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KIDNEY CANCER

Classifying the unclassified — molecular analysis of uRCC

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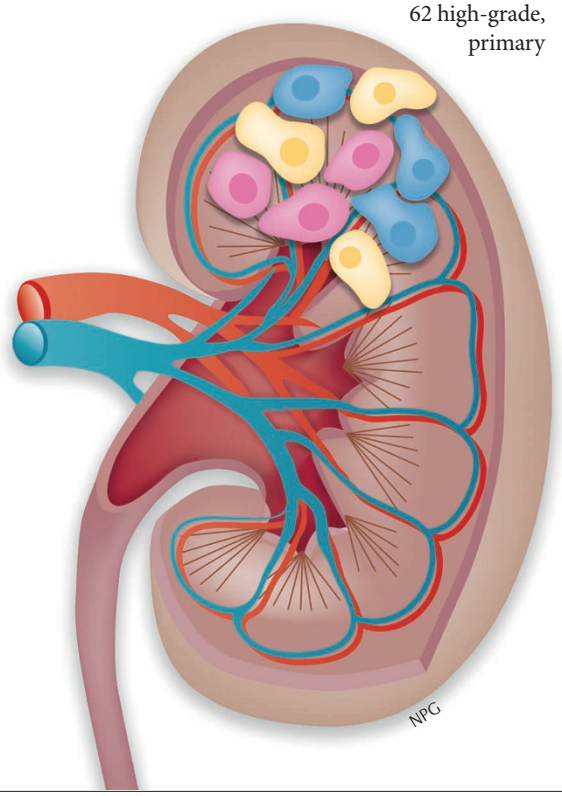
The first report on the molecular analysis of unclassified renal cell carcinoma (uRCC) has been published in *Nature Communications*. The study reveals distinct subtypes of cancer within a cohort of uRCC samples, which could have implications for the diagnosis and treatment of patients with this disease.

James J. Hsieh, a corresponding author for this investigation, explained "The incidence of kidney cancer that cannot be classified according to current morphological and molecular definitions is 5%, these cancers are categorized as uRCC." He continued "Patients with high-grade uRCC have poor clinical outcomes and no standard therapy exists for this disease." The research team, therefore, undertook molecular analysis of 62 high-grade, primary uRCC samples to elucidate genetic alterations associated with this rare form of kidney cancer.



Using the Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) assay, the team identified recurrent somatic mutations in 29 genes, including *NF2*, *SETD2*, *BAP1*, *KMT2C*, and *MTOR*.

Overall, 11 of 62 samples harboured *NF2* mutations and 14 tumours exhibited loss of chromosome 22q12, on which *NF2* resides, of which nine also had mutations in *NF2*. Genome-wide single nucleotide polymorphism analysis revealed that 13 of 15 samples with *NF2* mutations and/or 22q12 copy number loss had hemizygous loss of 22q.

NF2 is known to act as a tumour suppressor and has a key role in Hippo developmental pathways through suppressing YAP–TAZ nuclear translocation. Immunohistochemical analysis of uRCC tumours showed that those with *NF2* loss had significantly stronger nuclear staining for YAP–TAZ than those tumours with intact *NF2*. A significant enrichment of the YAP–TAZ transcription signature was also observed in tumours with *NF2* loss.

To assess the effect of *MTOR* mutations, the team generated *MTOR*-mutant 293T human embryonic kidney cells. Cells harbouring the Leu2427Arg mutant exhibited higher mTORC1 kinase activity than those with the Val2475Met mutation. Immunohistochemical analysis showed that tumours with the Leu2427Arg mutation had stronger staining for mTORC1 downstream substrates than those with the Val2475Met mutation. These data suggest that the Leu2427Arg mutant is pathogenic. Within this cohort, *NF2* loss and mTORC1 hyperactivity were found to be mutually exclusive, distinguishing two subsets of uRCC.

Three uRCC samples had *FH* somatic mutations, which have been described in hereditary leiomyomatosis RCC (HLRCC). FH loss was found to be inversely correlated with aberrant protein succination (detected using the 2-succino-cystein (2SC) assay) in four samples positive for 2SC and negative for FH. Genetic testing confirmed that three of these four samples were HLRCC.

IMPACT analysis also revealed that one sample had a *TPM3–ALK* fusion, which was confirmed using fluorescent *in situ* hybridization. Furthermore, recurrent mutations in genes associated with chromatin modulation and DNA damage response were observed in uRCC samples. Overall, seven of 62 samples harboured no significant molecular alteration according to the analyses undertaken, but could not be classified as oncocytomas.

Of the molecular subsets identified, patients with cancers harbouring FH deficiency and *NF2* loss had the worst clinical outcomes in this cohort. Hsieh told *Nature Reviews Urology* "We were able to classify 76% of our cohort into five groups — *NF2* loss, mTOR pathway activation, chromatin and/or DNA damage pathway mutations, *FH* mutations, and *ALK*-translocation. Importantly, this classification distinguished clinical outcomes." He concluded "Uncovering these molecular mechanisms could help stratify patients with uRCC and has pathogenic, diagnostic, and therapeutic implications for patients with this rare disease."

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