 1, 4, 6, 8, 9, 13,14, 15, and 22, and absence of trisomy 7 and 17.

- THIS STUDY-moderate expression of VSTM2A in all 4 MTSCC cases with high-grade cytological atypia

- VSTM2A expression ---tumor grade?

phenotypic classification for MTSCC

- IRX5 was previously identified to be specifically expressed in the loop of Henle in rats

- Taken together, our results suggest that MTSCC displays an overlapping phenotypic expression pattern with the loop of Henle region of normal nephrons.

RNA ISH

- RNA ISH is a sensitive and specific tool for assessing gene expression in malignancies, is a reliable and cost-effective alternative to RNA-seq for the detection of both VSTM2A and IRX5 markers, and can be easily applied in a laboratory or clinical setting
- -RNA integrity of tissue samples.
  - RNA ISH on core biopsy specimens in this study
  - a semiquantitative scoring method (ISH score) to interrogate VSTM2A expression.

summary

- our results demonstrate VSTM2A overexpression to be a sensitive and specific marker for MTSCC.
- VSTM2A overexpression by RNA ISH may serve as a diagnostic marker to clinically distinguish MTSCC from PRCC with overlapping histologic features.
- Furthermore, our results suggest that MTSCC displays an overlapping phenotypic expression pattern with the loop of Henle region.