New VHL Exon and Complex Splicing Alterations in Familial Erythrocytosis or von Hippel-Lindau Disease

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The *VHL* gene has been reported to contain three exons (E1, E2, E3). The commonly described *VHL* transcript is expressed ubiquitously and contains three spliced exons that encodes a 213 amino-acid (aa) protein (pVHL213 also termed pVHL30). A smaller isoform (pVHL160 or pVHL19) is initiated from an in-frame internal translation start site. These VHL proteins are involved in a variety of functions, the most studied being the regulation of the hypoxia inducible factor (HIF). A naturally occurring splice variant, comprises E1 directly spliced to E3 and is translated into a protein termed pVHL172 (pVHLΔE2), the functions of which are still under investigation.

Recently, we described a more complex regulation of *VHL* splicing. We identified new *VHL* transcripts that contained a cryptic-exon that we termed E1'. E1' is located in intron 1 and is spliced between E1 and E2 or E3. In addition, the sequence located upstream of E1' represents a transcriptionally active region and transcripts are initiated from this potential alternative promotor. A weak expression of these mRNA isoforms is detected in normal human tissues.

More importantly, we identified germline mutations in the new *VHL* cryptic exon that cause VHL-related disease. We identified heterozygous mutations in E1' in one large family presenting a typical VHL disease and in patients with isolated pheochromocytoma or multiple hemangioblastoma without any alteration in the other *VHL* exons. In addition, we identified compound heterozygous or homozygous E1' mutations in ten families with erythrocytosis.

We performed comprehensive studies of E1' and we showed that dysregulation of its splicing causes VHL-related disease. We demonstrated that mutations in E1' induce its excessive retention in the transcripts with a resulting decrease of functional VHL proteins expression. Comparative studies of mutations in E1' identified in patients with erythrocytosis or multiple tumors showed a strong correlation between the severity of the impact on splicing and the severity of the developed disease.

This discovery highlights the importance of thoroughly studying all the regulatory mechanisms of the *VHL* gene in order to better understand its functions.

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