Fumarate Hydratase Deficiency and Cancer: Activation of Hypoxia Signaling?

Peter J. Ratcliffe[1,*]
[1]The Henry Wellcome Building for Molecular Physiology, University of Oxford, Headington Campus, Roosevelt Drive, Oxford OX3 7BN, United Kingdom
*Correspondence: pjr@well.ox.ac.uk
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Molecular genetic analysis of hereditary leiomyomatosis and renal cell cancer (HLRCC) unexpectedly revealed germline defects in the gene encoding the Krebs cycle enzyme fumarate hydratase (FH), stimulating great interest in the underlying mechanism of oncogenesis. It has been proposed that the associated accumulation of fumarate competitively inhibits the 2-oxoglutarate-dependent dioxygenases that regulate hypoxia-inducible factor (HIF), thus activating oncogenic hypoxia pathways. In this issue of *Cancer Cell*, Pollard and colleagues describe a genetic mouse model of FH deficiency that recapitulates aspects of the human disease, including HIF activation and renal cysts, enabling further insights into this unusual cancer syndrome.

The association of tumor hypoxia with aggressive cancer phenotypes is well established, with parenchymal hypoxia within solid tumors predicting not only resistance to treatment but also adverse natural history. Nevertheless, precise understanding of the mechanisms underlying these associations is lacking, and the distinction of cause and effect remains problematic. Thus, the involvement of genetic defects in hypoxia signaling pathways in predisposition to cancer is of great interest. Considerable attention has been focused on defining the role of HIF in von Hippel-Lindau (VHL)-associated cancer, where inactivation of the VHL ubiquitin E3 ligase blocks proteolytic degradation of HIF and upregulates HIF target genes (Kaelin, 2002). Against this background, reports linking hereditary cancer syndromes to defects in Krebs cycle enzymes that also appear to perturb HIF degradation, but by different mechanisms, have attracted widespread attention (Gottlieb and Tomlinson, 2005).

In mammalian cells, hypoxia-induced changes in gene expression extend to several hundred transcripts, the majority of which respond directly or indirectly to the α/β heterodimeric transcription factor HIF. The oxygen-sensitive signal is generated by enzymatic hydroxylations of specific prolyl and asparaginyl residues within HIFα subunits (HIF1α, HIF2α, and HIF3α) that promote proteolytic destruction and transcriptional inactivation of HIF in the presence of oxygen. These hydroxylations are catalyzed by a series of nonheme Fe (II) and 2-oxoglutarate-dependent dioxygenases that split molecular oxygen and couple the hydroxylation of HIFα to the oxidative decarboxylation of the Krebs cycle intermediate 2-oxoglutarate (Schofield and Ratcliffe, 2004). These findings suggested that, in addition to oxygen dependence, the system might be perturbed by changes in intermediary metabolism affecting Krebs cycle metabolites, providing an appealing link between hypoxic and metabolic signals.

Two quite distinct hereditary cancer syndromes have been shown to be caused by mutations in genes encoding Krebs cycle enzymes. Hereditary pheochromocytoma and paraganglioma are associated with mutation in succinate dehydrogenase (*sdh*) subunits B, C, and D, while HLRCC, a syndrome characterized by multiple benign skin and uterine leimyomata, occasional leiomyosarcoma, and in some families, aggressive renal cell carcinoma, is associated with muta-

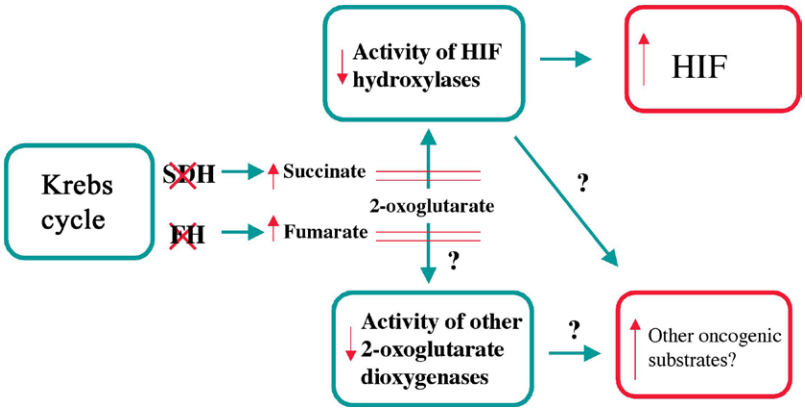

Figure 1. Postulated Oncogenic Pathways in Cells Deficient in Fumarate Hydratase or Succinate Dehydrogenase
Accumulation of the Krebs cycle intermediates fumarate and succinate is proposed to inhibit HIF hydroxylases or other 2-oxoglutarate-dependent dioxygenases, leading to upregulation of HIF or other oncogenic substrates.

tions in *FH* (Gottlieb and Tomlinson, 2005). In each case, associated tumors manifest upregulation of HIF, and based on the established role of particular Krebs cycle intermediates (including fumarate and succinate) in inhibiting other 2-oxoglutarate-dependent dioxygenases (procollagen prolyl and lysyl hydroxylases), it was proposed that upregulation of HIF might be due to inhibition of the HIF hydroxylases by Krebs cycle intermediates, and that activation of the HIF pathway might be the cause of the tumor predisposition (Figure 1).

If true, this would have interesting general implications. First, it raises the possibility that other circumstances in which intermediary metabolism is perturbed (either in neoplasia or nonneoplastic conditions) might modulate HIF signaling. Second, it adds to accumulating evidence that the generally observed associations between activation of the HIF pathways and tumor growth might, in some circumstances, be causal. However, the hypothesized model is by no means proven, and each of the links in the proposed sequence of events requires careful consideration. The mouse model of *fh* inactivation generated by Pollard and colleagues should therefore be of substantial utility (Pollard et al., 2007). Moreover, using biallelic genetic inactivation in embryonic stem cells, Pollard et al. provide secure evidence that *fh* inactivation does indeed upregulate HIFα, consistent with data reported previously using pharmacological inhibition of FH and *fh*-directed siRNAs (Isaacs et al., 2005; Pollard et al., 2007). But is the proposed mechanism of HIF activation by fumarate accumulation correct?

Recent in vitro data confirm that fumarate is an effective inhibitor of the HIF prolyl hydroxylases and that inhibition is competitive with 2-oxoglutarate, with IC_{50} values reported at 6.6 μM and 80 μM in different assays (Isaacs et al., 2005; Koivunen et al., 2007). Studies of tumor extracts from FH-associated myomata reveal large increases in fumarate—to concentrations well in excess of these levels (Pollard et al., 2005). Thus, notwithstanding uncertainties arising from cellular compartmentalization (the Krebs cycle reactions takes place within the mitochondrion, whereas the most likely sites of HIF hydroxylation are within the cytosol and the nucleus), kinetic measurements are consistent with *fh* inactivation impairing HIF hydroxylation through fumarate accumulation. Succinate accumulation in SDH-associated tumors is less clearly in excess of the reported IC_{50} for HIF prolyl hydroxylases (Koivunen et al., 2007; Pollard et al., 2005). However, reversal of HIFα accumulation in SDH-deficient cells following application of esterified (cell-permeant) 2-oxoglutarate strongly suggests that a similar process of competitive inhibition of HIF hydroxylases is taking place (Mackenzie et al., 2007). Thus, it seems likely that severe dysregulation of the Krebs cycle can upregulate hypoxia signaling in these rare disorders. Might more subtle metabolic dysregulation also have significant effects in more commonly encountered situations? Demographic observations of altitude dependence of paraganglioma incidence in families bearing *sdh* mutations suggests an interaction with hypoxia. Conceivably, changes in intermediary metabolism that affect the entry to the Krebs cycle via acetyl CoA, or via anaplerotic pathways that allow metabolite entry at other points in the cycle, might affect the levels of relevant metabolites in a manner that modulates hypoxia signaling. Moreover, correction of HIF upregulation in SDH-deficient cells by exogenous 2-oxoglutarate suggests that therapeutic correction might be feasible (Mackenzie et al., 2007). The mouse model of *fh* deficiency reported by Pollard and colleagues should now enable testing of these interesting possibilities (Pollard et al., 2007).

What of the link between activation of HIF and cancer? Pollard et al. describe proliferation of the renal epithelium and renal cysts following inactivation of *fh* (Pollard et al., 2007). This pathology was associated with life-limiting polyuric renal failure. Since in some (but by no means all) disease settings renal cysts are considered to be precursors of renal cell cancer, it is possible that, had the animals survived longer, renal cancer would have developed. The situation is thus similar to RCC-associated VHL disease, where inactivation of *vhl* in mouse renal epithelial results in proliferation and cystic change but not overt RCC. In the mouse model of VHL disease, renal cyst development can be corrected by inactivation of HIF1β, though interestingly not HIF1α (Rankin et al., 2006), and in experimental tumors derived from human RCC, genetic manipulation strongly implicates a causal role for HIF2α in promoting growth (Kaelin, 2002). Thus, it is tempting to suggest a similar mode of oncogenesis in VHL and HLRCC disease, with HIF activation as the common factor. However, there are differences. In VHL-associated RCC, upregulation of the HIF target gene carbonic anhydrase 9 is a consistent feature, and attention has focused on upregulation of the HIF2α targets TGFα and Cyclin D1 as likely contributors to cellular proliferation (Kaelin, 2002). In the report by Pollard et al., cyclin D1 expression was not measured, and renal cysts expressed neither CA9 not TGFα (Pollard et al., 2007). Thus, if HIF activation does underlie cyst development in both VHL disease and HLRCC the transcriptional targets that mediate the changes may well be different. Furthermore, the proposed mechanism does not explain the tissue-specific differences in tumor predisposition observed in VHL-, FH-, and SDH-associated cancer syndromes.

Thus, though *fh* inactivation can upregulate HIF, the causal role of HIF in renal cyst development (and other manifestations of FH and SDH deficiency) is less clearly established. Combining *FH*- and *HIF*α-defective alleles in the new mouse model should now permit this to be tested. Meantime, it is important to recognize first, that the HIF hydroxylases may have other oncogenic substrates in these diseases (Lee et al., 2005), and second, that fumarate or succinate accumulation might (in parallel with HIF hydroxylation) inhibit any of several dozen known or predicted 2-oxoglutarate-dependent dioxygen-

ases encoded by the human genome (Schofield and Ratcliffe, 2004). Other 2-oxoglutarate-dependent dioxygenases that have credible roles in cancer biology include those with long-established functions in extracellular matrix formation, such as procollagen prolyl and lysyl hydroxylases, and those with recently established functions in histone demethylation (Shi and Whetstine, 2007). Thus, it remains conceivable that HIF activation is a marker rather than the cause of oncogenic predisposition and/or that different types of tumor predisposition reflect effects on different enzyme/substrate systems. Either way, the new findings open a range of possible insights into the association of hypoxia and cancer progression.

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Modeling Synovial Sarcoma: Timing Is Everything

Sean R. Davis[1] and Paul S. Meltzer[1,*]
[1]Genetics Branch, Center for Cancer Research, National Cancer Institute, 37 Convent Drive, Bethesda, MD 20892, USA
*Correspondence: pmeltzer@mail.nih.gov
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Synovial sarcoma is characterized by the presence of a fusion protein involving *SYT* and *SSX2*. In this issue of *Cancer Cell*, Haldar et al. have genetically engineered a mouse model of this disease. They show that expression of the *SYT-SSX2* fusion gene yields a highly penetrant and representative model of human synovial sarcoma, but only if expression occurs in a particular biologic context. The mouse model will be a valuable resource for studying tumor biology but is also a striking example of how important understanding of normal tissue and developmental biology is to our understanding of cancer.

Sarcomas comprise a diverse group of malignant tumors of mesenchymal origin, arising in connective tissues such as fat, muscle, fibrous tissue, and bone (Helman and Meltzer, 2003). These tumors are relatively rare, with an annual incidence of seven to eight per 100,000. They account for less than 1% of all malignancies overall, but about 15% of malignancies in children. Sarcomas have historically been classified based on their site of origin, morphology, and immunohistochemical properties. Although some sarcomas can be classified easily according to these criteria and their clinical features, many present significant diagnostic challenges. However, more recently, many soft-tissue sarcomas have been shown to carry recurrent, characteristic chromosomal translocations, several of which generate fusion proteins that act as transcription factors (Table 1) (Mackall et al., 2002). As in leukemia and lymphoma, these causative genetic events provide a point of departure for sarcoma researchers. Importantly, the translocations are likely to reveal biological mechanisms whose importance transcends the rarity of these diseases. However, despite the opportunities provided by the discovery of sarcoma translocations, biological understanding of many sarcomas remains limited. The rarity of sarcomas and their clinical heterogeneity, even within a single histologic classification, present the greatest obstacles.