and Fuks, 2003; Paris et al., 2001), suggesting that this represents a generic response mechanism for mammalian tissue damage by large single-dose irradiation. The possibility that a similar crosstalk between microvasculature and tumor clonogens occurs during fractionated radiotherapy when the HIF-1-mediated endothelial protection is removed, such as reported by Moeller et al., represents a testable hypothesis.

In principle, the studies of Moeller et al. support the notion that fractionated radiotherapy, like single-dose radiation, engages a vascular component of the tumor response. In the case of fractionated radiotherapy, however, this response is largely attenuated by adaptive signals generated by HIF-1 activation. Hence, Moeller et al. suggest that HIF-1 may represent a valid target for radiosensitization via derepression of endothelial cell death. However, they caution that HIF-1 inactivation, if it is to be therapeutically efficacious, should be scheduled to optimize tumor cell radiosensitization. In contrast, the endothelial death signal produced by large-dose exposure (>8–10 Gy) may precede or be of sufficient magnitude to overcome HIF-1 anti-death protection. These provocative studies should open up new avenues for basic research into mechanisms of endothelial cell damage and the role of the microvascular response in therapy, potentially providing new pharmacologic targets for improving radiation and other anticancer treatments.

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A common pathway for genetic events leading to pheochromocytoma

Mutations in *VHL*, *RET*, *NF1*, *SDHB*, *SDHC*, and *SDHD* can give rise to pheochromocytoma/paraganglioma. These different genetic lesions may all act by decreasing the activity of a 2-oxoglutarate-dependent oxygenase, SM-20/EglN3/PHD3, resulting in reduced apoptosis of neural crest cells during development.

Hereditary tumor syndromes have given numerous insights into cancer biology. Often it is straightforward to see a link between the genetic defect and tumor predisposition. This is the case if the gene product impinges on pathways involved in cell proliferation or cell death. In other examples, maintenance of the genome is compromised, so that the likelihood of developing mutations that lead to an increase in proliferation or death is increased. But the link between the genetic defect and tumor predisposition is not always clear. Arguably, these cases are most likely to lead to truly novel insights into tumor development. A paper from Bill Kaelin's group in this issue of *Cancer Cell* suggests that apparently unlinked genes implicated in paraganglioma act in a single common pathway (Lee et al., 2005). The authors provide evidence that all the genetic defects act by decreasing the likelihood of apoptosis of neural crest cells at the time during development when levels of nerve growth factor (NGF) become limiting. The study gives particular insight into the interesting issue of how mutations affecting succinate dehydrogenase components could be tumorigenic.

Paragangliomas are tumors of the autonomic nervous system. Non-chromaffin paragangliomas are rare, are described as "chemodectomas," and are located in the head and neck. Chromaffin paragangliomas are much commoner, have endocrine activity, and are referred to as "pheochromocytomas." These are usually located in the adrenal medulla but sometimes occur in the pre- and paravertebral thoracoabdominal regions. Familial paraganglioma syndromes can

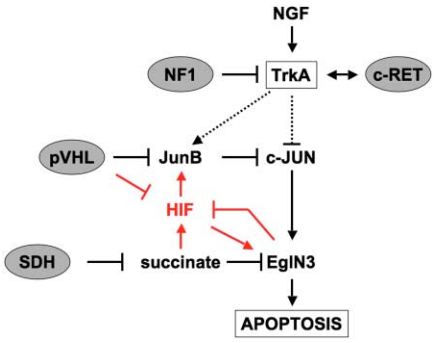

Figure 1. As NGF levels become limiting during development, mutations affecting *NF1*, *RET*, *VHL*, and SDH subunits all decrease apoptosis mediated by SM-20/EglN3/PHD3. Adapted from Lee et al. (2005) to show interactions with HIF.

manifest as nonchromaffin head and neck tumors only, adrenal and/or extra-adrenal pheochromocytomas only, or a combination of the two types of tumors. When pheochromocytomas occur in families, they may be associated with other abnormalities as part of three hereditary multitumor syndromes: von Hippel-Lindau (VHL) disease, type I neurofibromatosis, and type II multiple endocrine neoplasia (Maher and Eng, 2002). These are, respectively, caused by inactivating mutations in *VHL* or *NF1*, or activating mutations in *RET*. Other families have paraganglioma alone, with mutations in succinate dehydrogenase subunits B, C, or D, or mutations in *VHL* or *RET*. Importantly, about 25% of individuals with apparently sporadic pheochromocytoma have germline mutations in one of the genes involved in familial paraganglioma syndromes (Neumann et al., 2002). An intriguing puzzle has been how these apparently very different genetic lesions all lead to a similar phenotype.

The Kaelin group has made a number of seminal contributions to understanding the function of the *VHL* gene, beginning with their demonstration that it suppressed the growth of clear cell renal cell carcinoma (CCRCC) cells in xenografts (Iliopoulos et al., 1995). They and others went on to show that *VHL*-defective CCRCC cells exhibit constitutively high expression of certain gene products normally upregulated in low oxygen. Our own studies established that this is because *VHL* is necessary for the oxygen-dependent inactivation of hypoxia-inducible factor (HIF) (Maxwell et al., 1999). HIF activation is necessary and sufficient for many of the manifestations of *VHL* loss of function, including growth of CCRCC cells as xenografts (Kondo et al., 2003). However, there are cogent lines of evidence that *VHL* has other functions besides its role in regulating HIF. In *C. elegans*, there are a subset of genes that are dysregulated in *vhl-1* mutants that are not normalized in *vhl-1*/*hif-1* double mutants (Bishop et al., 2004). In humans, certain mutations in *VHL* have been associated with familial pheochromocytoma alone (type IIC VHL disease), and these mutations do not appear to alter the ability to regulate HIF. This strongly suggested that *VHL*-related pheochromocytoma might not be related to HIF activation and that missense mutations in *VHL* associated with type IIC disease would offer a powerful tool to identify HIF-independent actions of *VHL*.

The study in this issue of *Cancer Cell* shows that *VHL* suppresses JunB and that all mutations tested—including those associated with type IIC VHL disease—abrogate its ability to do so (Lee et al., 2005). JunB acts as an antagonist of c-Jun, blunting c-Jun-mediated apoptosis as NGF levels become limiting. There is previous evidence to suggest that *RET* (the receptor for glial-derived neurotrophic factor [GDNF]) and *NF1* could act through this pathway by modulating the action of the NGF receptor TrkA. The study goes on to examine the downstream effector pathway, using a variety of approaches to investigate the role of a protein initially identified as SM-20 in rat smooth muscle cells and subsequently implicated in apoptosis in neural crest-derived cells. SM-20 is now known to be a 2-oxoglutarate-dependent prolyl hydroxylase (see below). The current study provides clear evidence that this enzyme is necessary and sufficient for increased apoptosis on NGF withdrawal, and that the action is abolished by a mutation that will abrogate oxygenase activity. The 2-oxoglutarate-dependent oxygenases produce succinate as an end product, providing a tantalizing potential link to succinate dehydrogenase. This is because decreased SDH activity has been shown to result in accumulation of succinate, which inhibits SM-20 activity. Thus, all the genetic lesions associated with pheochromocytoma are proposed to act on a single common pathway that is responsible for culling precursor cells during development (Figure 1).

One satisfying aspect is that these findings provide an explanation as to why the mutations in tumor suppressors that are found in familial paraganglioma syndromes are rare in sporadic pheochromocytoma, except as occult germline mutations. This is because the pathway is no longer critical once development is completed. From a therapeutic perspective, it suggests that premalignant lesions will be present in at-risk individuals and could be ablated before they develop into pheochromocytoma. A second clinically relevant aspect is that it would explain why mutations are not found in more than one of these genes in individual cases/kindreds, since they would not have additive effects. The study also confirms previous findings that VHL regulates atypical protein kinase C (aPKC) activity and shows that this effect is independent of HIF activation. It seems likely that this involves selective ubiquitylation of phosphorylated aPKC, and it will be interesting to understand the molecular interaction underlying this.

An important issue is whether HIF—which could almost be regarded as VHL's alter ego—has any role in the pheochromocytoma pathway. Although HIF doesn't seem to be involved, potential links keep cropping up (Figure 1). First, part of the effect of VHL status on JunB is mediated by HIF. Second, SM-20 is itself involved in the HIF/VHL pathway. Some years after SM-20 was first identified, it was isolated as one of a family of three iron- and 2-oxoglutarate-dependent oxygenases that hydroxylate HIF α subunits at conserved prolyl residues—termed PHD, HIF-PH, or EglN enzymes (Schofield and Ratcliffe, 2004). This hydroxylation provides the recognition signal that enables HIF α subunits to be captured by VHL. Indeed, it was previously reported that decreased activity of SDH is sufficient to activate HIF (Selak et al., 2005). Nevertheless, under standard conditions PHD2/EglN1 is the predominant HIF hydroxylase, and RNAi knockdown of SM-20/PHD3/EglN3 alone does not generally lead to HIF activation (Berra et al., 2003). Third, it is intriguing that SM-20/EglN3/PHD3 is a transcriptional target of HIF, with expression increasing in low oxygen tension by as much as two to three orders of magnitude. *VHL* inactivation results in HIF activation in all cell types examined to date, so it would have been predicted that *VHL* loss of function would result in *increased* expression of SM-20/EglN3/PHD3. Importantly, Lee et al. find that amongst VHL mutations that ablate its ability to regulate HIF, there are differential effects on the expression of SM-20/EglN3/PHD3 (Lee et al., 2005). Thus, the mutations tested that are associated with a high risk of pheochromocytoma suppressed SM-20/EglN3/PHD3, even if they failed to suppress HIF. Overall, the potential interactions of the proposed pathway with HIF suggest that it would be premature to exclude a role in pheochromocytoma.

Understanding how mutations affecting succinate dehydrogenase are tumorigenic is likely to have implications beyond pheochromocytoma. Mutations in another tricarboxylic acid cycle enzyme, fumarate hydratase (FH), were

recently found to underlie a different hereditary tumor syndrome, familial leiomyomatosis and renal cell cancer (Eng et al., 2003). Isaacs et al. report in this issue that decreased activity of FH inhibits HIF hydroxylase activity (Isaacs et al., 2005). So there is an emerging picture that TCA cycle defects can impact key enzyme reactions by altering the concentration of fumarate and succinate. This is an alternative to the hypothesis that loss of function of FH or SDH subunits acts through mitochondrial dysfunction and increased generation of reactive oxygen species (Eng et al., 2003). So far, studies have focused on HIF hydroxylases, but these form only one branch of an extensive family of 2-oxoglutarate-dependent oxygenases whose activity might be modulated.

In any event, a spotlight is now on *EglN3*/*PHD3*, and it will be interesting to determine if mutations in this gene occur in pheochromocytoma, or in other tumors. An important challenge is identifying the link between the enzyme and apoptosis, which presumably involves hydroxylation of a protein other than HIF.

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Metastasis gets site specific

Organ-specific homing and colonization of cancer cells are important and interesting features of metastasis. Molecular programs that contribute to this tropism may be elucidated through gene expression profiling with DNA microarrays. Using experimentally derived breast cancer cells that home specifically to bone or to lung, several investigators have concluded that distinct alterations in gene expression underlie metastasis to these sites. Minn et al. (2005) report a set of genes involved in lung-specific metastasis of breast cancer; the authors have determined the functional contribution of several genes to the metastatic cascade, as well as the relevance of these genes to human disease.

Metastasis, the spread of cancer from the primary site of tumor growth to other organs, is the leading cause of cancer-related morbidity and mortality. It is a sequential process, contingent on tumor cell acquisition of the following capabilities: invasion, survival and arrest in the bloodstream, extravasation, and colonization at a distant site. Layered onto these general requirements of metastasis, tumor cells may acquire the capacity to preferentially colonize distinct organs. Over a century ago, Paget compared metastatic tumor cells to widely disseminated seeds, which will grow only on fertile soils (Paget, 1889). This vision propelled the hypothesis that tumor cell-host interactions serve as the prime contributor to organ-specific metastasis. An understanding of metastatic colonization, both general and organ specific, may be key to the development of antimetastatic therapies; the final step of colonization has not been completed in the majority of cancer patients at the time of diagnosis and surgery and is therefore amenable to therapeutic targeting (Steeg, 2003).

Tissue tropism or organ-specific homing of cancer cells depends on both the histology and the stage of cancer. Ocular melanomas stand as a stunning example of this tropism, metastasizing preferentially to the liver. Other cancers, such as breast, metastasize to multiple sites, most commonly lung and bone tissue and with less frequency the liver and the adrenal glands. Several factors are thought to influence the site of cancer metastasis, and these include (1) the pattern and direction of blood flow from the primary tumor, (2) mechanical trapping of tumor cells at a secondary site by small capillary beds, (3) tumor cell adhesion at a secondary site by the expression of appropriate cell surface proteins,