

Symposium on New Scientific Research Related to the Health Effects of Trichloroethylene
Washington, DC, February 26-27, 2004

VHL Alterations and Renal Tumorigenesis

Yih-Horng Shiao, Ph.D.

Laboratory of Comparative Carcinogenesis,
National Cancer Institute at Frederick, MD

The *VHL* gene

- The von Hippel-Lindau (*VHL*) disease: A hereditary syndrome with predisposition to various tumors, including renal cell carcinomas, because of *VHL* gene alterations.
- Sporadic clear cell renal carcinomas (the common form of kidney tumors): LOH at 3p25 (>90%), *VHL* mutations (30-60%), and hypermethylation in the *VHL* promoter (up to 19%).

The von Hippel-Lindau disease

Phenotype

Genotype

Type 1

- Renal cell carcinomas or cysts (25-60%)

Deletion
Frameshift

Type 2A

- CNS hemangio-
- blastomas (44-72%)

Missense

Type 2B

- Retinal hemangio-
- blastomas (25-60%)

Deletion
Frameshift
Missense

Type 2C

- Pancreatic tumors or cysts (35-70%)
- Pheochromocytomas (10-29%)

Missense

Sporadic renal cell carcinomas

Phenotype

Genotype (*VHL* mutations)

Clear cell (70-80%)

Deletion/frameshift (>50%)
Missense (<50%)

Papillary (10-15%)

Rare

Chromophobe (5%)

Rare

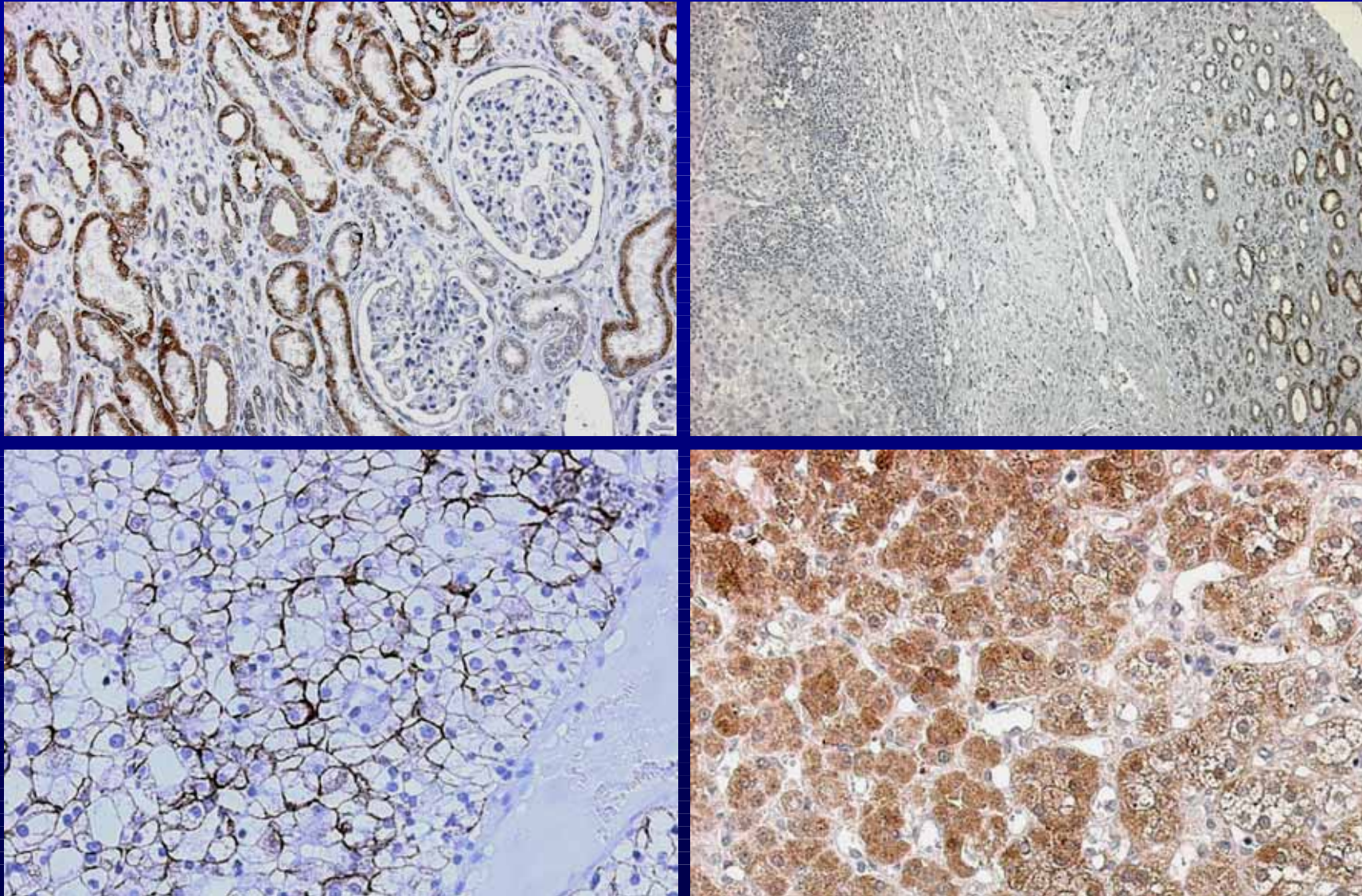
Oncocytoma (5%)

Rare

Association of *VHL* gene alteration with renal clinicopathological data

- **Tumor stage: Inconsistent**
- **Nuclear grade: Inconsistent**
- **Metastasis: Inconsistent**
- **Cancer-free or cancer-specific survival:
“Better” with *VHL* gene alterations**

VHL protein in renal cell carcinomas

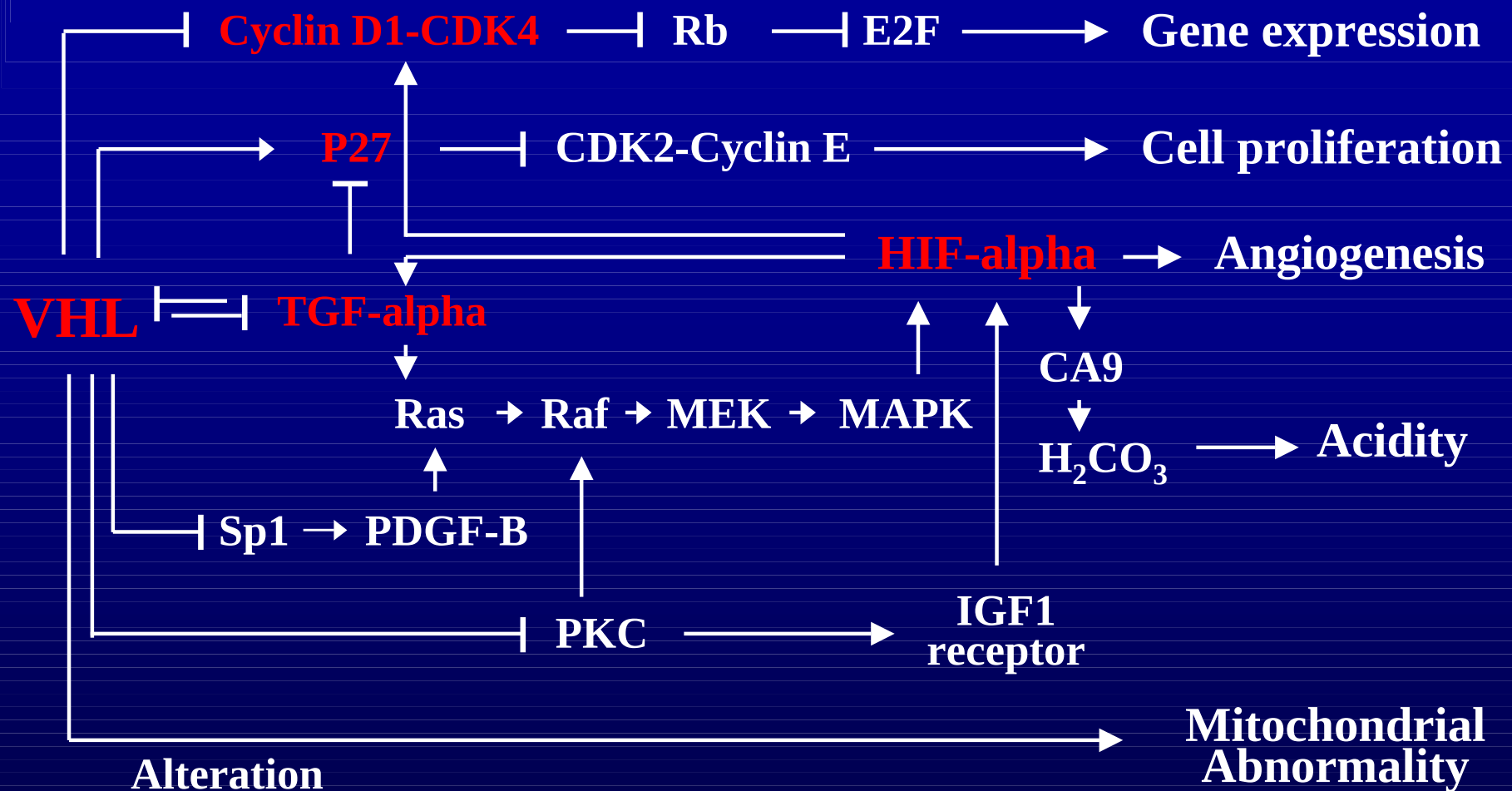


Association of VHL protein expression with renal clinicopathological data

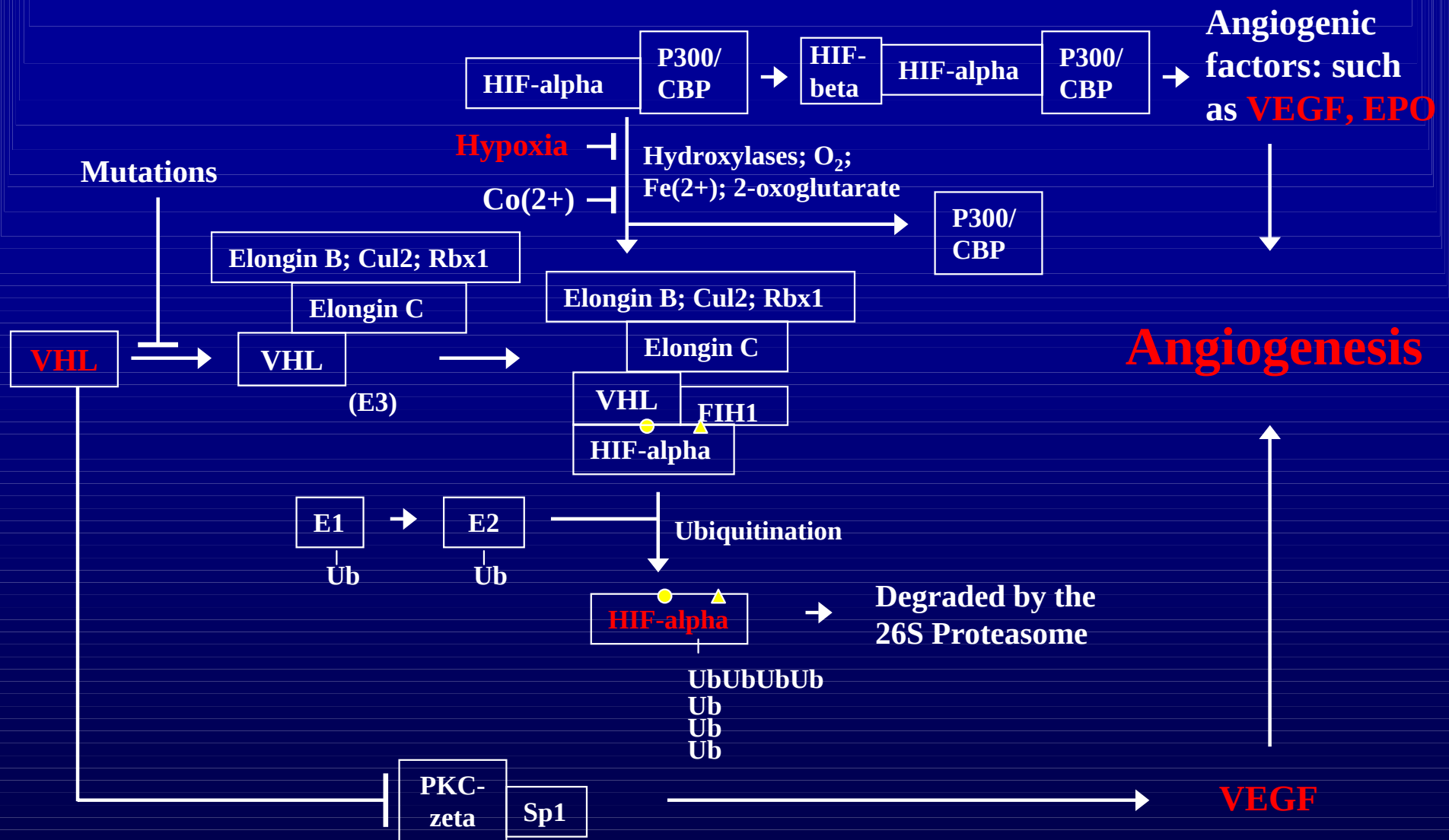
	Membrane	Cytoplasm /Negative	P ^a value	Nuclei/ Cytoplasm	Negative	P ^b value
Missense	9 (64%)	5 (36%)	0.0025	-	-	-
Others	14 (23%)	47 (77%)				
Grade 1	6 (50%)	6 (50%)	0.2214	40 (87%)	6 (13%)	<0.0001
Grade 2	11 (31%)	25 (69%)		171 (76%)	54 (24%)	
Grade 3/4	6 (22%)	21 (78%)		64 (50%)	63 (50%)	
TI	23 (38%)	37 (62%)	0.0034			
TII/TIII	0 (0%)	15 (100%)				
TI/TII				131 (76%)	42 (24%)	0.0121
TIII				144 (64%)	81 (36%)	
Survival (Cox model)	-	-	-	Better	Poor	0.04

- **Does VHL alteration initiate renal tumorigenesis?**
- **Do different VHL alterations have diverse tumorigenic potentials?**

Evidence for tumorigenesis *in vitro*



Evidence for angiogenesis *in vitro*

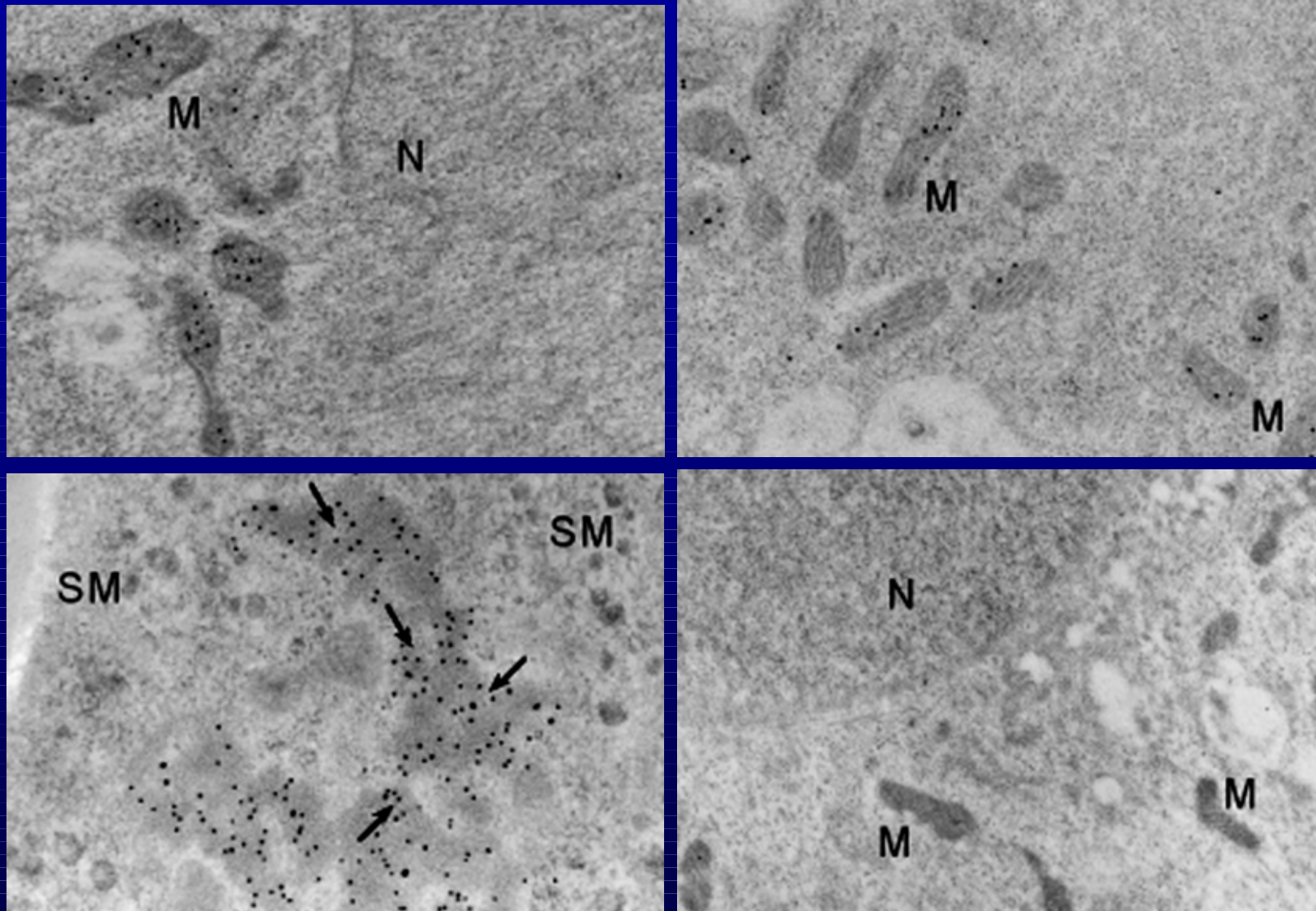


Evidence from *VHL*-knockout animals

- *VHL*^{-/-} mice: Embryonic lethality
- *VHL*^{+/-} mice: Susceptible to vascular lesions in the liver (21%)
- Mice with conditional *VHL*^{lox/-} and *Cre* alleles: Vascular lesions in the liver (>90% over 12 months of age), heart, kidney, and pancreas

Tumor initiation
vs
Tumor progression

VHL immunogold electron microscopy



Mutation spectra indicative of exposures

Missense	Possible causes
Transitions	
GC to AT	Deamination of 5-methyl-C (CpG sites) or C Alkylation of G at O ⁶ position
AT to GC	Deamination of A; alkylation of T at O ² or O ⁴ position
Transversions	
GC to TA	Mispairing of A with 8-OH-G or with apurinic G
AT to TA	Mispairing of A with apurinic A site
AT to CG	Misincorporation of 8-OH-G; error-prone repair of O ² - or O ⁴ -alkyl T
GC to CG	Mispairing of G with oxidatively-damaged G

VHL mutations and TCE exposure

TCE ^a exposure	Base # 454	Mutation(s)			GC to AT	Missense
		No	1	≥2		
High	7/17 (41%)	2 (11%)	4 (24%)	11 (65%)	21/27 (78%)	27/50 (54%)
Medium	6/24 (25%)	6 (25%)	15 (63%)	3 (13%)		
Low	0/3 (0%)	3 (100%)	0 (0%)	0 (0%)		
No	0/107 (0%)	31/73 (42%)	42/73 (58%)	0/73 (0%)	~25% ^b	~30% ^b
P value	<0.0001	<0.0001			-	-

^aPatients working in metal-processing plant

^bBeroud et al., Human Mut. 15:86, 2000.

Brauch et al., JNCI. 91:854, 1999

Conclusion

- There is still lack of direct evidence that VHL alterations initiate renal tumorigenesis, although they may be involved in tumor progression.
- Different VHL alterations have distinct tumorigenic potential: Higher tumorigenicity tends to associate with frameshift mutations and protein down-regulation.
- Comparison of mutation spectra in renal cell carcinomas may be able to identify specific base changes associated with TCE exposure but more population-based studies are needed to have sufficient statistical power.
- Co-exposures, such as metals, smoking, hypertension, obesity, and chronic renal disease, need to be examined.