

## Abstract

Upregulation of hypoxia-inducible factors HIF-1 and HIF-2 is frequent in human cancers and may result from tissue hypoxia or genetic mechanisms, in particular the inactivation of the von Hippel–Lindau (VHL) tumour suppressor gene (TSG). Tumours with VHL inactivation are highly vascular, but it is unclear to what extent HIF-dependent and HIF-independent mechanisms account for pVHL tumour suppressor activity. As the identification of novel pVHL targets might provide insights into pVHL tumour suppressor activity, we performed gene expression microarray analysis in VHL-wild-type and VHL-null renal cell carcinoma (RCC) cell lines. We identified 30 differentially regulated pVHL targets (26 of which were 'novel') and the results of microarray analysis were confirmed in all 11 novel targets further analysed by real-time RT–PCR or Western blotting. Furthermore, nine of 11 targets were dysregulated in the majority of a series of primary clear cell RCC with VHL inactivation. Three of the nine targets had been identified previously as candidate TSGs (DOC-2/DAB2, CDKN1C and SPARC) and all were upregulated by wild-type pVHL. The significance for pVHL function of two further genes upregulated by wild-type pVHL was initially unclear, but re-expression of GNG4 (G protein gamma-4 subunit/guanine nucleotide-binding protein-4) and MLC2 (myosin light chain) in a RCC cell line suppressed tumour cell growth. pVHL regulation of CDKN1C, SPARC and GNG4 was not mimicked by hypoxia, whereas for six of 11 novel targets analysed (including DOC-2/DAB2 and MLC2) the effects of pVHL inactivation and hypoxia were similar. For GPR56 there was evidence of a tissue-specific hypoxia response. Such a phenomenon might, in part, explain organ-specific tumorigenesis in VHL disease. These provide insights into mechanisms of pVHL tumour suppressor function and identify novel hypoxia-responsive targets that might be implicated in tumorigenesis in both VHL disease and in other cancers with HIF upregulation