HIF and fumarate hydratase in renal cancer

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Hereditary leiomyomatosis and renal cell cancer is a recently described hereditary cancer syndrome in which affected individuals are predisposed to the development of leiomyomas of the skin and uterus. In addition, this clinical entity also can result in the development of biologically aggressive kidney cancer. Affected individuals harbour a germline mutation of the fumarate hydratase (FH) gene, which encodes an enzyme that catalyses conversion of fumarate to malate in the Kreb’s cycle. Thus far, proposed mechanisms for carcinogenesis associated with this syndrome include aberrant apoptosis, oxidative stress, and pseudohypoxic drive. At this time, the majority of accumulating data support a role for pseudohypoxic drive in tumour development. The link between FH mutation and pseudohypoxic drive may reside in the biochemical alterations resulting from diminished/absent FH activity. These biochemical derangements may interfere with oxygen homeostasis and result in a cellular environment conducive to tumour formation.

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Kidney cancers represent a heterogeneous group of malignancies linked by the primary site of pathology. Despite originating in the kidney, renal cell carcinoma (RCC) is a disease with varying genetic basis. Biologic characterisation of this disease has been greatly aided by the investigation of families with multiple members affected by RCC. Probably the earliest and best-characterised familial form of kidney cancer is von-Hippel Lindau (VHL). von-Hippel Lindau RCC. Probably the earliest and best-characterised familial form of kidney cancer is von-Hippel Lindau (VHL). von-Hippel Lindau families are susceptible to the development of clear cell histology RCC owing to inherited alterations of the VHL gene (Latif et al, 1993). Moreover, the VHL gene has also been implicated in the development of a significant proportion of sporadic clear cell kidney cancers (Gnarra et al, 1994). In addition to VHL, other genes have been identified to be involved with inherited kidney cancer, for example, c-met in hereditary papillary renal carcinoma (Schmidt et al, 1997).

More recently, an inherited neoplastic syndrome has been identified and referred to as hereditary leiomyomatosis and renal cell cancer (HLRCC) (Launonen et al, 2001). Families affected by HLRCC possess a germline-inactivating mutation in the gene encoding the Kreb’s cycle enzyme, fumarate hydratase (FH) (Tomlinson et al, 2002). These individuals are predisposed to development of benign leiomyomas of the skin and uterus as well as highly aggressive kidney cancers (Launonen et al, 2001; Tomlinson et al, 2002; Wei et al, 2006). Somatic genetic events inactivating the second FH allele in leiomyomas and kidney tumours have been identified (Tomlinson et al, 2002). Kidney cancers are less penetrant than leiomyomatous manifestations in HLRCC-affected families (Wei et al, 2006). Renal tumours have been identified in approximately one-third of HLRCC families evaluated at the National Cancer Institute; however, family recruitment criteria may have favoured selection for families affected by kidney cancer (Wei et al, 2006). Kidney tumours that develop in patients with HLRCC are biologically very aggressive. Of 13 individuals identified with kidney cancer in the first reported cohort of North American families, nine patients succumbed to metastatic disease within 5 years from initial diagnosis (Toro et al, 2003). As a result, regular screening of affected HLRCC individuals is advocated. Although FH mutation has been linked to the development of RCC in the context of HLRCC, its role in sporadic kidney cancers appears to be limited (Kiuru et al, 2002). Morris et al (2004) examined DNA from RCC samples. They were unable to detect FH mutations in all nine cell lines tested. Of the 46 tumour samples examined, no mutations could be identified in 42. In the remaining four tumour samples, the alteration identified was a silent single-nucleotide polymorphism (Morris et al, 2004). In addition, Kiuru et al (2002) were unable to identify FH mutations in 52 sporadic RCCs. These data suggest that biallelic loss of FH is necessary to promote HLRCC-associated renal tumorigenesis. As complete loss of FH activity is developmentally disfavoured owing to the severe energy deprivation imposed on the developing brain by such a genetic defect, appearance of HLRCC appears to be restricted to those otherwise healthy individuals hemizygous for FH in whom a somatic mutation has inactivated the second allele. Despite a potentially limited role for FH in sporadic RCC, investigation of the molecular basis of HLRCC provides a unique opportunity to identify signalling pathways that may be important for kidney cancer aetiology.

ROLE OF FH IN KIDNEY CANCER

The role of FH alterations in the development of HLRCC is under intense investigation. As mentioned, FH is an enzyme of the Kreb’s cycle that catalyses the conversion of fumarate to malate. It

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appears that FH acts as a tumour suppressor gene and fits Knudson’s two-hit model (Tomlinson et al., 2002). Patients with HLRCC inherit a mutant copy of the FH gene as well as a wild-type copy. Tumour formation appears to occur when there is somatic alteration of the wild-type copy (Tomlinson et al., 2002). Although allelic loss appears to be the most common alteration, insertion and missense mutations have also been described in kidney tumours from patients with germline mutations of FH (Tomlinson et al., 2002).

Given the reliance of cells on the mitochondrion to generate adenosine triphosphate by oxidative phosphorylation, it seems that a cell with a defective tricarboxylic (TCA) cycle would be at a disadvantage. However, FH is not the only TCA enzyme whose inactivation is linked to neoplasia. Mutations of succinate dehydrogenase (SDH) have been implicated in the development of a cancer syndrome referred to as hereditary paraganglioma and pheochromocytoma (HPGL). Succinate dehydrogenase catalyses the conversion of succinate to fumarate. In contrast to FH, which functions as a homotetramer (Wei et al., 2006), SDH consists of four different subunits, named A, B, C, and D (Gottlieb and Tomlinson, 2005). Mutations of SDH-B, SDH-C, and SDH-D have all been identified with HPGL (Baysal et al., 2000; Niemann and Muller, 2000; Astuti et al., 2001).

Postulated mechanisms for these inherited cancer syndromes include aberrant apoptosis, oxidative stress, and pseudohypoxic drive (Gottlieb and Tomlinson, 2005). Of these, pseudohypoxic drive has the most supportive evidence based on both clinical and basic investigations. Pseudohypoxic drive refers to the activation of hypoxia response signalling pathways under normal oxygen conditions. The concept of pseudohypoxia is best understood in the context of the VHL pathway.

THE VHL PATHWAY AND HIF DYSREGULATION

VHL is a tumour suppressor gene involved in the development of clear cell RCC. The VHL protein product, hereafter referred to as pVHL, is part of a complex signalling cascade that modulates a cell’s oxygen-dependent gene expression. In normoxic conditions, pVHL is known to associate with several proteins including Cul-2, Elongin B, and Elongin C to form the VHL complex (Figure 1) (Kibel et al., 1995; Linehan et al., 2003). Intact VHL complex possesses ubiquitin ligase activity that targets proteins for degradation (Pause et al., 1997). Hypoxia inducible factor 1α (HIF-1α) and HIF-2α are two of the proteins targeted for degradation by the VHL complex under normoxic conditions (Ivan et al., 2001; Kaelin Jr, 2005). These two proteins are key regulators of oxygen homeostasis (Kaelin Jr, 2005). They are transcription factors involved in the expression of genes involved in nutrient catabolism, angiogenesis, as well as cell growth and differentiation (Semenza, 1999). Target genes of the HIF transcription factors include vascular endothelial growth factor (VEGF), glucose transporter 1 (GLUT1), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and erythropoietin (EPO). VHL complex recognition of HIF requires the hydroxylation of conserved proline residues of the HIF protein (Ivan et al., 2001). Hydroxylation of HIF is carried out by a family of enzymes referred to as HIF prolyl hydroxylases (HPHIs), also referred to as prolyl hydroxylases (Kaelin, 2005). The enzymatic hydroxylation of HIF requires molecular oxygen and 2-oxoglutarate (2-OG) as cosubstrates (Kaelin Jr, 2005). In addition, ascorbate and iron are required cofactors for HPH catalytic activity (Kaelin Jr, 2005). Under hypoxic conditions, molecular oxygen is limited. As a result, HIF remains unhydroxylated. In the unhydroxylated state, HIF avoids ubiquitination by the VHL complex, and is therefore available to activate the transcription of the aforementioned genes. In instances where VHL mutations are present, HIF is able to avoid degradation, thus creating a pseudohypoxic state. Indeed, upregulation of both HIF-1α and HIF-2α has been identified in clear cell RCCs along with concomitant expression of HIF downstream target genes (Wiesener et al., 2001; Zhang et al., 2006). Yet there may be differential contribution of the HIF proteins to tumorigenesis. This is in part supported by the contrasting findings in the RCC cell line 786-O, which lacks pVHL that are tumorigenic in mouse xenograft models. Reintroduction of pVHL diminishes tumour formation in xenograft models consistent with the tumour suppressor function of pVHL (Ilipoulos et al., 1995). However, the tumour suppressor function of pVHL is mitigate in xenograft models with introduction of an HIF-2α mutant that avoids ubiquitination and degradation (Kondo et al., 2002). On the other hand, the tumour-suppressor function of pVHL remains intact despite the presence of the corresponding HIF-1α mutant (Maranchie et al., 2002). These and other studies have contributed to the current belief that HIF-2α may be more important to the formation of clear cell RCC.

HLRCC AND PSEUDOHYPOXIC DRIVE

Several reports provide evidence of HIF accumulation in both leiomyomas and renal tumours from HLRCC individuals. Concurrent studies in HPGL have also implicated pseudohypoxic drive and by analogy support involvement of pseudohypoxia in the aetiology of HLRCC kidney tumours. Given that both FH and SDH represent TCA cycle enzymes, it seems reasonable to consider the possibility that their inactivation may uncover a similar mechanism linking mitochondrial dysfunction with oncogenesis.

The notion of TCA cycle dysregulation and pseudohypoxic drive was initially derived from studies in paraganglioma. The increased frequency of carotid body paragangliomas in individuals who resided in higher altitudes (Pacheco-Ojeda et al, 1988) suggested a role for chronic hypoxia (Gimenez-Roqueplo et al, 2001). The similarity between HPGL tumours from patients with germline SDHD mutations and normal carotid body tissue exposed to chronic hypoxia led Baysal et al (2000) to suggest that SDHD was a critical gene involved in oxygen sensing. Furthermore, they...
proposed that loss of SDHD would lead to what they referred to as 'hypoxic stimulation' and cellular proliferation. Tumours from HPGL patients with SDHD mutations revealed enhanced expression of HIF as well as VEGF (Gimenez-Roqueplo et al., 2001). These and other studies helped formulate the rationale for evaluating the role of pseudohypoxic drive in HLRCC.

Pollard et al. (2005b) examined HIF-1α expression in kidney tumours (both papillary and collecting duct histologies) in patients with HLRCC. Their findings in kidney tumours were compelling for activation of hypoxic pathways. In all renal tumours examined, they noted strong HIF-1α staining in the nuclei of tumour cells. This finding was also confirmed by immunoblotting, as HIF-1α was easily detected in tumour cell lysate with no detectable HIF-1α in normal kidney tissue. Pollard et al. (2005b) also examined HIF-1α expression in kidney tumours from patients with HLRCC. Interestingly, six of the seven tumours were from patients less than 40 years of age. HIF expression, evaluated by immunohistochemistry, was determined in cancerous tissues in relation to normal, matched renal parenchyma from the same patient. Both HIF-1α and HIF-2α were found to be significantly overexpressed in HLRCC renal tumours, but HIF-1α expression seemed to be preferentially increased compared to HIF-2α.

Further supportive evidence comes from observation of the upregulated downstream HIF targets in tumour tissues isolated from patients with HLRCC. Pollard et al. (2005a) examined microvessel density in HLRCC uterine leiomyomas. Vascular density, determined by immunohistochemical detection of the vascular endothelial expression of VEGF, was significantly higher in HLRCC uterine leiomyomas as compared to non-leiomyomatous myometrium from HLRCC women. Furthermore, there was a statistically significant higher vascular density in HLRCC leiomyomas compared to sporadic leiomyomas or normal myometrium procured from women without HLRCC (Pollard et al., 2005a). Interestingly, sporadic uterine leiomyomas actually had diminished vascular density as compared to normal myometrium controls from matched patients. In concordance with this finding, in situ hybridisation studies revealed upregulation of VEGF transcripts in HLRCC uterine leiomyomas in comparison to normal myometrium (from HLRCC and non-HLRCC women) as well as sporadic uterine leiomyomas (Pollard et al., 2005a). These findings were confirmed by quantitative real-time PCR data that revealed enhanced expression of VEGF (1.4 – 3.5-fold) as compared to patient-matched normal myometrium. In addition, other hypoxia-responsive gene changes were also found in HLRCC leiomyomas including downregulation of TSP1, a known antiangiogenesis factor (Pollard et al., 2005a). This investigation suggests significant differences between sporadic leiomyomas and HLRCC leiomyomas with regard to mechanism of tumorigenesis. Indeed, complementary evidence for different mechanisms is indicated by the finding that biallelic FH alterations are found in an extremely small subset of sporadic uterine leiomyomas (Lehtonen et al., 2004). Further investigation subsequently revealed moderate HIF-1α expression in HLRCC fibroids with weak/moderate staining of surrounding myometrium (Pollard et al., 2005b). Although overexpression of HIF-1α in HLRCC fibroids is less than kidney the concomitant upregulation of these proteins suggests that pseudohypoxic activation contributes to the genesis of uterine leiomyomas in HLRCC patients. There is also supportive evidence for activation of hypoxic pathways in kidney tumours from HLRCC patients. Isaacs et al. (2005) identified enhanced GLUT1 expression by immunohistochemistry in multiple HLRCC kidney tumours as compared to normal renal tissue.

Although there is clear evidence of activation of pseudohypoxic pathways in HLRCC tumours, the molecular mechanism underlying this phenomenon remains obscure. Unlike the involvement of pVHL in HIF degradation, there is no direct link between the FH and HIF proteins. The answer may lie in the biochemical alterations that result from the absence of an intact FH enzyme.

As noted earlier, FH catalyses the hydration of fumarate to form malate. The absence of FH could presumably result in chronically elevated levels of fumarate and altered levels of other TCA intermediates and molecules indirectly linked to the TCA cycle. There is direct evidence to support elevation of intracellular fumarate secondary to reduced FH activity as a proximal cause of pseudohypoxia. Isaacs et al. (2005) transfected the FH wild-type lung carcinoma cell line A549 with small interfering RNA (siRNA) targeting FH. The intracellular fumarate level rapidly doubled in treated A549 cells. Although these data suggest the feasibility of using siRNA expression in kidney tumours examined for activation of hypoxic pathways. In all renal tumours examined, they noted strong HIF-1α staining in the nuclei of tumour cells. This finding was also confirmed by immunoblotting, as HIF-1α was easily detected in tumour cell lysate with no detectable HIF-1α in normal kidney tissue. Pollard et al. (2005b) also examined HIF-1α expression in kidney tumours from patients with HLRCC. Interestingly, six of the seven tumours were from patients less than 40 years of age. HIF expression, evaluated by immunohistochemistry, was determined in cancerous tissues in relation to normal, matched renal parenchyma from the same patient. Both HIF-1α and HIF-2α were found to be significantly overexpressed in HLRCC renal tumours, but HIF-1α expression seemed to be preferentially increased compared to HIF-2α.

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CONCLUSION

Hereditary leiomyomatosis and renal cell cancer is a hereditary cancer syndrome predisposing individuals to the development of aggressive kidney cancers. These individuals are known to harbour a germline mutation of FH, which codes for the enzyme that catalyses the conversion of fumarate to malate. Although multiple mechanisms of tumorigenesis have been proposed, the preponderance of data thus far supports a role for pseudohypoxic drive involving fumarate-dependent HIF activation. The link between FH inactivation and HIF appears to involve inhibition of HPH with concomitant upregulation of hypoxia response genes including VEGF and GLUT-1.

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