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Extramitochondrial Fumarate Inhibits Multiple 2-OG Oxygenases in Fumarate Hydratase Deficient Cells

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Fumarate Hydratase (FH) mutations predispose to hereditary leiomyomatosis and renal cell cancer (HLRCC). The mechanisms(s) underlying tumorigenesis remain unclear though strong evidence links intracellular fumarate accumulation with inhibition of 2-OG dependent dioxygenases, such as HIF prolyl-hydroxylases (PHDs) that regulate HIF{alpha}. Herein, we describe a novel cellular model of FH-deficiency in which prolyl but not asparaginyl hydroxylation is severely impaired. We have corroborated these observations in renal cysts and cancer cells lacking functional FH. Furthermore, our cellular model and FH-mutant cancer cells exhibit hypermethylation of histone H3 which can be reversed by either exogenous 2-OG or stable expression of wildtype FH. Finally, we expressed and assayed function of the obscure cytoplasmic FH isoenzyme in Fh1-deficient cells. Remarkably, despite the cells being respiration deficient, cytosolic FH reversed defects in both proline hydroxylation and lysine demethylation. In summary, these data provide evidence for a link between FH-deficiency and epigenetic regulation and a novel role of cytosolic FH that may be a paradigm for the study of other metabolic genes and early events leading to cancer.

1

What is the Best Treatment for Renal Lesions in VHL?

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Approximately 40% of patients with VHL have multifocal, bilateral RCC. Interventions are usually performed for lesions approaching 3 cm in order to balance prevention of metastatic progression with the number of interventions. Acceptance of partial nephrectomy has also resulted in preservation of renal function in VHL patients and the advent of ablative therapy has allowed many of these tumors to be approached percutaneously. These have been proposed as first-line treatments for patients with VHL. However, cystic tumors are considered contraindications to ablative therapy, as are larger tumors or extensive multifocality with tumors greater than 3 cm in size. A review of the literature and our own experience shows the feasibility for primary and repeat ablative therapy for amenable tumors, and the feasibility of repeat partial nephrectomy in experienced hands, but the latter is associated with increasing rates of complications and major adverse events. The majority of VHL patients with renal involvement require interventions for their kidneys. Open partial nephrectomy is the primary modality used, performed successfully both as a primary and secondary procedure, but becomes successively more difficult. Ablative therapy is also successful but is only possible in a smaller proportion of patients due to limitations of current technology.

2

Characterization of the VHL-ECM Pathway

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The VHL tumor suppressor regulates several HIF-indpendent pathways, one of them regulating collagen IV extracellular matrix assembly. We have shown recently that pVHL binds to COL4A2. We will present new data on the nature of the pVHL-collagen binding. Furthermore, recent evidence from VHL tumors has pointed to role of the p53 tumor suppressor gene in the pathogenesis of VHL tumorigenesis. We therefore mated VHL knockout mice with p53 knockout mice and observed an acceleration of tumorigenesis. We will present a detailed analysis of this phenotype.

3

Induction of Extreme Metabolic Depression by the Nucleolus

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Sustained cellular function and viability of a eukaryotic cell relies on the intricate balance of ATP-demand and supply pathways. Eukaryotic cells switch from the high-energy producing aerobic oxidative phosphorylation pathway to low-energy producing anaerobic metabolism in low oxygen tension. This poses an unviable energy disequilibrium as anaerobic metabolism cannot sustain the intensity of energy-demanding aerobic biochemical processes. Several animal species, such as frogs and turtles, suppress cellular energy demand by more than 95% to maintain energy equilibrium and viability during phases of low ATP production. It is generally agreed upon that humans, and most other mammals, have lost the evolutionary ability to significantly depress metabolism and thus are unable to survive low oxygen tension for a prolonged period of time. This study describes an unexpected homeostatic program whereby the mammalian nucleolus captures and inactivates core proteins involved in DNA synthesis, transcription and translation, to instigate a systemic reconfiguration of biochemical pathways. This reversible process is regulated by extracellular hydrogen protons, an end-product of anaerobic metabolism, and achieves a degree of metabolic suppression sufficient for cellular viability during periods of low ATP supply. Nucleolar sequestration of ATP- consuming proteins is regulated by methylation of key arginine residues of their nucleolar detention sequence (NoDS) and intergenic spacer RNAs. These findings provide evidence that mammalian cells are equipped with a program that induces a state of metabolic dormancy to maintain viability during hypoxia-induced anaerobic metabolism.

4

Mutation of SDHB and Inherited RCC Susceptibility

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Familial Renal Cell Carcinoma (RCC) is a heterogeneous disorder most commonly caused by germline mutations in the VHL, MET, FLCN and FH genes or constitutional chromosome 3 translocations. However in many cases the genetic basis is undefined. Interestingly, the fumarate hydratase gene (FH) encodes for an enzyme in the Krebs cycle, loss of which results in a build up of substrate and induction of pseudohypoxic signalling. Mutations of the preceding enzyme, succinate dehydrogenase (SDH), are associated with pheochromocytoma and head and neck paraganglioma, symptoms which are also associated with the loss of VHL. Therefore, we investigated whether germline mutations in the succinate dehydrogenase subunit genes (SDHB, SDHC, SDHD) might cause apparently non-syndromic inherited RCC susceptibility (Familial, Bilateral or Early Onset). No mutations in SDHC or SDHD were identified but 3/68 (4.4%) probands had a germline SDHB mutation. Patients with a germline SDHB mutation presented with familial RCC (n=1) or bilateral RCC (n=2) and no personal or family history of phaeochromocytoma or head and neck paraganglioma. Mean age at diagnosis of RCC was 37.8 years (range 24-73). These findings (a) demonstrate that patients with suspected inherited RCC should be examined for germline SDHB mutations, (b) suggest that all SDHB mutation carriers should be offered surveillance for RCC and (c) provide a further link between familial RCC and pseudohypoxic signalling. Since the initial observation, several new patients that presented with inherited RCC susceptibility have been shown to have SDHB mutations.

5

Metabolic Links to Renal Cancer

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Introduction: *FH* is a recently identified tumor suppressor gene. Germline mutations of this gene results in a rare inherited form of renal cancer referred to as Hereditary Leiomyomatosis and Renal Cell Cancer. *FH* encodes for the tricarboxylic acid enzyme fumarate hydratase (also referred to as fumarase). Thus far, the role of *FH* in clear cell renal cancer, the most common histological variant of RCC, has not been determined. We thus present our findings with regard to the expression of *FH* in clear cell kidney cancer. **Methods:** Matched tumor and normal tissue were harvested from patients undergoing surgical resection for presumed renal cancer. Tumors were pathologically confirmed to be of clear cell histology. Tumor lysates as well as patient-matched normal renal tissue were immunoblotted for FH. In addition, RNA was extracted from biological samples and analyzed by real time RT-PCR analysis to assess for FH mRNA levels. **Results:** Immunoblot analysis revealed diminished levels of FH protein in clear cell kidney cancer as compared with normal renal tissue. In addition, real-time RT-PCR analysis demonstrated that FH mRNA levels were also reduced in tumor tissue.
Conclusion: These data indicate that FH expression is reduced at the transcriptional level in sporadic clear cell renal cancer. These are the first data to examine FH expression in the most common histological variant of renal cancer. The mechanism by which FH is downregulated and the biological significance of these findings remain to be determined.

6

Radiosurgery for Cerebellar Hemangioblastomas

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Introduction: Surgery is the main treatment of cerebellar hemangioblastomas and radiosurgery (RS) may have a role as a complementary or alternative approach. **Object:** To assess the role of RS in the management of primary or recurrent cerebellar hemangioblastomas. Methods: Retrospective analysis of 11 consecutive patients with cerebellar hemangioblastomas (22 lesions) treated with RS between 1999 and 2008 with a 6 months minimum follow-up. RS was delivered with Gamma Knife for primary (17 lesions) or recurrent (5 lesions) hemangioblastomas. Seven patients had von Hippel-Lindau disease-associated hemangioblastomas (15 lesions) and 4 had sporadic hemangioblastomas (7 lesions). **Results:** The median follow-up was 45 months (11-113 months). The median marginal dose was 15 Gy (13–24 Gy) and median target volume was 0.12 mL (0.01–11.5 mL). Two patients died from disease progression, and one of them due to progression in the central nervous system. The overall survival after RS was 100% at 1 year and 90% at 3 and 6 years. Tumor growth was controlled in 86.4% (19 in 22) of cases. The progression-free survival after RS at 1, 3 and 6 years was 100%, 90% and 60%, respectively. No complication such as radiation-induced peritumoral edema or radiation necrosis occurred. **Conclusions:** These early results show that RS provides a high local control rate of cerebellar hemangioblastomas, is associated with a low risk of adverse radiation effects and is an attractive alternative to multiple surgical procedures for patients with von Hippel-Lindau disease.

7

Neoplastic Diagnosis Timing Profile in von Hippel-Lindau´s Patients in a Personal Series

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Introduction: Von Hipple-Lindau patients present nervous system and retinal hemangioblastomas (HGB) associated with paragangliomas/ pheocromocytomas (PGL), endolymphatic sac tumors (ELST), renal cell carcinoma (RCC) and pancreatic neuroendocrine tumors (PNT). The object of this study is to evaluate the presenting timing profile of these tumors. **Material and Method:** Age and sequence of imaging confirmed diagnosis of every new tumor were evaluated in a series of 62 patients with a total of 291 diagnosed tumors from 30 families, studied and followed in a neuro-oncological syndrome center. **Results:** Among HGBs, Retinal were precocious (initial diagnosis at age 8, median 29); Cerebellar follows (first diagnosed at 13, median 35); Then, Brainstem follows (begins at 8, median 41), and Spinal have a later diagnosis (beginning at age of 9, median 42). PGLs begin at age 11, with median 34. ELSTs begin at 17 years, with median 34. PNTs diagnosis starts at 14, median 42, and RCCs were diagnosed the latest, starting at 20 years with median at 45. No relation has been observed among age of presentation and other clinical or molecular characteristics. **Conclusions:** In von Hippel-Lindau´s disease, the neoplastic occurrence begins at early age, 10% of cases before age of 19. A precocious first diagnosis doesn´t predict an aggressive clinical course for subsequent tumors. The temporal profile is not predictable by molecular diagnosis. Molecular diagnosis and clinical and imaging studies should be performed since pediatric age, in order to obtain an early diagnosis and adequate management of these neoplasms.

8

Regulation of the VHL Tumor Suppressor

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Introduction: The von Hippel-Lindau (VHL) gene is a classical tumor suppressor in that it is inactivated in patients with von Hippel-Lindau disease, as well as the majority of patients with sporadic clear cell renal carcinoma (RCC). The VHL protein (pVHL) is an E3 ubiquitin ligase with many functions, including regulating the hypoxia response and microtubule stability. **Objectives:** While the involvement of pVHL in targeting of HIFalpha subunits for ubiquitin-mediated degradation is well understood, we wanted to understand more about regulation of pVHL itself. Methods: We examined hypoxia-induced cell cycle arrest in RCC cells, and determined pVHL levels in normoxic and hypoxic cells, as well as in cells at defined points of the cell cycle. **Results:** Hypoxia-induced cell cycle arrest is associated with pVHL expression in RCC cells. pVHL levels are decreased in hypoxia and this is controlled through a post-transcriptional mechanism. In addition, pVHL levels fluctuate during the cell cycle, with decreased levels during mitosis and G1. The pVHL sequence contains Destruction consensus box sequences between amino acids 60-67 and 82-90, which are recognized by the anaphase promoting complex/cyclosome (APC/C) E3 ubiquitin ligase, and pVHL associates with Cdh1, an activator of APC/C. **Conclusions:** Down regulation of pVHL in hypoxia suggests a novel and complementary mechanism to account for stabilization of HIF alpha subunits. In addition, our results suggest that pVHL is a novel substrate of APC/CCdh1 and that pVHL degradation is mediated through Destruction box-dependent as well as –independent mechanisms.

9

Destructive Targeting via VHL Beyond HIF

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Polycythemia is a condition characterized by a net increase in the total number of red blood cells. The most common form of acquired polycythemia, polycythemia vera (PV), is caused by activating mutations in *Janus kinase 2 (JAK2)* and is typified by hypersensitivity of the erythroid progenitors to erythropoietin (EPO). Intriguingly, Chuvash polycythemia (CP), a rare congenital form of polycythemia, despite overlapping clinical features with PV is not associated with *JAK2* mutations. Rather, CP is caused by homozygous R200W and H191D mutations in the *von Hippel-Lindau (VHL)* tumour suppressor gene whose gene product is the principal negative regulator of the hypoxia-inducible factor (HIF), which governs critical adaptive responses to compromised oxygen availability. However, the molecular mechanisms underlying some of the hallmark features of CP such as hypersensitivity to EPO are unclear and unexplained by the current knowledge of VHL tumour suppressor functions. Here, wedescribe an intriguing twist to the E3 ubiquitin ligase activity of VHL that brings new insight into the biological function of VHL.

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Folliculin Functional Studies and Mouse Models of Birt-Hogg-Dube' Syndrome

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Introduction: Birt-Hogg-Dube´syndrome (BHDS) is an inherited disorder in which patients develop fibrofolliculomas, lung cysts/spontaneous pneumothorax and renal tumors. Germline mutations (insertions/deletions, nonsense and splice site) in *FLCN*, a novel gene on 17p11.2, were identified in BHD patients and predicted to truncate the FLCN protein. Somatic "second hit" mutations have been identified in BHD-derived renal tumors suggesting that FLCN is a tumor suppressor. **Objective:** Our aim is to understand FLCN function and the mechanism by which FLCN inactivation leads to BHD-associated renal cancer. **Methods:** Co-immunoprecipitations were performed to identify FLCN interacting proteins, which were characterized *in vitro*. Several mouse models were developed in which *Flcn* was inactivated homozygously in the kidney or heterozygously in the whole animal. A xenograft model was generated with a BHD-derived renal tumor cell line, UOK257, providing a system for therapeutic drug testing. **Results:** A novel FLCN-interacting protein, FNIP1, was identified that interacts with 5'-AMP activated protein kinase (AMPK), an important cellular energy sensor and negative regulator of mTOR. The Akt-mTOR pathway was activated in polycystic kidneys that developed in kidney-targeted Flcn conditional knockout mice, which showed a partial response to the mTOR inhibitor rapamycin. Moreover Akt, mTORC1 and mTORC2 were activated in solid tumors from aged *Flcn* heterozygous mice. Tumors developed in NOD/SCID mice injected with *FLCN-null* UOK257 cells, but not *FLCN*-restored cells confirming tumor suppression by FLCN. **Conclusion:** Our data support a model in which the FLCN/FNIP1 complex through AMPK interacts with the Akt-mTOR pathway. Loss of FLCN tumor suppressor function may contribute to dysregulation of this pathway and BHD-associated renal tumorigenesis. Supported in part with federal funds under contract HHSN261200800001E.

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A Zebrafish Model for VHL

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The small tropical zebrafish has been gaining rapid popularity as a model organism for human disease. As a vertebrate, it has all the organs mammals have (lungs and breasts excepted!) but in a simpler form. We generated two zebrafish lines with germline mutations of VHL. The resultant fish lack VHL and survive through embryonic development into the larval stages. The vhl mutant zebrafish manifests problems in its kidneys, pancreas, liver, vasculature, eyes, and cerebellum similar to neoplasms experienced by VHL patients. Furthermore, the vhl mutant zebrafish develop polycythemia, a blood disorder also known to be caused by VHL mutations in humans. Adding a human VHL gene back into the zebrafish will rescue these phenotypes; however, if we reconstitute the vhl-mutant zebrafish with a mutant human VHL, some or none of the phenotypes will be rescued. Using this approach we have begun testing VHL mutant alleles for genotype-phenotype correlations. The vhl-mutant zebrafish is also very amenable to testing new drugs and drug combinations. We conclude that the zebrafish is an exciting new model that faithfully recapitulates VHL disease and opens avenues of experimentation that have been previously impossible in mice.

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Relief of Intractable Nausea after Resection of Brainstem Hemangioblastoma in Patients with von Hippel-Lindau Disease: a Clinical Series

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Symptoms of hemangioblastomas in VHL depend on location, and the common predilection for cerebellum and brainstem lead to issues with balance, strength, coordination, and cranial nerve palsies. We present five patients who suffered intractable nausea from hemangioblastoma located in or near the distal medulla. All had VHL, and 4/5 had a family history for that condition. Age ranged from 19 to 48 years at time of surgery, 4/5 patients were female, and 2/5 had an associated cyst. Relief in all cases occurred within one week after tumor removal. The physiological basis of the nausea is hypothesized to be disruption of function of the area postrema. This circumventricular organ is situated just cranial to the obex in the floor of the distal fourth ventricle, and induces nausea and vomiting when visceral afferents or afferents from extramedullary brain centers are stimulated or when chemical changes in blood activate chemoreceptors there. In all patients here reported, tumor either sat immediately adjacent to or within the area postrema, or tumor cyst compressed it. Etiologies of the nausea include direct disruption or compression of the area postrema, or shifts in perfusion of this region caused by tumor hypervascularity and AV shunting. Nausea in patients with VHL can be caused by other conditions associated with the disease (e.g., pancreatic cysts or tumors), but this symptom should trigger a search for hemangioblastoma of the distal medulla, which can easily be shown by MRI. As such patients are resistant to anti-emetic medications, surgery can offer much benefit.

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Somatic Alteration of the VHL Gene in Sporadic Renal-Cell Carcinomas as a Potential Biomarker

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VHL alterations have been described in a high percentage of sporadic clear-cell renal-cell carcinomas (ccRCC). In most recent years, drugs targeting the pVHL/HIF pathway, such as sunitinib, sorafenib, temsi-rolimus and bevacizumab have proven beneficial for the treatment of ccRCC. We previously described that VHL mutation or promoter hypermethylation was associated with advanced tumor stage. As loss of the VHL function contributes to the pathogenesis of ccRCC, it is reasonable to speculate that VHL inactivation might be a prognostic or predictive factor and could be applied as a bio-marker in ccRCC. Since the publication of this data, there have been at least 11 other studies on alteration status of VHL, some of which support the prognosis hypothesis, some do not. This could be due to several reasons, such as different detection methods applied, the number of ds studied or the limited observation time. We have improved our detection rate of VHL alterations significantly by introducing the MLPA (Multiplex Ligation-dependent Probe Amplification) technique. Applying the now-a-day most comprehensive analysis techniques, including whole gene sequencing, MLPA, promoter methylation analysis and 3 p loss of het-erozygosity studies, we extended out initial study by the number of cases studied and the observation time. As a single center study, we included 129 cases of RCC for a follow-up time of 12 to 17 years. Genotype-phenotype association and survival curves demonstrate the prognostic importance of VHL al-teration for ccRCC. These findings will be discussed in the light of the results of recent studies.

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VHL Tumor Suppressor Protein Regulates Oncogenic Macroautophagy in Renal Clear Cell Carcinoma (RCC)

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Introduction: *VHL* gene is lost in a 60% to 80% of human renal clear-cell carcinoma (RCC), the most prevalent and malignant histological type of kidney cancer. MicroRNAs (miRs) are short noncoding RNAs that bind to specific elements on mRNAs to repress translation of target proteins with regulatory functions in cancer. **Objectives:** To identify VHL-regulated miRs which paly functional role in RCC. Methods: MiR levels were measured using Q-RT-PCR. Macroautophagic activity was determined using LC3B flux, LC3B immunofluorescence and TEM. RCC cell viability was determined using trypan blue exclusion test and formation of tumors was determined using orthotopic xenografts in nude mice models. **Results:** Expression of mir204 was universally decreased in RCC tumors, as compared with normal kidneys, and the degree of reduced expression correlated with tumor grade and cancer progression. MiR204 was cytotoxic to VHL(-) RCC cells in vitro and inhibited growth of tumors formed by these cells in nude mice. VHL positively regulated expression of miR204, and protected non-tumorigenic cells from cytotoxic activity of miR204. We further established that tumor suppressing activity of miR204 was due to inhibition of macroautophagy and starvation, and we identified relevant targets. **Conclusions:** Loss of VHL during RCC promotes loss of mir204 and derepression of specific targets, thus activating macroautophagy, which gives cancer cells access to intracellular nutrients that contribute to their survival and to tumor growth. In contrast, the presence of VHL stimulates expression of endogenous mir204 and leads to suppression of macroautophagy, rendering the cells insensitive to the activity of exogenous mir204.

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Identification of Germline Mutations in the VHL Gene of Families with the von Hippel-Lindau Disease

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The von Hippel-Lindau disease is an autosomal dominant pattern hereditary cancer syndrome caused by germline mutations in the *VHL* gene, and predisposes the patient to the development of a variety of malignant and benign tumors in various organs. The molecular analysis of germline mutation is a tool that enables early diagnosis of tumors in carriers before they develop clinical symptoms of the disease. The disease clinical diagnosis is based on clinical criteria that consider the family history and the clinical presentation of characteristic VHL lesions. In this work, peripheral blood genomic DNA was used as template for germline mutational analysis. After PCR the 3 exons of the *VHL* gene, Sager sequencing was used for point mutation detection. Southern-blot and MLPA were used to detect partial or complete deletion of the VHL and neighbor genes. Among 40 families completely studied, 32 point mutations, and eight rearrangements were identified in the probands. The predictive test has been applied to asymptomatic relatives under risk and was used to identify candidates to follow a specific program of screening. In summary, we could characterize the VHL germline mutation in all probands studies. The high detection of *VHL* mutation in our laboratory might result of the stringent clinical criteria (based on the documented evidence of the lesions) adopted by the clinicians of our group. A high level of accuracy could be also helpful in the molecular screening of apparently sporadic forms of VHL-related lesions, and in cases when the family history is limited, making improbable the diagnosis of VHL when a patient tested negative for *VHL* mutation.

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Expression Profile in von Hippel-Lindau Disease-Associated and Sporadic Clear-Cell Renal Cell Carcinomas

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The renal cell carcinoma (RCC) is the ninth most common type of cancer in world. The most frequent histology is clear-cell RCC, and can be sporadic or associated with hereditary syndromes, like von Hippel-Lindau Disease (VHL), an autosomal dominant syndrome that results from germline mutations in the *VHL* gene. The aim of this study was to analyze the expression profiles of ccRCCs from VHL patients compared with sporadic. Seven sporadic ccRCC paired with normal kidney tissue, and 21 paired ccRCC from six VHL patients were included. Total RNA was extracted using commercial kits, and microarray experiments were performed using Human Gene 1.0 ST Array (Affymetrix). Partek Suite Software was used for statistical analysis of differential expressed genes between sporadic and VHL tumors, and hierarchical clustering. We found 60 differentially expressed genes which discriminate VHL from sporadic tumors: 45% of them associated with protein binding, 30% related to nucleic acid binding, and 27.5% involved in ion binding. The hierarchical cluster analysis showed that normal samples (VHL + sporadic) grouped together, but in two distinct clusters; and separate of tumor samples (VHL + sporadic). We could define a molecular profile that distinguish normal kidney from VHL patients from normal kidney from patients with sporadic ccRCC. In conclusion, our results highlight new mechanisms of ccRCC carcinogenesis in VHL, and define genes that are exclusively expressed in normal and tumor tissues trigged by germline *VHL* mutations.

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Proposed Changes to the VHL Handbook

Joyce Wilcox Graff - VHL Family Alliance

The VHL Handbook is a tool for people with VHL and their health care teams, providing an overview of what VHL is, how it behaves, and how to watch out for problems. It includes a screening protocol, the common manifestations, and some treatment suggestions. Its last major revision was in 2005. While the information in it is still valid, we now have additional information which might be included, especially in the areas of ELST, pancreas, and kidney. Some words about clinical trials and drug therapies are needed, both to encourage participation and to restrain enthusiasm, as there is still no "magic bullet". We would particularly appreciate the input of physicians from all countries on the screening protocol. Data has been assembled in several countries on the effectiveness of the current protocol in guiding treatment recommendations, and the level of patient compliance. This screening protocol serves also to guide healthcare payers as to what is required for responsible management of this condition. Once an issue is identified, the clinician will modify the requirements as needed to follow the progress of a particular tumor.

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Evaluation of the Somatic Alterations of the VHL Gene in Renal Cell Carcinoma Associated with von Hippel-Lindau Disease (VHL)

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The clear cell renal carcinomas (ccRCC) are aggressive tumors, found sporadically or associated with hereditary syndromes. In VHL, 25% of patients will develop a ccRCC during their lifetime. Germline mutation in the *VHL* gene is now detected in virtually 100% of the VHL patients following stringent clinical criteria. In ccRCCs associated with VHL, a second mutational event occurs in somatic cells of tumors. The aim of this study was to evaluate the somatic mutational events involving the *VHL* gene of ccRCCs removed from eight VHL patients. In total, 30 ccRCCs and 8 paired normal renal tissues were included after pathology review. *VHL* gene methylation was addressed by MS-PCR; Sanger sequencing of the *VHL* gene was used to detect point mutation, and we use MLPA to evaluate VHL locus deletions. In six patients, SNP Array 6.0 (Affymetrix) data of their tumors and normal kidneys were available, and CNV of 3p was assessed for further validation. Interestingly, germline point mutation could also be detected in the tumors, and sometimes helped to confirm LOH of the *VHL* locus. We could characterize new somatic alterations of the *VHL* gene in 25 of the 30 tumors studied, including one point mutation, and 23 large deletions of 3p-ter. *VHL* gene methylation was not detected in any of the tumors. High density SNP Array analysis showed to be a useful tool to evaluate cytogenetic alterations in ccRCC, with an advantage of defining accurately the extension of the loss of 3p-ter. In conclusion, a number of methods combined are necessary to accurately assess somatic alterations of the *VHL* gene in VHL RCC.

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Copy Number Variation Analysis of a Pancreatic Neuroendocrine Tumor (NET) from a Patient with von Hippel-Lindau (VHL)

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Von Hippel-Lindau disease (VHL) is an autosomal dominant syndrome that results from germline mutations in the *VHL* gene, predisposing to multiples tumors, including neuroendocrine tumor (NET) of the pancreas detected in 11% to 17% during lifetime. Little is known about the cytogenetic anomalies presented in pancreatic NETs, and the identification of commonly imbalanced genes may contribute for the understanding of the somatic steps of the carcinogenesis. The aim of this study was to detect chromosomal imbalances in a pancreatic NET of a patient with VHL disease. We used GeneChip Human Mapping 50K Set Xba240 (Affymetrix) to assess genomic CNVs; Genotyping Console Software (Affymetrix) was used for analysis. We detected three deletions in 3p25.3-p11.2, 3q26.33-qter, and 8q21.11-q24.3, and one duplication in 7q22.1-qter. All genes located in these regions were analyzed according to their biological processes using DAVID Functional Annotation Tool. This analysis revealed a duplication of the oncogene *MET*, and other genes involved in cell proliferation, resistance to apoptosis and angiogenesis. The *PPARG* gene, from the *PTEN/PI3K/AKT/mTOR* pathway, was deleted; *BRAF* gene, component of the *RAS/RAF/MAPK* pathway, was duplicated. The involvement of known pathways already described in other VHL-related tumors, as RCCs and hemangioblastomas, suggests common steps in the tumorigenesis and progression of pancreatic NETs related with VHL disease.

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Molecular Dynamics Study of the Mutant pVHL Phe76del and its Interactions with Components of the pVHL Complex

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The von Hippel-Lindau tumor suppressor protein (pVHL) is 213 aminoacids protein belonging to the E3 ubiquitin ligase complex. pVHL plays a central role in oxygen-sensing pathway by regulating the degradation of hypoxia-inducible factor (HIF). The capture of HIF1 alpha by pVHL is regulated by hydroxylation of a specific conserved prolyl residue on HIF, a modification that is oxygen-dependent. The VHL gene is mutated in the germline of patients with VHL syndrome, which is characterized by the development of highly vascular tumors and constitutively high levels of HIF1 alpha. The VHL Phe76del mutation is the most common germline mutation in Brazilians, predisposing carriers to an increased risk of hemangioblastomas and renal carcinomas. The disturbance of the dynamic coupling of HIF and pVHL(Phe76del) was experimentally confirmed in vitro, but the mechanism of such complex disruption is still not clear. To investigate this, a model for the mutant pVHL(Phe76del) was built, and the interaction with HIF and elongins were monitored during molecular dynamics. Our results detected unstable interactions along the pVHL(Phe76del)/HIF interface, with an overall reduction in the interface area, lost of hydrogen bonds, and a disassembly of the pVHL binding pocket for the conserved hydroxyproline residue. Our work was able to clarify the mechanism of lost of affinity between the mutant pVHL(Phe76del) and HIF. Future studies will address if molecular dynamics of mutant pVHL correlates with family phenotypes and individual risks.

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Genomic Copy Number Variation Analysis in VHL-Associated Renal Cell Carcinomas Suggests Clonality

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The von Hippel-Lindau disease (VHL) is an autosomal dominant syndrome that results from germline mutations in the gene VHL, located at 3p25-p26. VHL predisposes individuals to develop multiples benign and malignant tumors in different organs, including the kidney. The clear cell renal cell carcinomas (ccRCC) occur in about 25% of the patients and are usually multiple. No studies have explored if multiple ccRCC in the same kidney might be originated from independent events or from one tumor cell progenitor. The analysis of somatic mutations in the VHL gene is usually limited to address this matter. We used whole genome analysis Copy Number Variation (CNV) in multiple ccRCC from patients with VHL to compare the profile of chromosomal gains and losses from multiple tumors of a same patient and among different VHL patients. We analyzed 21 ccRCC removed from seven VHL patients and paired normal renal tissue, and seven sporadic ccRCC, using the CNV-array techniques and the Genome-Wide Human SNP array 6.0 (Affymetrix). The analysis of genomic gains and losses was performed using Genotyping Console Software (Affymetrix). Losses in chromosome 3 were the most common aberration detected in ccRCCs associated with both VHL and sporadic tumors, followed by gains on chromosome 5. However, global chromosomal aberrations and breakpoints were more similar among synchronous tumors of a patient than among tumors from different patients. We suggest that, at least a subset of synchronic ccRCCs share global structural alterations at the DNA level, and therefore, might be clonal.

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Management of Brainstem and Spinal cord Hemangioblastomas in Patients with von Hippel-Lindau Disease

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Introduction: Spinal cord hemangioblastomas are associated with von Hippel-Lindau disease (VHL) in more than 75% of cases. Brainstem hemangioblastomas are present in up to 20% of VHL patients, and are almost pathognomonic of the disease. Management of these tumors is controversial, as patients are not affected from isolated hemangioblastomas, but by a genetic multineoplastic condition. The aim of this paper is to present the surgical results of spinal and brainstem hemangioblastomas in a VHL referral center. **Material and method:** We reviewed a series of 12 patients (age range 15-66) who underwent 14 surgical procedures to remove 20 hemangioblastomas: 4 in the brainstem, 4 in bulbo-medullary junction, and 12 in the spinal cord. The indication for surgery was stablished by initiation of symptoms or evident growth of tumor. **Results:** Preoperative sensory deficit was present in 10 patients (76.9%), followed by motor deficit in 7 (53.8%). Complete resection was possible in all cases. In the postoperative functional assesment, improvement was obtained in 1 (7.7%), clinical stability in 12 (84.6%) and clinical deterioration in 1, from functional grades I to II (7.7%). Early in the postoperative assesment, a functional deterioration occurred in 4 (30.8%) patients, all fully recovered after 3 months, excepting the abovementioned case. **Conclusions:** Complete microsurgical resection of spinal cord and brainstem hemangioblastomas in VHL patients is achieved with good results and at very low rate of complications, when performed by surgeons particularly involved in the management of patients with VHL disease.

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The Benefits of Ultrasound in Resection of CNS Hemangioblastomas

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Introduction: CNS hemangioblastomas can be permanently cured by complete surgical removal. However, the vascular nature of these lesions and difficulties in localizing the tumors account for operative morbidity and recurrence. Color Doppler sonography has been proven useful during surgical removal of other vascular lesions. **Objective:** In the present study we evaluate the usefulness of this technique for hemangioblastoma. We used the SonoWand Invite™ intraoperative navigation system in a consecutive series of hemangioblastomas operated at our institution. Patients with von Hippel-Lindau disease as well as sporadic hemangioblastomas were included. **Results:** The system was used on n= 64 consecutive hemangioblastomas operated at our institution from 2007 to 2009. The tumors were localized in the cerebellum (n=26), spinal cord (n=27), brainstem (n=10) and supratentorial (n=1). In n=53 cases the patients were diagnosed with VHL disease and germline mutations of the VHL tumor suppressor gene were identified in 98%. Average tumor size was 1,782 mm^3 and 45% of the tumors were cystic. 42/64 tumors could be localized by grayscale sonography. All tumors were visible on color Doppler sonography. However, in 40 cases, only the pathologic vessels and not the solid tumor itself enhanced on color Doppler. Postoperative MRI follow-up revealed recidive tumors in 5 cases. Three of these had had diffuse hemangioblastomatosis and one was a spinal nerve tumor. **Conclusions:** Color Doppler sonography is a sensitive intraoperative tool to guide the surgical approach and resection and provides reliable resection control in surgery of CNS hemangioblastoma.

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Management of Central Nervous System Hemangioblastomas in von Hippel-Lindau Disease

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Central nervous system (CNS) hemangioblastoma (HB) is the most frequent tumor in von Hippel-Lindau disease (VHL). The tumor occurs in cerebellum, brain stem, and spinal cord. However, management of CNS HBs in VHL has been controversial. Here, we discuss the management of CNS HBs in VHL.Surgically treated 62 CNS HBs (cerebellar 41, brain stem 3, spinal 18) in 34 VHL patients from 1992 to 2010 were retrospectively examined in diagnosis and surgical treatment. Diagnosis of CNS HBs depended on symptoms, neurological examination, laboratory data, CT, MRI, and gene diagnosis. Symptoms in cerebellar HB were faintness, gait disturbance, nausea, and headache while those in spinal ones were pain, numbness, and weakness in extremities. Neurological examination revealed cerebellar dysfunction for cerebellar HB and regional hypesthesia or muscular weakness for spinal one. Laboratory data often reveal polycythemia due to secretion of erythropoietin from the tumor. Gene diagnosis for VHL gene mostly revealed ger-mline mutation in VHL gene. Treatment for CNS HBs in cerebellum as well as spinal cord after symptomatic was surgical resection in the first choice. Surgical outcomes was mostly excellent or good except for large tumors (cerebellar HB >4cm, spinal HB>2 cm). In the second choice, stereotactic irradiation did not always control the tumor. VHL patients bearing CNS hemangioblastoma mostly developed other CNS ones in a different site. In conclusion, surgical resection is the first-choice treatment for CNS HB in VHL, and it should be done at appropriate timing without neurological deterioration.

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Neuronal Differentiation of Stem cells by Transfer of a VHL Peptide and Regenerative Therapy

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We previously demonstrated that von Hippel-Lindau tumor suppressor (VHL) protein has a function of neural induction in neural stem cells (NSCs) without any neurotrophic factors. Then, we hypothesized that a neural induction domain potentially exists in the VHL protein. Here we identify a neural induction domain for pluripotent somatic stem cells at elonign BC binding site in the VHL protein, and show neuronal differentiation of the cells by transfer of the domain peptide linked to protein transduction domain (PTD). It is suggested that the neuronal differentiation of stem cells by the transfer of the domain peptide is caused by competition with the domain peptide and elongin A in binding to elongin C followed by the inhibition of Stat 3. In addition, we show that the domain has the same function for other somatic stem cells except for neural stem cells, and that an evolutionally conserved short amino-acid sequence within SOCS-box proteins as well as VHL protein, which is termed the BC-box motif, has an ability to induce neuronal differentiation in somatic stem cells. Furthermore, when the domain peptide-transferred stem cells are grafted into recipient nervous tissue in the spinal cord injury model, the grafted cells differentiate to neurons and recovery of the neuronal symptom is recognized. Thus, the neuronal differentiation of pluripotent somatic stem cells is occurred by transfer of the neural induction domain peptide linked to PTD, and would contribute to neuronal regenerative therapy for VHL patients with impaired spinal cord and cerebellum.

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Endolymphatic Sac Tumors (ELST) in VHL Patients - Evaluation of Screening Methods in a National Study

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Introduction: ELSTs are found in 11-16% of vHL patients, but it is uncertain whether recommended audiologic screening is adequate for tumour detection. The study aims at evaluating ELST screening strategies and patterns in audiometric parameters in vHL patients. **Material and Methods:** We included 40 out of 42 VHL mutation carriers over 15 years of age known in Denmark. Subjects were interviewed about ELST-related symptoms and referred to audiologic examination and inner ear MRI. Audiological work-up comprised otoscopy, pure tone and speech audiometry, tympanometry, determination of stapedial reflex thresholds, and in some cases auditory brainstem response and vestibular testing. MRIs were carried out by a 3T MR scanner with high resolution imaging. 3D T2 weighted TSE images were acquired of the 8. cranial nerve, inner ear and endolymphatic sac with 0.6 mm isotropic resolution. Results: were blinded and evaluated independently by two Ear-Nose-Throat- and Radiology specialists. Median age at examination was 39 years (range: 15-65). **Results:** So far, prevalence of MRI-visible ELST among VHL mutation carriers is 5% (2/39). One ELST patient had subjective and objective audio-vestibular symptoms prior to MRI. The second ELST patient, however, had no symptoms and unremarkable audiometric findings. Assuming MRI findings as a measure of true ELST occurrence, sensitivity of audiometry in ELST diagnosis was 50% and specificity 19%. **Conclusion:** The study is ongoing and results including all subjects will be presented. Preliminary results indicate that MRI is superior to other methods of ELST detection.

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Delineating Genotype-Phenotype Correlations Among Brazilian Families with von Hippel-Lindau Disease

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VHL disease is a hereditary cancer syndrome that predisposes to a myriad of benign and malignant tumors, mainly cerebellar hemangioblastomas, retinal angiomas, renal cell carcinomas(RCC) and pheochromocytomas(PC). These manifestations are due to germline and somatic mutations in the VHL gene and disruption of VHL product. Families are classified into types 1 and 2, showing an evident genotype-phenotype correlation, as type 2 is associated with PC and missense mutations. Famili es with large deletions have now been subclassified according to the contiguous genes status and the risk of RCC. **Objective:** To better characterize genotype-phenotype correlations among Brazilian families with VHL disease. **Methods:** Ten unrelated families underwent genetic testing, eight of them fulfilled clinical criteria and two had sporadic cerebellar hemangioblastoma. PCR, RT-PCR, RFLP, MLPA, DNA sequencing were performed. **Results:** Four different germline mutations were found: c.226_228delTTC; c.217C>T; IVS1-1G>A and IVS2-1G>C. The first three mutations were correlated with type 1 disease whereas the last one with type 2B, which had not been identified in the germline so far. The transcriptional processing of a novel splice-site mutation was characterized. Three type 1 families presented large VHL deletions, two of them including the neighbouring FANCD2/C3orf10 genes, and did not develop any renal lesions. We suggest that both families be reclassified into type 1B (low RCC risk). Conclusion: The present study delineated the genotype-phenotype correlation of seven Brazilian families with VHL disease and characterized a novel mutation.

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Endothelial Fenestrations Associated with VHL Gene Alteration is a Potent Target of Anti-VEGF Therap

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Introduction: VEGF-targeted therapy shows anti-tumor effects for mRCCs. However, neither precise mechanisms nor potent target of this therapy had been largely unknown. Experimental studies using VEGF neutralization in mice model revealed that VEGF-dependent capillaries were characterized by the existence of fenestrations in endothelium on electron microscopy study. **Objectives and Methods:** We examined the microcapillaries of human ccRCC specimens for the existence of fenestrations and analyzed possible mechanisms of developing of microvasculature with distinctive phenotype by using the mouse xenograft model. **Results:** Abundant endothelial fenestrations were found in the majority of sporadic CC-RCCs with VHL mutation. This finding was also recapitulated in mice xenograft models in that tumor microvasculature from VHL-/- pRC3 cells harbored more abundant endothelial fenestrations compared to those from VHL restored WT8 or even WT8/HIF2a P531A cells. Accordingly, treatment with Bevacizumab resulted in a significant repression of tumor size in pRC3 tumors with the reduction of MVD and the number of endothelial fenestrations but not that of WT8 or WT8/HIF2a P531A cells. **Conclusions:** Our results suggest that sporadic RCCs with VHL mutation harbor VEGF-dependent tumor vessels with abundant fenestration on their endothelium. Unexpectedly, HIF independent pathways of pVHL mainly regulate the development of tumor vasculatures with distinctive structure. Importantly, our preliminal results from shRNA mediate JunB knockdown in 786-O cells highly suggest that JunB, at least in part regulate the ECM remodeling including the endothelial fenestrations. Collectively, aPKC/JunB pathway would be a potent therapeutic target for VHL-/- RCC cells.

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Role of Pregnancy on Hemangioblastomas in von Hippel-Lindau Disease: a Retrospective French Study

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Von Hippel-Lindau disease (VHL) is a dominantly inherited disorder predisposing to highly vascularized tumors including hemangioblastomas of the central nervous system (CNS) and the retina. The disease results from germ-line mutations in the VHL tumour-suppressor gene that plays a key role in the cellular response to tissue hypoxia and angiogenesis. CNS and retinal hemangioblastomas occur in about 75% and 60% patients, respectively. Only a few case reports and one study on a small population of VHL patients (*Grimbert et al., Am. J. Gyn. Obst. 1999,180:110-1*) were previously interested in a potential deleterious role of pregnancy on hemangioblastomas which express progesterone receptors. We present a retrospective and comparative French study in 269 women from 172 families coming from the national VHL clinical database. The aim was to analyse the onset of new hemangioblastomas and potential tumoral complications in patients according to their gestational status. Available data of imaging follow-up of CNS and retina were collected in 176 women with at least one pregnancy (group 1) and 93 women with none (group 2). More complications of hemangioblastomas in group 1 (p=0.031) were noted. This appeared not to be correlated with cerebrospinal or retinal topography. To our knowledge, this work represents the first study analysing the effect of pregnancy in a very large series of women with VHL. This study underlies the necessity of follow-up patients closely during pregnancy. Thus, a magnetic resonance imaging without injection is systematically required during the fourth month of pregnancy in each patient with previously known CNS lesions.

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An Evaluation of the Danish National Clinical Guidelines for Von Hippel-Lindau (VHL)

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Purpose: Von Hippel-Lindau (VHL) is a rare hereditary and potentially fatal cancer syndrome. Because of its unpredictable manifestations from various organ systems, surveillance is not linked to a single department and may therefore be incomplete. As one of the first countries in the world, Denmark published national guidelines for the surveillance of patients with manifest and possible VHL in 2005. The present study is the first of its kind where patients with suspected and manifest VHL are followed according to national guidelines at a single institution, and the purpose was to evaluate 1) to what extend the guidelines were being followed and 2) what findings were disclosed. **Methods:** The study included 27 individuals with diagnosed (14 patients) or suspected (13 patients) VHL, observing the Danish VHL-guidelines at the Department of Neurosurgery, Rigshospitalet, Denmark from October 2002 to April 2008. The data were collected by reviewing the patients records. **Results:** 48% of the patients had manifestations revealed that influenced the treatment, and 26% of the patients had asymptomatic manifestations demonstrated. All investigations were conducted with a lower frequency than recommended. **Conclusions:** The study shows that the national clinical guidelines were not being fully complied with. The investigations revealing the most serious VHL manifestations were carried out with a frequency closest to the recommendations. Many investigations lead to clinical consequences. Therefore we recommend that all patients suspected with or suffering from VHL are monitored according to structured clinical guidelines.

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Vitreoretinal Surgery for Severe Retinal Capillary Hemangiomas

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Retinal capillary hemangioblastoma (RCH) is one of the most common expressions of von Hippel-Lindau (VHL) disease and may lead to blindness in the affected eyes. Although small and middle sized RCH can be efficiently treated by laser photocoagulation, cryotherapy or a combination of both, some patients present, at their first ocular examination, with large, multiple, complicated RCH, which cannot be treated conventionally. We evaluated the long-term success rate of vitreoretinal surgery in a series of 23 eyes presenting with complex cases of RCH due to VHL disease. The most common indication for surgery was the impossibility of eradicating RCH, due either to their large size or to their combination with an exudative or tractional detachment, epiretinal membrane proliferation, or a vitreous hemorrhage. All eyes underwent pars plana vitrectomy with epiretinal membrane dissection and silicone oil or gas injection. In 9 eyes, retinectomy was performed to remove the RCH. The other 14 were treated by laser endophotocoagulation, alone or combined with trascleral cryotherapy. Mean follow up was 8 years. Six months after surgery, the retina was flat in 22 eyes. Long-term complications included RCH reproliferation in 14 eyes and neovascular glaucoma in 5 eyes. Seven eyes became blind. In the remaining 16 eyes, final visual acuity ranged from 20/20 to counting fingers. Large RCH were satisfactorily treated by either vitrectomy with epiretinal dissection and endolaser photocoagulation, or retinectomy for RCH resection, although a high rate of vision-threatening RCH recurrence was observed in the long term.

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Oncololytic Targeting of Renal Cell Carcinoma via Encephalomyocarditis Virus

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Apoptosis is a fundamental host defence mechanism against invading microbes. Inactivation of NF-□B attenuates encephalomyocarditis virus (EMCV) virulence by triggering rapid apoptosis of infected cells, thereby pre-emptively limiting viral replication. Recent evidence has shown that hypoxia-inducible factor (HIF) increases NF-□B-mediated anti-apoptotic response in clear-cell renal cell carcinoma (CCRCC) that commonly exhibit hyperactivation of HIF due to the loss of its principal negative regulator, von Hippel-Lindau (VHL) tumour suppressor protein. Here, we show that EMCV challenge induces a strong NF-□B-dependent gene expression profile concomitant with a lack of interferon-mediated anti-viral response in VHL-null CCRCC, and that multiple established CCRCC cell lines, as well as early-passage primary CCRCC cultured cells, are acutely susceptible to EMCV replication and virulence. Functional restoration of VHL or molecular suppression of HIF or NF-□B dramatically reverses CCRCC cellular susceptibility to EMCV-induced killing. Notably, intratumoural EMCV treatment of CCRCC in a murine xenograft model rapidly regresses tumour growth. These findings provide compelling pre-clinical evidence for the usage of EMCV in the treatment of CCRCC and potentially other tumours with elevated HIF/NF-□B-survival signature.

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Emerging Therapeutic Options for VHL Patients: a Tale of three Studies

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Background: Von Hippel-Lindau (VHL) disease induces vascular neoplasms in multiple organs. We evaluated the safety and efficacy of targeted molecular intervention in VHL patients and examined the expression of various candidate receptors in the endothelium of archived tissue. **Methods:** Patients with genetically confirmed VHL and measurable lesions were enrolled and given oral sunitinib. The primary end point was toxicity. Modified RECIST were used for efficacy assessment. We evaluated 20 archival renal cell carcinomas (RCC) and 20 hemangioblastomas (HB) for endothelial biomarker expression levels using laser-scanning cytometry (LSC). **Results:** Fifteen patients were treated. Grade 3 toxicity included fatigue in five patients. Eighteen RCC, and 21 HB lesions were evaluable. Six of the RCCs (33%) responded partially, whereas none of the HBs did (P=0.014). LSC revealed mean phosphorylated FRS2 levels in HB endothelium were 12.5 (0.49) vs. 11.9 (0.99) in RCC (P=0.059) and in the whole tumor sample, were 11.45 (0.26) for HB vs. 11.26 (0.089) for RCC (P=0.003). **Conclusions:** Sunitinib treatment in VHL patients showed acceptable toxicity. We confirmed a significant response to sunitinib in RCC but not in HB. Greater expression of pFRS2 in HB tissue than in RCC raises the hypothesis that treatment with FGF pathway–blocking agents may benefit patients with HB. We will be treating 14 VHL patients with VHL and hemangioblastomas with TKI258, a small molecule inhibitor of FGFR and VEGFR. Additionally, we will be opening a multicenter 40 patient study treating VHL patients with pazopanib, a well tolerated small molecule VEGFR inhibitor.

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Altering the Stability of Mutant VHL: Potential Therapeutic Consequences

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Background: VHL can acquire missense, nonsense, truncation and full length mutations. Approximately one third of known mutations are missense, generating full length protein with altered functionality. As part of VHL protein (pVHL) maturation, binding to the protein folding nanomachine TRiC occurs, which folds pVHL into a mature conformation, and facilitates association with the hypoxia-inducible factor binding proteins elongins C and B. Disease causing point mutations exist which prevent TRiC binding, or decrease Elongin-BC association with nascent pVHL. Mutated proteins have decreased functionality, and considerably lower stability, and are rapidly degraded via heat shock protein (HSP) 70 and HSP 90 mediated proteasomal degradation. By refolding mutated pVHL into a native configuration, improving pVHL's ability to associate with the Elongin B-C complex, or by decreasing the degradation of mutated pVHL with residual functionalirty, the cancer phenotype can be reduced or reversed. Methods: Venus tagged wild type and W117A mutant VHL were transduced into 786-0 cell lines. Lines were used in a Prestwick library screen to identify compounds that raised fluorescence levels. **Results:** Several compounds were discovered that altered VHL levels in W117A mutated 786-0 lines, altered cell morphology, and resulted in compound specific intracellular VHL aggregations. **Conclusions:** Fluorescence based screening tools can be used to identify compounds that alter mutated VHL homeostasis. Secondary screens and biochemical characterization of compound effect on the VHL biosynthetic and degradative pathways are underway to better define the role of these compounds as potential therapeutic modalities in patients with specific VHL mutations.

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Tale of the Tail: Clinical and Functional Properties of Novel VHL Mutation (X214L) Consistent with Type 2A Phenotype and Low Risk of Renal Cell Carcinoma

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This report describes the clinical and functional properties of a novel von Hippel Lindau variant (X214L) in families with a Type 2A phenotype. The same or similar VHL point mutations were identified in several Type 2A families. This stop codon mutation predicts the translation of a "run-on" protein, extending the length of the normal VHL protein by 14 amino acids. We identified four families with pheochromocytoma-dominant VHL phenotypes who carry stop codon mutations. Although the elongated VHL protein length is the same, the amino acid encoded by the stop codon mutation is different in three of the four families, making shared family origin very unlikely. Western blot was conducted using VHL null renal clear carcinoma cell lines that expressed wild type or X214L mutant pVHL. The predicted 14 amino acid extended protein was stably expressed. The X214L mutant pVHL downregulated HIF□ expression in a normal, canonical hydroxylation-dependent, manner. Defective variant regulation of JunB protein expression was identified. Thus we concluded that the gene variant, X214L, appears to be a deleterious mutation associated with a Type 2A VHL phenotype. Jun B seems to be the targeted part of the VHL machinery that leads to tumor development in germline carriers of this variant. The variant's ability to regulate HIF, predicts for a low risk of clear cell renal carcinoma.

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Knife for Intracranial Hemangioblastomas in von Hippel-Lindau Patients. When and How?

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Introduction: Hemangioblastomas (HGBs) are a distinctive feature of Von Hippel-Lindau (vHL) disease, with successive surgical treatments being a major cause of morbidity and mortality. Radiosurgery has become an option in their treatment.**Objective:** The analysis of our results and those published of Gamma Knife (GK) Radiosurgery for intracranial (IC) HGBs, focusing on vHL´s patients. Method: Between 1994 and 2010, 18 treatments in 14 patients (7 males/7 females) with a total of 40 HGBs have been performed. Fourteen treatments have a complete follow-up. Mean age was 37.4 years. The mean marginal dose was 13.9 Gy, with a mean prescription isodose of 59.4% and a mean treated volume of 4.4 cm3. Six patients had a vHL diagnosis. **Results:** The mean follow up time has been 4 years. The local volumetric control was obtained in all but three patients. In all vHL patients other location HGBs appeared during follow up. **Conclusions:** Gamma Knife radiosurgery is an effective option to surgery in the treatment for vHL patient´s hemangioblastomas. Due to the steep dose decay and minimal peripheral irradiation of healthy tissues, it should be the preferred radiotherapeutic technique. Having in mind the genetic condition of this disease, where the potential oncogenic effect of radiotherapy should be taken into account, any therapeutic decision must be evaluated individually. This treatment must be used in patients with lesions with evident growth or with progressive symptoms, when surgery is not a safe option in a vHL experienced neurosurgical unit.

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VHL in Brazil: Genetics, Biobanking and VHL Family Care

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The von Hippel-Lindau disease is a genetic disorder caused by germline mutation in the *VHL* gene. Although the clinical manifestations are limited to a particular number of organs and systems, including hemangioblastomas in the CNS and retina, pheochromocytomas and paragagliomas, clear cell renal cell carcinomas (ccRCC), pancreatic NETs, ELSTs, among others, the variety of manifestations vary not only between families, but among members in a family. The first study of VHL in Brazil was published by our group in 2003 presenting the germline *VHL* mutations of the first 20 families studied. In order to characterize the spectrum of manifestations of VHL patients of Brazilian families, give them appropriated support, and free access to genetic testing, this study was extended at the Brazilian National Cancer Institute (INCA). In 2009 the Brazilian Alliance on VHL (ABVHL) was founded. So far, around 50 VHL families have been identified, and 44 of them have their germline *VHL* mutation characterized. Interesting, the germline mutation Phe76del was identified in three distinct VHL families in Brazil. A network of reference medical specialists and medical centers has been developed, and genetic services around the country have started seeing VHL patients and families. Orientation and second opinion are remotely accessible to the public, doctors, and researchers. In 2007, a hereditary tumor bank was created with the collaboration of VHL patients and their doctors providing high quality tissue to the ongoing genetics studies of our and other labs.

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Understanding VHL Disease: Molecular Characterization of a Spanish Series

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Genetic characterization of VHL patients has leaded a VHL family's classification based on a genotype–phenotype correlation. While type 1 VHL families (lacking pheochromocytoma though presenting other VHL clinical features) are mainly related to protein truncating mutations, type 2 families (with at least one case of pheochromocytoma) are mostly associated with missense mutations (Maher et al., 1996). The latter group is sub-classified into three different groups based on phenotypes. VHL 2A cases are associated with a low risk of ccRCC, although retain predisposition for developing the remaining VHL-tumors. Generally, subjects with VHL 2B are affected by all the clinical manifestations of the disease, and VHL 2C subgroup includes patients with only familial pheochromocytoma. In these last years, we have witnessed great changes in this field, mainly due to the application of new technologies to diagnostic procedures. The structural analysis of VHL missense mutations has allowed us to understand the phenotypic differences among families carrying mutations affecting the same residue (Ruiz-Llorente S, et al., 2004), and we know now that the risk for CCRC and hemangioblastoma varies among different surface missense mutations (Ong et al., 2007). Regarding type 1 VHL, it has been suggested that complete deletions of the VHL gene that also encompass the BRK1 gene (also known as HSPC300) confers a low risk of CCRC (Maranchie et al., 2004; Cascon et al., 2007). These families could be designated as VHL Type 1B (McNeill A, et al., 2009). A summary of genotype-phenotype correlation found in a large Spanish VHL series molecularly characterized will be presented.

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Case Report: Radiosurgery for Endolymphatic Sac Tumor in a Patient with von Hippel-Lindau Disease

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A 27-year-old man with von Hippel-Lindau (VHL) disease presented with vertigo and tinnitus in the left ear. Neuroimaging demonstrated an extra axial mass with erosion of the postero-medial petrous part of the left temporal bone that was suggestive of endolymphatic sac tumor (ELST) and 2 cerebellar nodules that were suggestive of hemangioblastomas. Audiograms revealed a slight high-frequency hearing loss. He was treated with definitive Gamma Knife radiosurgery (RS) for both lesions. After 9 months, the tinnitus and vertigo were relieved, with significant reduction on symptoms intensity and frequency. Serial imaging evaluations from treatment until 12 months post-RS showed stable lesions and no evidence of new lesions. Audiogram at 17 months post-RS revealed moderate high-frequency hearing loss. Clinical evaluation at 22 months after RS revealed maintenance of tinnitus and worsening of vertigo pattern. After 28 months he underwent surgery for excision of the left temporal bone and the pathological findings were consistent with ELST. We suggest that RS may be an effective and safe therapeutic option for VHL patients with ELST regarding tumor control, but with probably little benefit to audiovestibular function.

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Primary Cilium: a Tumor Suppressor Organelle

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Renal cystic disorders are commonly associated with mutations in genes such as *VHL, TSC2* and *PKD1* that are commonly referred to as 'cystoproteins'. Loss of function of these cystoproteins promotes the development of renal cysts and often, renal cell carcinoma (RCC), as observed in the setting of VHL disease. Importantly, these cystoproteins localize to the primary cilium, an organelle, that in epithelial cells functions as an environmental sensor and participates in spatial regulation of several signaling pathways, and cell polarity. Loss of primary cilia resulting from cystoprotein deficiency is directly linked to cytsogenesis, although it is clear that additional events are required for the progression of the 'pre-cystic' lesions to cysts and renal cell carcinoma. We are investigating how formation of cilia is regulated by VHL, and the oncogenic consequences of loss of this key signaling organelle in kidney epithelial cells. For example, the primary cilium is required for the maintenance of planar cell polarity (PCP), which refers to the polarization of cells perpendicular to their apical/basal axis. During development, loss of PCP leads to widening rather than elongation of renal tubules, resulting in the development of renal cysts. Given that the kidneys are exposed to a wide variety of renal insults and are constantly undergoing repair and regeneration, one of the key hypotheses we are testing is that pre-neoplastic cysts arise in the setting of VHL deficiency, and that these cysts arise as a reactive response to injury.

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