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Erythropoietin (Epo) is a glycoprotein hormone that is the principal regulator of red blood cell production. In the adult, it is produced primarily in the kidney in response to hypoxia or reduced hemoglobin. Hypoxic regulation of Epo production is due to activation of the Epo gene by HIF-1 (**h**ypoxia **i**nducible **f**actor-1), a heterodimeric transcription factor comprising HIF-1 α and HIF-1 β subunits. In the presence of oxygen, the HIF-1 α subunit is rapidly proteolysed by a mechanism involving hydroxylation of specific prolyl residues and interaction with von Hippel-Lindau protein (pVHL). In reduced oxygen, HIF-1 α is stabilized, resulting in enhanced gene transcription. The Epo gene is but one of numerous genes, such as the pro-angiogenic VEGF, that are regulated in this manner. The inherited cancer syndrome von Hippel-Lindau disease is characterized by a mutated VHL gene leading to the absence of hypoxic gene regulation. The result is the constitutive expression of normally hypoxically regulated genes, including Epo. Some cases of renal cysts and renal cancer are caused by somatic mutations of the VHL gene in the renal tissue, and these cases often exhibit increased Epo production and the paraneoplastic syndrome of erythrocytosis. Those renal cancers resulting from other mutations but possessing normal VHL genes do not produce Epo. Also, there is heterogeneity of VHL gene deletions within individual renal cell carcinoma tumors, leading to a more complex clinical picture.

Key words: erythropoietin, von Hippel-Lindau, hypoxia, gene regulation.

Erythropoietin, von Hippel-Lindau protein and renal cancer: molecular basis for the paraneoplastic syndrome of erythrocytosis

Erytropoetyna, białko von Hippel-Lindau i rak nerki: molekularne podstawy towarzyszącego chorobie nowotworowej zespołu erytrocytozy

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A striking characteristic of neoplastic processes is the partial or total release of the involved tissue from control by those factors that regulate development and differentiation. A consequence of this release from control is the occurrence of several paraneoplastic syndromes due to secretion by the tumor cells of one or more substances. The secretion of these substances may be the result of the expression of a gene not normally expressed in the tissue, and, hence, a totally foreign molecule may be synthesized, e.g., ACTH secretion by bronchogenic carcinoma ("ectopic" hormone production). On the other hand, the substance may be secreted by the tissue normally, but the tumor, being independent of feedback processes, elaborates an abnormally large amount of the particular hormone and/or its precursors. One well-characterized example is the insulinoma, which secretes insulin at a rate often independent of plasma glucose levels and, moreover, may secrete large quantities of the precursor molecule proinsulin so as to achieve a plasma proinsulin/insulin ratio far greater than that resulting from normal secretion by the pancreatic islets.

Renal cancer, or more specifically adenocarcinoma or hypernephroma, frequently can be associated with such paraneoplastic syndromes [1], a point of substantial clinical importance since a significant fraction of patients with this tumor present with systemic but without specific genitourinary symptomatology. Like bronchogenic carcinoma, renal adenocarcinoma may secrete hormones or other substances not secreted by the normal organ parenchyma, i.e., ectopic production. Examples include parathyroid hormone, enteroglucagon and chorionic gonadotropin. But it must be emphasized that the kidney has several endocrine functions of its own, and excess production of renin, prostaglandins and erythropoietin (Epo) have all been reported in patients with renal adenocarcinoma. It is the molecular mechanism of Epo production by renal adenocarcinoma that will now be addressed.

Epo is a glycoprotein hormone that is the prime regulator of red blood cell production. It also has numerous non-hematopoietic actions (reviewed in [2]). In the adult, it is produced primarily by interstitial fibroblasts in the renal cortex. Its rate of secretion (corresponding to expression of the Epo gene) is regulated by hypoxia (see below). Renal tumors may secrete excess Epo by at least two different mechanisms. Firstly, the expanding mass may cause

Erytropoetyna (Epo) jest hormonem glikoproteinowym i pełni funkcję głównego regulatora wytwarzania czerwonych krwinek. U osób dorosłych hormon ten jest wytwarzany głównie w nerkach w odpowiedzi na niedotlenienie lub obniżenie poziomu hemoglobiny. Regulacja wytwarzania erytropoetyny w warunkach obniżonego stężenia tlenu polega na aktywacji genu kodującego erytropoetynę przez HIF-1 (ang. ***h**ypoxia **i**nducible **f**actor-1*) – czynnik transkrypcyjny o budowie heterodimeru, składający się z 2 podjednostek: HIF-1α i HIF-1β. W obecności tlenu podjednostka HIF-1α ulega szybkiej proteolizie. Mechanizm tej proteolizy obejmuje hydroksylację specyficznej reszty prolinowej i interakcję z białkiem von Hippel-Lindau (pVHL). W warunkach obniżonego stężenia tlenu następuje stabilizacja HIF-1α, co powoduje wzrost transkrypcji genów. Gen kodujący erytropoetynę jest jednym z wielu genów regulowanych w ten sposób (innym genem z tej grupy jest np. gen kodujący proangiogenny czynnik VEGF). Charakterystyczną cechą dziedzicznego zespołu von Hippel-Lindau jest mutacja w genie VHL, powodująca brak regulacji ekspresji genów w warunkach niedotlenienia. Wynikiem tej sytuacji jest konstytutywna ekspresja genów, takich jak gen kodujący erytropoetynę, które normalnie są regulowana przez obniżone stężenie tlenu. Niektóre przypadki torbieli nerkowych i raka nerki są spowodowane przez somatyczne mutacje genu VHL w komórkach nerki. Te przypadki często charakteryzują się zwiększonym wytwarzaniem Epo i zespołem erytrocytozy, towarzyszącym chorobie nowotworowej. Inne przypadki raka nerki, które powstały na skutek mutacji w innych genach i posiadają normalny gen VHL, nie wytwarzają Epo. Zatem istniejąca heterogenność w delecjach genu VHL w indywidualnych przypadkach raka nerki leży u podstaw większej złożoności obrazu klinicznego tej choroby.

Słowa kluczowe: erytropoetyna, von Hippel-Lindau, niedotlenienie, regulacja ekspresji genów.

partial obstruction of adjacent blood vessels resulting in localized areas of hypoxemia. This mechanism is the likely cause of increased Epo levels found in some patients with benign renal cysts. It may, of course, also be operative in some patients with malignant renal lesions. However, the second mechanism, that of true synthesis of Epo by the malignant tissue itself, is the more intriguing one, despite the difficulty in establishing it in a given case.

Identification of hypoxia inducible factor-1 (HIF-1)– hypoxia regulates more than the erythropoietin gene

In 1993, Wang and Semenza described hypoxia inducible factor 1 (HIF-1) [3, 4]. It is a nuclear factor from Hep3B cells, an Epo-producing hepatoma cell line, that was detected when cells were cultured in 1% oxygen but not in 20% oxygen. Hypoxia also induced it in several cell lines that did not express Epo. HIF-1 DNA binding activity disappeared rapidly when hypoxic cells were exposed to increased oxygen. HIF-1 rapidly associates with and dissociates from its DNA binding site *in vitro* ($T_{1/2}$<1 min for both processes). The authors also showed that the iron chelator desferrioxamine induced both Epo expression and HIF-1 binding activity [5]. Desferrioxamine induced Epo gene expression in the kidneys of mice *in vivo*. Importantly, desferrioxamine also induced HIF activity in Epo non-producing cells, suggesting "a common hypoxia signal transduction pathway leading to HIF-1 induction in different cell types."

HIF-1 activates the transcription of numerous genes other than erythropoietin (see [6]). Semenza *et al.* showed that transcripts encoding the glycolytic enzymes aldolase A, phosphoglycerate kinase 1 and pyruvate kinase M were induced in cells by exposure to HIF inducers (1% oxygen, cobalt chloride or desferrioxamine). Partial gene sequences from these enzymes contained nucleotide sequences related to the HIF-1 binding site of the Epo enhancer and bound HIF-1 specifically. These glycolytic enzyme gene sequences containing HIF-1 binding sites were shown to mediate hypoxia inducible transcription, further demonstrating the importance of hypoxic regulation outside of the erythropoietin system.

HIF-1 is a heterodimer [7]. Both subunits of HIF-1 (α and β) are basic helix-loop-helix (bHLH) proteins containing a PAS domain. The PAS domain is common to the *Drosophila* PER and SIM proteins and the mammalian ARNT (aryl hydrocarbon receptor nuclear translocator) and AHR proteins. HIF-1 α is related to SIM and HIF-1 β is one of a series of ARNT gene products. Thus, the designations "HIF-1 β" and "ARNT" are often used interchangeably throughout the literature.

The importance of HIF-1 action in hypoxic regulation of gene expression was further emphasized when it was shown that vascular endothelial growth factor (VEGF) gene transcription was activated by HIF-1. Forsythe et al. observed that VEGF induced angiogenesis in several clinically important situations including myocardial ischemia, retinal disease and tumor growth and that HIF-1 was responsible [8]. Interestingly, in demonstrating that a 500 nt region of the 3' UTR of VEGF mRNA was critical for stabilization of VEGF mRNA [9, 10], Levy et al. observed that the protein mRNA complex was elevated in cells lacking the von Hippel-Lindau tumor suppression gene. This observation was to have great importance in the elucidation of the oxygen sensor.

Other interacting proteins and the regulation Of HIF-1

The regulation of Epo gene expression as well as other hypoxia inducible genes requires several regulatory proteins in addition to HIF-1 α and β. In a study of 11 different orphan nuclear receptors, Galson et al. screened their ability to bind to elements in the Epo promoter and enhancer by electrophoretic mobility shift assay [11]. Four of these receptors bound

specifically to the response elements in the Epo promoter and enhancer, namely, hepatic nuclear factor 4 (HNF-4), TR2-11, ROR α 1 and EAR3/COUP-TF1. By ectopically expressing HNF-4 in HeLa cells, the authors observed an 8-fold increase in hypoxic induction of a reporter gene construct containing the minimal Epo enhancer and promoter. Thus, HNF-4 is an important positive regulator in tissue specific and hypoxia inducible expression of the Epo gene.

The homologous transcription adaptors p300/CRB and CBP also play a role in hypoxic regulation. Arany *et al*. searched for specific p300 binding proteins and found that HIF-1 α interacted with p300 [12] and that hypoxia induced the formation of a DNA binding complex containing both HIF-1 α and p300/CBP. p300/CBP-HIF complexes are important in the regulation of hypoxia induced genes.

Ebert and Bunn provided an important insight into the complex role played by p300/CRB in hypoxic regulation [13]. They analyzed the protein multimer that binds to the lactate dehydrogenase A (LDH-A) promoter and demonstrated the involvement of HIF-1, p300/CRB and CREB-1/ATF-1. Also , Bunn and his colleagues demonstrated the mechanism of regulation of HIF-1 α. Huang *et al*. identified an **oxygen dependent degradation domain (ODDD)** within HIF-1 α that controlled degradation by the ubiquitin-proteasome pathway [14]. Deletional mutagenesis of the domain yielded a HIF-1 α that was stable and active in the absence of hypoxic induction.

The von Hippel-Lindau protein, proline hydroxylation and the oxygen sensor

The von Hippel-Lindau protein was the seminal clue that led to the identification of the oxygen sensor. Von Hippel-Lindau (VHL) disease is an inherited disorder in which individuals have a predisposition to develop clear cell renal carcinoma, pheochromocytoma, spinal cord cerebellar and retinal hemangioblastoma [15-21]. Hemangiomas of other organs (adrenals, lungs, liver) and multiple pancreatic and renal cysts occur as well. There is a clear pro-angiogenic phenotype. Earlier, Levy *et al*. described the association of VEGF expression and the absence of VHL protein (pVHL) [10], and Iliopoulus *et al*. observed that VHL deficient cells lacked hypoxic regulation that could be restored by expression of VHL protein [22].

It was discovered that pVHL played a role in oxygen dependent proteolysis of HIF [23]. In VHL deficient cells, HIF-1 α protein was constitutively stabilized in normoxia, and HIF-1 was activated. Expression of pVHL restored oxygen dependent instability of HIF-1 α. pVHL and HIF-1 α interacted directly, and pVHL was detected in the hypoxic HIF-1 DNA binding complex. pVHL and HIF-1 dissociated in cells treated with desferrioxamine or cobalt. Thus, the pVHL/HIF-1 interaction is iron dependent, and it is required for oxygen dependent degradation of HIF-1 α.

Cockman et al. demonstrated that pVHL was essential for an oxygen dependent degradation domain (ODDD) mediated destruction of HIF-1 α by the ubiquitin-proteasome pathway [14, 24]. HIF-1 α ubiquitinylation was defective in VHL deficient renal carcinoma cells and that exogenous expression of pVHL complemented this defect. This effect was specific for HIF-1 α subunits. They went on to demonstrate that short sequences within the internal transactivation domains of HIF α were sufficient for recognition by pVHL. Mutagenesis studies delineated the structural requirement for this interaction. The authors concluded that pVHL regulated HIF α degradation by functioning as a "recognition component of a ubiquitin ligase complex".

In a critical discovery, Jaakkola *et al.* showed that HIF-1 α targeting to the pVHL ubiquitin E3 ligase complex was dependent upon oxygen regulated prolyl hydroxylation [25]. Studies of HIF-1 α demonstrated that proline 564 hydroxylation was critical for this interaction [26, 27]. The enzyme responsible for this reaction was designated as **HIF α prolyl hydroxylase (HIF-PH)**. The absolute requirement for oxygen as a cosubstrate and iron as a cofactor suggested that HIF-PH functioned directly as a cellular oxygen sensor. Hydroxylation of proline 402 provides yet another site for pVHL binding [28].

In addition to prolyl hydroxylation that regulates the hypoxia inducibility/stabilization of HIF-1 α, hydroxylation at another site in HIF-1 α plays a distinct role. The C-terminal activation domain (CAD) of HIF-1 α and its regulation involve hydroxylase activity that is not dependent upon pVHL [29]. This CAD hydroxylation is on asparagine 803 of HIF-1 α. Indeed, a hypoxia inducible HIF asparagine hydroxylase, identical to a previously identified **HIF interactor designated factor inhibiting HIF (FIH)** [30], was shown to downregulate HIF α transactivation and was later shown to interact with HIF-1 α and pVHL, thus mediating repression of HIF-1 transcriptional activity. This asparagine hydroxylation abrogated p300 binding to HIF-1 α. Figure 1 depicts the results of HIF-1 α proline and asparagine hydroxylation.

Von Hippel-Lindau (VHL) disease and acquired VHL mutations

Von Hippel-Lindau disease is an autosomal dominant cancer syndrome resulting from a germ line mutation in the VHL gene [16-21]. Mutation or loss of the VHL gene results in a propensity to develop numerous benign or malignant tumors, as well as cysts in several organ systems [20]. Within the central nervous system, individuals may develop retinal hemangioblastomas, endolymphatic sac tumors and cranial spinal hemangioblastomas of the cerebellum, brain stem, spinal cord, lumbar sacral nerve roots and supratentorial structures. Disorders of the viscera include renal cell carcinoma and cysts, pheochromocytomas, pancreatic tumors or cysts, epididymal cystadenomas and broad ligament cystadenomas. Table 1 (modified from reference [20]) shows the age of onset and frequency of VHL disease lesions.

Since VHL protein plays a critical role in the regulation of the Epo gene, it is not surprising that some VHL disease

Table 1. Von Hippel-Lindau disease lesions
Tabela 1. *Zmiany patologiczne w chorobie Von Hippel-Lindau*

	Mean (range) age of onset, years	Frequency in patients (%)
CENTRAL NERVOUS SYSTEM		
Retinal hemangioblastomas	25 (1-67)	25-60%
Endolymphatic sac tumors	22 (12-50)	10%
Craniospinal hemangjoblastomas		
Cerebellum	33 (9-78)	44-72%
Brain stem	32 (12-46)	10-25%
Spinal cord	33 (12-66)	13-50%
Lumbosacral nerve roots	Unknown	<1%
Supratentorial	Unknown	<1%
VISCERA		
Renal cell carcinoma or cysts	39 (16-67)	25-60%
Pheochromocytoma	30 (5-58)	10-20%
Pancreatic tumor or cyst	36 (5-70)	35-70%
Epididymal cystadenoma	Unknown	25-60%
Broad ligament cystadenoma	Unknown	Unknown

tumors can produce Epo constitutively. Renal cell carcinomas or cysts and cerebellar hemangioblastoma are most frequently associated with elevated circulating Epo levels [31-39]. There is no clear reason why other VHL disease lesions may not also produce Epo. Perhaps they do, but not at levels high enough to be detected clinically. There appears to have been no systematic assessment of this phenomenon among VHL disease patients or their pathological tissues.

Acquired VHL mutations also occur most frequently in clear cell renal carcinoma [40-46]. Loss of chromosome 3p or loss of heterozygosity can result in inactivation of VHL. The incidence of erythrocytosis associated with renal adenocarcinoma has been reported to be between 1-5% [39, 47] and has not been correlated with the frequency of VHL mutations. Besides loss or mutation of VHL, other genes have been associated with renal cell carcinoma including FHIT, FOXP1 and others [40, 46, 48-53]. This potential

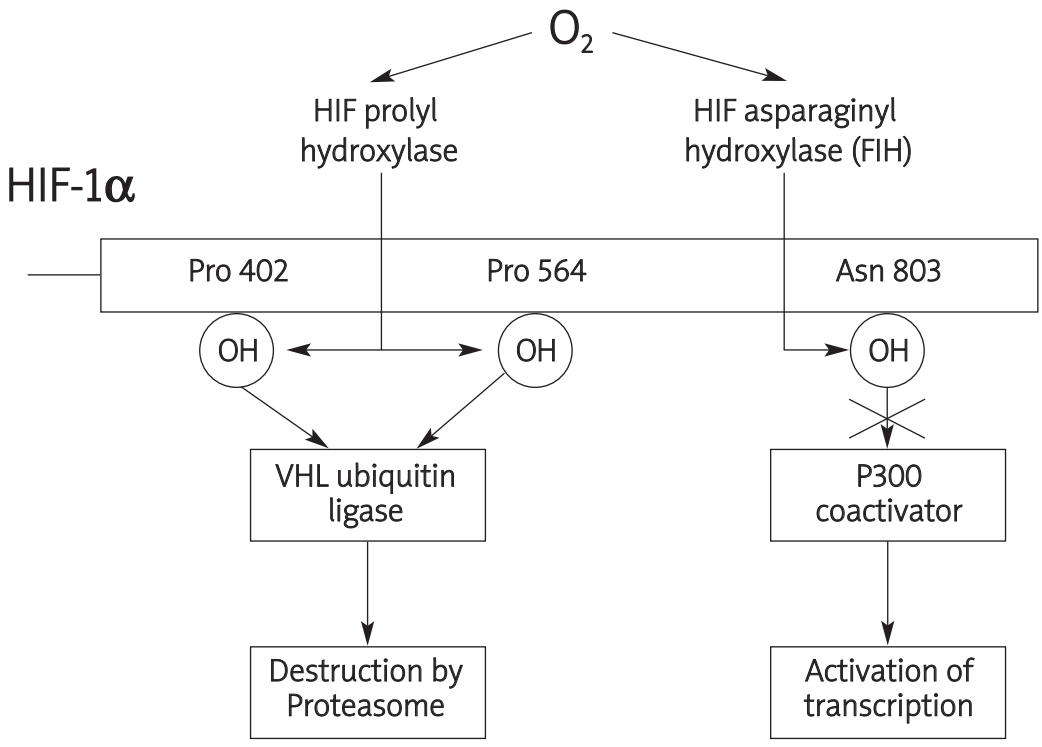

Fig. 1. Regulation of HIF-1 α by hydroxylation of proline 402 and 564 and asparagine 803
Ryc. 1. *Regulacja HIF-1 alfa przez hydroksylację proliny 402 i 564 oraz asparaginy 803*

multiplicity of causes of renal cell carcinoma may account for the relatively low incidence of Epo production by this tumor. Also, there is heterogeneity of VHL gene deletions within individual renal cell carcinoma tumors [41].

Other erythropoietin-producing tumors

Other, non-VHL associated tumors, may also produce Epo. One of the most common is hepatocellular carcinoma [54-56]. Epo gene expression by these cells may simply reflect "de-differentiation" from the adult to a more fetal phenotype (the liver is the principal source of Epo production in the fetus). Also, Wilms' tumors may produce Epo [57-62]. More unusual types of Epo-producing tumors include pancreatic ductal carcinoma [63], renal capillary hemangioma [64-79] and uterine leiomyoma [65-79]. In most of these more unusual situations, an investigation for VHL mutations was not carried out.

Summary

Our present understanding of the mechanism of hypoxic regulation of the Epo gene (and numerous other genes) provides a direct link to the cause of some forms of renal cancer, namely, the von Hippel-Lindau protein. Mutation or loss of this gene, either in an inherited fashion or in a tissue-specific acquired fashion, can lead to renal cysts and carcinoma as well as neoplasms of several other organs. Loss of hypoxic regulation of several genes plays a role in the neoplastic process, and increased production of Epo is recognized as the paraneoplastic syndrome of erythrocytosis.

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References

1. Sufrin G, Chasan S, Golio A, Murphy G. Paraneoplastic and serologic syndromes of renal adenocarcinoma. Semin Urol 1989; 7 (3): 158-71.
2. Sytkowski AJ. Erythropoietin: Blood, Brain and Beyond. 2004, Weinheim: Wiley-VCH.
3. Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A 1993; 90 (9): 4304-8.
4. Wang GL, Semenza GL. Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia. J Biol Chem 1993; 268 (29): 21513-8.
5. Wang GL, Semenza GL. Desferrioxamine induces erythropoietin gene expression and hypoxia-inducible factor 1 DNA-binding activity: implications for models of hypoxia signal transduction. Blood 1993; 82 (12): 3610-5.
6. Semenza GL. Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol 2002; 64 (5-6): 993-8.
7. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92 (12): 5510-4.
8. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996; 16 (9): 4604-13.
9. Levy AP, Levy NS, Goldberg MA. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. J Biol Chem 1996; 271 (5): 2746-53.
10. Levy AP, Levy NS, Goldberg MA. Hypoxia-inducible protein binding to vascular endothelial growth factor mRNA and its modulation by the von Hippel-Lindau protein. J Biol Chem 1996; 271 (41): 25492-7.
11. Galson DL, Tsuchiya T, Tendler DS, Huang LE, Ren Y, Ogura T, Bunn HF. The orphan receptor hepatic nuclear factor 4 functions as a transcriptional activator for tissue-specific and hypoxia-specific erythropoietin gene expression and is antagonized by EAR3/COUP-TF1. Mol Cell Biol 1995; 15 (4): 2135-44.
12. Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, Bunn HF, Livingston DM. An essential role for p300/CBP in the cellular response to hypoxia. Proc Natl Acad Sci U S A 1996; 93 (23): 12969-73.
13. Ebert BL, Bunn HF. Regulation of transcription by hypoxia requires a multiprotein complex that includes hypoxia-inducible factor 1, an adjacent transcription factor, and p300/CREB binding protein. Mol Cell Biol 1998; 18 (7): 4089-96.
14. Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci U S A 1998; 95 (14): 7987-92.
15. Clifford SC, Cockman ME, Smallwood AC, Mole DR, Woodward ER, Maxwell PH, Ratcliffe PJ, Maher ER. Contrasting effects on HIF-1alpha regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel-Lindau disease. Hum Mol Genet 2001; 10 (10): 1029-38.
16. Clifford SC, Maher ER. Von Hippel-Lindau disease: clinical and molecular perspectives. Adv Cancer Res 2001; 82: 85-105.
17. Iliopoulos O. Von Hippel-Lindau disease: genetic and clinical observations. Front Horm Res 2001; 28: 131-66.
18. Sims KB. Von Hippel-Lindau disease: gene to bedside. Curr Opin Neurol 2001; 14 (6): 695-703.
19. Singh AD, Shields CL, Shields JA. Von Hippel-Lindau disease. Surv Ophthalmol 2001; 46 (2): 117-42.
20. Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, Linehan WM, Oldfield EH. Von Hippel-Lindau disease. Lancet 2003; 361 (9374): 2059-67.
21. Kim WY, Kaelin WG. Role of VHL Gene Mutation in Human Cancer. J Clin Oncol 2004; 22 (24): 4991-5004.
22. Iliopoulos O, Levy AP, Jiang C, Kaelin WG, Jr., Goldberg MA. Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. Proc Natl Acad Sci U S A 1996; 93 (20): 10595-9.
23. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis.[comment]. Nature 1999; 399 (6733): 271-5.
24. Cockman ME, Masson N, Mole DR, Jaakkola P, Chang GW, Clifford SC, Maher ER, Pugh CW, Ratcliffe PJ, Maxwell PH. Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. J Biol Chem 2000; 275 (33): 25733-41.
25. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation [comment]. Science 2001; 292 (5516): 468-72.
26. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG, Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing [comment]. Science 2001; 292 (5516): 464-8.
27. Yu F, White SB, Zhao Q, Lee FS. HIF-1alpha binding to VHL is regulated by stimulus-sensitive proline hydroxylation [erratum appears in Proc Natl Acad Sci U S A 2001; 98 (25): 14744]. Proc Natl Acad Sci U S A 2001; 98 (17): 9630-5.
28. Masson N, Willam C, Maxwell PH, Pugh CW, Ratcliffe PJ. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO Journal 2001; 20 (18): 5197-206.

29. Sang N, Fang J, Srinivas V, Leshchinsky I, Caro J. Carboxyl-terminal transactivation activity of hypoxia-inducible factor 1 alpha is governed by a von Hippel-Lindau protein-independent, hydroxylation-regulated association with p300/CBP. Mol Cell Biol 2002; 22 (9): 2984-92.
30. Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, Elkins JM, Oldham NJ, Bhattacharya S, Gleadle JM, Ratcliffe PJ, Pugh CW, Schofield CJ. Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. J Biol Chem 2002; 277 (29): 26351-5.
31. Sherwood JB, Goldwasser E. Erythropoietin production by human renal carcinoma cells in culture. Endocrinology 1976; 99 (2): 504-10.
32. Shouval D, Anton M, Galun E, Sherwood JB. Erythropoietin-induced polycythemia in athymic mice following transplantation of a human renal carcinoma cell line. Cancer Res 1988; 48 (12): 3430-4.
33. Da Silva JL, Lacombe C, Bruneval P, Casadevall N, Leporrier M, Camilleri JP, Bariety J, Tambourin P, Varet B. Tumor cells are the site of erythropoietin synthesis in human renal cancers associated with polycythemia. Blood 1990; 75 (3): 577-82.
34. Gross AJ, Wolff M, Fandrey J, Miersch WD, Dieckmann KP, Jelkmann W. Prevalence of paraneoplastic erythropoietin production by renal cell carcinomas. Clin Invest 1994; 72 (5): 337-40.
35. Tachibana O, Yamashima T, Yamashita J, Takinami K. Immunohistochemical study of basic fibroblast growth factor and erythropoietin in cerebellar hemangioblastomas. Noshuyo Byori 1994; 11 (2): 169-72.
36. Shiramizu M, Katsuoka Y, Grodberg J, Koury ST, Fletcher JA, Davis KL, Sytkowski AJ. Constitutive secretion of erythropoietin by human renal adenocarcinoma cells in vivo and in vitro. Exp Cell Res 1994; 215 (2): 249-56.
37. Clark D, Kersting R, Rojiani AM. Erythropoietin immunolocalization in renal cell carcinoma. Modern Pathology 1998; 11 (1): 24-8.
38. Gold PJ, Fefer A, Thompson JA. Paraneoplastic manifestations of renal cell carcinoma. Semin Urol Oncol 1996; 14 (4): 216-22.
39. Thorling EB. Paraneoplastic erythrocytosis and inappropriate erythropoietin production. A review. Scan J Haematol Suppl 1972; 17: 1-166.
40. Chino K, Esumi M, Ishida H, Okada K. Characteristic loss of heterozygosity in chromosome 3P and low frequency of replication errors in sporadic renal cell carcinoma. J Urol 1999; 162 (2): 614-8.
41. Moch H, Schraml P, Bubendorf L, Richter J, Gasser TC, Mihatsch MJ, Sauter G. Intratumoral heterogeneity of von Hippel-Lindau gene deletions in renal cell carcinoma detected by fluorescence in situ hybridization. Cancer Res 1998; 58 (11): 2304-9.
42. Duffy K, Al-Saleem T, Karbowniczek M, Ewalt D, Prowse AH, Henske EP. Mutational analysis of the von hippel lindau gene in clear cell renal carcinomas from tuberous sclerosis complex patients. Modern Pathology 2002; 15 (3): 205-10.
43. Lott ST, Chandler DS, Curley SA, Foster CJ, El-Naggar A, Frazier M, Strong LC, Lovell M, Killary AM. High frequency loss of heterozygosity in von Hippel-Lindau (VHL)-associated and sporadic pancreatic islet cell tumors: evidence for a stepwise mechanism for malignant conversion in VHL tumorigenesis. Cancer Res 2002; 62 (7): 1952-5.
44. Shuin T, Kamata M, Ashida S. Development of human renal cell carcinoma (RCC) – the responsible genes for the development of hereditary and sporadic human RCCs. Gan to Kagaku Ryoho [Japanese Journal of Cancer & Chemotherapy] 2002; 29 (10): 1719-25.
45. Wiesener MS, Seyfarth M, Warnecke C, Jurgensen JS, Rosenberger C, Morgan NV, Maher ER, Frei U, Eckardt KU. Paraneoplastic erythrocytosis associated with an inactivating point mutation of the von Hippel-Lindau gene in a renal cell carcinoma. Blood 2002; 99 (10): 3562-5.
46. Sukosd F, Kuroda N, Beothe T, Kaur AP, Kovacs G. Deletion of chromosome 3p14.2-p25 involving the VHL and FHIT genes in conventional renal cell carcinoma. Cancer Res 2003; 63 (2): 455-7.
47. Murphy GP, Kenny GM, Mirand EA. Erythropoietin levels in patients with renal tumors or cysts. Cancer 1970; 26 (1): 191-4.
48. Lovell M, Lott ST, Wong P, El-Naggar A, Tucker S, Killary AM. The genetic locus NRC-1 within chromosome 3p12 mediates tumor suppression in renal cell carcinoma independently of histological type, tumor microenvironment, and VHL mutation. Cancer Res 1999; 59 (9): 2182-9.
49. Martinez A, Fullwood P, Kondo K, Kishida T, Yao M, Maher ER, Latif F. Role of chromosome 3p12-p21 tumour suppressor genes in clear cell renal cell carcinoma: analysis of VHL dependent and VHL independent pathways of tumorigenesis. Molecular Pathology 2000; 53 (3): 137-44.
50. Dreijerink K, Braga E, Kuzmin I, Geil L, Duh FM, Angeloni D, Zbar B, Lerman MI, Stanbridge EJ, Minna JD, Protopopov A, Li J, Kashuba V, Klein G, Zabarovsky ER. The candidate tumor suppressor gene, RASSF1A, from human chromosome 3p21.3 is involved in kidney tumorigenesis. Proc Natl Acad Sci U S A 2001; 98 (13): 7504-9.
51. Morrissey C, Martinez A, Zatyka M, Agathanggelou A, Honorio S, Astuti D, Morgan NV, Moch H, Richards FM, Kishida T, Yao M, Schraml P, Latif F, Maher ER. Epigenetic inactivation of the RASSF1A 3p21.3 tumor suppressor gene in both clear cell and papillary renal cell carcinoma. Cancer Res 2001; 61 (19): 7277-81.
52. Girolami F, Passerini I, Gargano D, Frusconi S, Villari D, Nicita G, Torricelli F. Microsatellite analysis of chromosome 3p region in sporadic renal cell carcinomas. Pathol Oncol Res 2002; 8 (4): 241-4.
53. Ramp U, Caliskan E, Ebert T, Karagiannidis C, Willers R, Gabbert HE, Gerharz CD. FHIT expression in clear cell renal carcinomas: versatility of protein levels and correlation with survival. J Pathol 2002; 196 (4): 430-6.
54. Kew MC, Fisher JW. Serum erythropoietin concentrations in patients with hepatocellular carcinoma. Cancer 1986; 58 (11): 2485-8.
55. Sakisaka S, Watanabe M, Tateishi H, Harada M, Shakado S, Mimura Y, Gondo K, Yoshitake M, Noguchi K, Hino T, et al. Erythropoietin production in hepatocellular carcinoma cells associated with polycythemia: immunohistochemical evidence. Hepatology 1993; 18 (6): 1357-62.
56. Hwang SJ, Lee SD, Wu JC, Chang CF, Lu CL, Tsay SH, Lo KJ. Clinical evaluation of erythrocytosis in patients with hepatocellular carcinoma. Chung Hua i Hsueh Tsa Chih - Chinese Medical Journal 1994; 53 (5): 262-9.
57. Murphy GP, Mirand EA, Sinks LF, Allen JE, Staubitz WJ. Ectopic production of erythropoietin in Wilms tumor patients in relation to clinical stage and disease activity. J Urol 1975; 113 (2): 230-3.
58. Slee PH, Blusse A, de la Riviere GB, den Ottolander GJ. Erythrocytosis and Wilms' tumour. Scan J Haematol 1978; 21 (4): 287-91.
59. Dreicer R, Donovan J, Benda JA, Lund J, Degowin RL. Paraneoplastic erythrocytosis in a young adult with an erythropoietin-producing Wilms' tumor. Am J Med 1992; 93 (2): 229-30.
60. Souid AK, Dubansky AS, Richman P, Sadowitz PD. Polycythemia: a review article and case report of erythrocytosis secondary to Wilms' tumor. Pediatr Hematol Oncol 1993; 10 (3): 215-21.
61. Lal A, Rice A, al Mahr M, Kern IB, Marshall GM. Wilms tumor associated with polycythemia: case report and review of the literature. J Pediat Hematol Oncol 1997; 19 (3): 263-5.
62. Chen CW, Huang SP, Li YC, Chou YH, Huang CH. Adult Wilms' tumor associated with polycythemia–a case report. Kaohsiung J Med Sciences 2001; 17 (2): 107-11.
63. Kawai H, Kojima M, Yokota M, Iguchi H, Wakasugi H, Jimi A, Funakoshi A. Erythropoietin-producing pancreatic ductal adenocarcinoma. Pancreas 2000; 21 (4): 427-9.
64. Leak BJ, Javidan J, Dagher R. A rare case of renal hemangioma presenting as polycythemia. Urology 2001; 57 (5): 975.
65. Fried W, Ward HP, Hopeman AR. Leiomyoma and erythrocytosis: a tumor producing a factor which increases erythropoietin production. Report of case. Blood 1968; 31 (6): 813-6.
66. Payne PR. Polycythemia and uterine fibroids. Proc R Soc Med 1968; 61 (12): 1279-80.
67. Payne P, Woods HF, Wrigley PF. Uterine fibromyomata and secondary polycythaemia. J Obstet Gynaecol Br Commonw 1969; 76 (9): 845-9.
68. Wrigley PF, Malpas JS, Turnbull AL, Jenkins GC, McArt A. Secondary polycythaemia due to a uterine fibromyoma producing erythropoietin. Brit J Haematol 1971; 21 (5): 551-5.

69. Spurlin GW, Van Nagell JR, Jr., Parker JC, Jr., Roddick JW, Jr., Uterine myomas and erythrocytosis. Obstet Gynecol 1972; 40 (5): 646-51.
70. Toyama K, Fujiyama N, Kikuchi M, Chen TP, Hasegawa M. Erythrocytosis associated with uterine leiomyoma. A report of the first case in Japan (author's transl). Rinsho Ketsueki - Japanese Journal of Clinical Hematology 1973; 14 (0): 1440-6.
71. Weiss DB, Aldor A, Aboulafia Y. Erythrocytosis due to erythropoietin-producing uterine fibromyoma. Am J Obstet Gynecol 1975; 122 (3): 358-60.
72. Naets JP, Wittek M, Delwiche F, Kram I. Polycythaemia and erythropoietin producing uterine fibromyoma. Scand J Haematol 1977; 19 (1): 75-8.
73. Desablens B, Steenkiste G, Boffa GA, Messerschmitt J. [Polycythaemia and uterine fibroma. A case with in vitro demonstration of an erythropoietic activity in the tumor (author's transl)]. Semaine des Hopitaux 1980; 56 (25-28): 1138-44.
74. Itoh K, Hiruma K, Wakita H, Nakamura H, Endoh N, Wong PM, Asai T, Yoshida S. Erythrocytosis associated with uterine leiomyoma: a case report. Rinsho Ketsueki - Japanese J Clin Hematol 1987; 28 (6): 897-901.
75. Clark CL, Wilson TO, Witzig TE. Giant uterine fibromyoma producing secondary polycythemia. Obstet Gynecol 1994; 84 (4 Pt 2): 722-4.
76. LevGur M, Levie MD. The myomatous erythrocytosis syndrome: a review. Obstet Gynaecol 1995; 86 (6): 1026-30.
77. Yoshida M, Koshiyama M, Fujii H, Konishi M. Erythrocytosis and a fibroid. Lancet 1999; 354 (9174): 216.
78. Kohama T, Shinohara K, Takahura M, Inoue M. Large uterine myoma with erythropoietin messenger RNA and erythrocytosis. Obstet Gynecol 2000; 96 (5 Pt 2): 826-8.
79. Suzuki M, Takamizawa S, Nomaguchi K, Suzu S, Yamada M, Igarashi T, Sato I. Erythropoietin synthesis by tumour tissues in a patient with uterine myoma and erythrocytosis. Brit J Haematol 2001; 113 (1): 49-51.

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