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KIDNEY CANCER

Programmed death ligand 1 regulation in ccRCC

The first evidence that *von Hippel–Lindau* (*VHL*) mutations positively correlate with programmed death ligand 1 (PD-L1) expression in clear cell renal cell carcinoma (ccRCC) has been presented in *European Urology*.

Tumours from 32 patients with ccRCC were assessed for *VHL* mutation status at both alleles, including somatic point mutations, loss of heterozygosity (LOH) and hypermethylation of the *VHL* promoter, and then classified according to type of *VHL* alteration. Loss of function (LOF) analysis was then conducted, which was based on the type of mutation present and the presence of LOH or promoter hypermethylation. Using these criteria, 71.9% of tumours had LOH, 65.6% had two *VHL* altered alleles and 43.8% had LOF. Quantitative real-time PCR was then used to assess *PDL1* (also known as *CD274*) expression, which was found to be significantly associated with *VHL* status regardless of classification.

In vivo analysis of six different *VHL* mutants transfected into the *VHL*-defective 786-O cell line (which overexpresses hypoxia-inducible factor 2α (HIF-2α (also known as endothelial PAS domain-containing protein 1)) showed that gradual truncated VHL protein (pVHL) LOF induces gradual dysregulation of HIF-2α expression, which positively correlates with PD-L1 messenger RNA (mRNA) and protein expression. Knock down of *HIF2A* in pVHL-deficient 786-O and A498 cells using short interfering RNA significantly decreased PD-L1 mRNA and protein expression.

Investigation of the mechanism by which HIF-2α regulates PD-L1 expression revealed putative hypoxia response elements (HREs) in human *PDL1*, with specific binding of HIF-2α to HRE-4 on the *PDL1* promoter in *VHL*-deficient, but not in *VHL*-corrected, 786-O cells, and HRE-4 was shown to be transcriptionally active.

HIF-1α was also observed to regulate PD-L1 in *VHL*-mutated RCC4 cells — knock down of HIF-1 and/or HIF-2 induced significant decreases in PD-L1 protein and mRNA expression.

These results show that PD-L1 is a novel target of HIF-2α and provide evidence of a link between *VHL* mutations, the HIF-2α signalling pathway and PD-L1 expression, indicating that the VHL–HIF-2α axis has a crucial role in the PD-L1 response in this disease.

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