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KIDNEY CANCER

Activation of oncogenes driven by VHL loss in ccRCC

> Knockdown of *ZNF395* inhibited colony formation, decreased cell viability, and increased apoptosis

The most comprehensive profiling of histone marks in clear cell renal cell carcinoma (ccRCC) undertaken to date has revealed that von Hippel–Lindau (VHL) loss contributes considerably to enhancer remodelling. Furthermore, a novel master regulator of ccRCC pathogenesis — *ZNF395* — has been identified. These results provide mechanistic insights into ccRCC development and progression and provide new potential treatment targets.

Histone profiling of three marks in ccRCC tumours, patient-matched, tumour-derived cell lines, and non-malignant kidney cell lines showed that the *cis* regulatory landscape of ccRCC is aberrant, having extensive genome-wide alterations. Both gain and loss of promoters and enhancers occurred, and gained promoters and enhancers had increased chromatin accessibility and long noncoding RNA expression and decreased DNA methylation.

Genes modified by tumour-specific regulatory elements were identified using three methods to assign enhancers. These analyses showed that gained enhancers have a ccRCC-specific signature, enriched in disease-specific features such as the HIF1α regulatory network and cell metabolism, whereas gained promoters were enriched at features involved in general cancer-promoting processes, such as the cell cycle and transcription.

Examination of the regulatory landscape of super-enhancers showed that 1,157 were gained and 294 were lost in ccRCC tumours. Among the putative targets of these super-enhancers were well-known oncogenes, but several lesser-known genes were also identified, which were overexpressed in tumours compared with nonmalignant samples and were unique to ccRCC.

ZNF395 was overexpressed in ccRCC tumours and kidney cancer cell lines. Knockdown of *ZNF395* inhibited colony formation, decreased cell viability, and increased apoptosis, but did not affect non-malignant kidney cells. The *ZNF395* super-enhancer was active in ccRCC cells, but not in nonmalignant cells, and it was exclusively overexpressed in ccRCC in the cancers profiled in TCGA. *In vivo*, ablation of *ZNF395* inhibited xenograft tumour growth.

Restoration of *VHL* in RCC cell lines delayed tumour growth *in vivo* and caused more pronounced changes to enhancers and super-enhancers than to promoters. Only gained enhancers with H3K27ac depletion were active in *VHL*-mutated ccRCC cell lines, and only these enhancers were associated with downregulation of their putative target genes on *VHL* restoration. Further analysis suggested that *VHL* loss contributes to enhancer malfunction in ccRCC and that *VHL* restoration can result in loss of enhancer identity caused by codepletion of histone marks.

HIF2α was the most enriched motif at *VHL*-responsive enhancers with H3K27ac depletion, and its expression was downregulated on *VHL* restoration. Gained enhancers had HIF2α occupancy twice that of tumour-specific promoters. HIF1α was not enriched at enhancers with H3K27ac depletion and localized to promoter-proximal regions, whereas HIF2α localized to distal regions. More overlap of HIF2α binding sites with gained enhancers was observed than for HIF1α binding sites; the opposite was found for gained promoters. Knockdown of *HIF2A* did not recapitulate *VHL* restoration in terms of gene expression and gained enhancers; however, correlation between the two conditions was increased for genes close to HIF2α and HIF2α-bound enhancers and super-enhancers.

Profiling of histone acetyltransferase p300 showed enrichment at gained enhancers compared with lost enhancers in RCC cell lines. Restoration of *VHL* in VHL-depleted cells decreased p300 binding; however, promoter–enhancer interactions were preserved. *HIF2A* knockdown also decreased p300 recruitment.

These results show that the *cis* regulatory landscape is abberant in RCC and identify the VHL-dependent enhancer that is required for *ZNF395* expression, which has a functional role in ccRCC tumorigenesis. These data provide new potential treatment targets for this disease.

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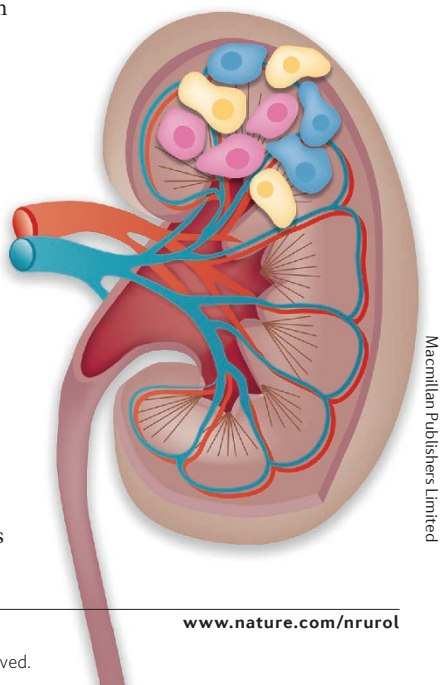

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