Dispatches

Cancer Metabolism: Feeding a Worm to Starve a Tumor

The tumor suppressor *Rb* is known to have its hand in many pots. New findings have added another pot to the mix — cell metabolism. This may lead to a better understanding of *Rb* mutant phenotypes and *Rb*'s roles in oncogenesis.

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It has been known for more than 80 years that cancer cells alter their use of metabolic pathways as a means for enhanced proliferation and survival [1,2]. In fact, changes in intracellular metabolism are now considered to be one of the hallmarks of oncogenic transformation, along with genomic instability, hyperproliferation, evasion of apoptosis, immortalization, metastasis induction, and activation of angiogenesis [3]. Such metabolic adjustments are essential for tumor cells to acquire sufficient sources of energy and to generate the biosynthetic building blocks required for cell growth and division. A paper recently published in *Current Biology* by Cui *et al.* [4] provides novel links between changes in metabolism and the role of the well-known tumor suppressor *Rb*.

The best-characterized metabolic change associated with tumors, known as the Warburg effect, refers to the strong preference of many cancer cells for glycolysis over oxidative phosphorylation [1,2]. Although less efficient in terms of producing ATP, glycolysis has the advantage of generating macromolecular precursors that are required for growth as well as antioxidants that are used to neutralize reactive oxygen species created during rapid proliferation. To offset potential deficits in energy production, cancer cells typically increase their uptake of extracellular glucose. In addition, cancer cells can display enhanced glutamine uptake and processing and exhibit increased lipid, protein, and nucleotide biosynthesis [1,2]. Also, many solid tumor cells undergo adaptive physiological changes triggered by hypoxia and acidosis [5].

Only recently have direct connections been established between the altered metabolism of tumor cells and the genetic changes associated with cancer progression. Oncogenes and tumor suppressors that have been implicated in metabolic transformation include several key players in the phosphatidylinositol 3-kinase (PI3K) pathway, including PTEN, AKT, and mTOR, mutations in which promote glycolysis and glucose uptake [1–3,6]. In addition, the transcription factor and tumor suppressor p53 regulates metabolism by several independent mechanisms, including activation of PTEN, and loss of p53 is thought to contribute significantly to the glycolytic phenotype [1,2,6]. Finally, one of the earliest identified oncogenes, *MYC*, stimulates glutamine uptake and metabolism and promotes glucose internalization and glycolysis via hypoxia-inducible factor 1 (HIF1), a target of mTOR [1,6].

One of the most intensively studied tumor suppressors is the retinoblastoma protein pRb [7,8], which is functionally inactivated in most human cancers and has been implicated in many cellular and developmental processes that contribute to its functions as a tumor suppressor [3,9]. This includes the well-established role of pRb in repressing cell-cycle progression but also encompasses functions relevant to genome stability, differentiation, quiescence, senescence, invasion, and angiogenesis. Notably, studies in non-mammalian systems, including *Caenorhabditis elegans*, have implicated pRb orthologs in biological processes that correlate well with reported roles of pRb in mammalian tumor suppression. In *C. elegans*, for example, the pRb ortholog, LIN-35, regulates progression through the cell cycle, differentiation, apoptosis, morphogenesis, and growth factor expression [10]. The recent paper by Cui *et al.* [4] now demonstrates a novel function for LIN-35/pRb in metabolic control, which may also be relevant to its role in humans as a tumor suppressor.

In their natural environment, *C. elegans* frequently encounter starvation conditions, which require compensatory changes to cellular metabolism and developmental programs. One key decision point occurs after hatching: worms in the first larval stage (L1) that are hatched in the absence of food undergo a developmental arrest referred to as the L1 diapause. The survival of starved L1 worms requires the activity of a conserved insulin–IGF-1 signaling (IIS) pathway, which indicates that the regulation of metabolism is conserved between *C. elegans* and higher eukaryotes [11]. Cui *et al.* [4] showed that the survival of starved L1 worms also depended on LIN-35/pRb because *lin-35/Rb* loss-of-function mutants were markedly sensitive to nutrient deprivation.

To gain insights into the mechanistic basis for LIN-35/pRb-mediated diapause survival, Cui *et al.* [4] performed a microarray analysis to compare wild-type and *lin-35/Rb* mutants. Rather surprisingly, the spectrum of genes that was regulated by LIN-35/pRb differed quite dramatically between fed and starved L1 worms, suggesting that there are categorically distinct functions for LIN-35/pRb under different growth conditions [12]. These findings indicate that, although LIN-35/pRb does not directly regulate most IIS pathway components, LIN-35/pRb and the IIS pathway co-regulate the expression of 58 genes in a manner that is largely antagonistic. However, genetic and microarray data suggest that LIN-35/pRb also promotes diapause survival through several mechanisms that are independent of the IIS pathway. This latter contention is supported by phenotypic analysis showing that glucose supplementation dramatically improved the survival of starved IIS pathway mutants but had only weak effects on *lin-35/Rb* mutants.

With respect to other relevant metabolic targets, LIN-35/pRb repressed the expression of many genes induced by toxins, pathogens, and oxidative stress. However, LIN-35/pRb also positively regulated

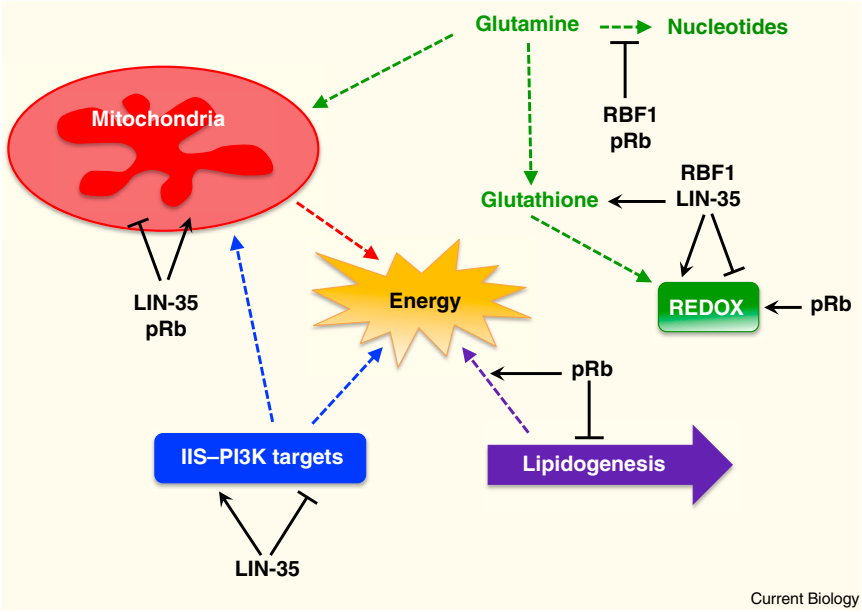

Figure 1. Diagram of reported pRb family links to cellular metabolism.
pRb family members are: LIN-35, *C. elegans*; RBF1, *D. melanogaster*; pRb, mouse and human. IIS, insulin–IGF-1 signaling; PI3K, phosphatidylinositol 3-kinase.

glutathione transferase genes that are critical for redox homeostasis, and *lin-35/Rb* mutants were hypersensitive to oxidative stress during L1 diapause. In addition, LIN-35/pRb controls the expression of many genes that encode mitochondrial respiratory chain components, which may be important mediators of the response to starvation. More globally, under conditions of nutrient deprivation, LIN-35/pRb is required for maintaining a transcriptional profile that is consistent with starvation. In the absence of LIN-35/pRb, starved animals displayed expression profiles that were more reminiscent of well-fed worms, which (perhaps not surprisingly) was deleterious for survival.

Interestingly, the role of LIN-35/pRb in L1 diapause survival is dependent to a large extent on the expression of LIN-35/pRb in neurons and, to a lesser degree, in the intestine. In contrast, expression of LIN-35/pRb in one of the major tissues of the worm, the hypodermis (epidermis), was not essential for diapause survival. The data did, however, suggest that tissues other than neurons and the intestine must also contribute to the role of LIN-35/pRb in the starvation response. Taken together, their findings indicate that LIN-35/pRb normally promotes diapause survival through a number of distinct metabolic pathways and in multiple tissue types.

One limitation of this current study is the reliance on microarray data in accounting for the mechanistic role of LIN-35/pRb in regulating the starvation response. Given that LIN-35/pRb differentially regulates more than 1,200 genes in starved L1 animals, it would, however, be difficult to functionally assess the contribution of individual genes or gene classes in this process. An important open question is whether or not LIN-35/pRb directly regulates genes implicated in the starvation response. One hint that at least some of the targets are direct is the observation that worms that had reduced function in the *C. elegans* E2F ortholog, *efl-1*, were also somewhat hypersensitive to L1 starvation. E2F transcription factors are conserved binding partners of pRb family members and together form the core of several multisubunit transcriptional repressor complexes [7]. In addition, a comparison of LIN-35/pRb transcriptional targets in starved L1s with experimentally verified LIN-35/pRb genomic binding sites showed a significant overlap, suggesting that regulation by LIN-35/pRb may in some cases be direct [13].

Admittedly, the role of LIN-35/pRb in the starvation response could be a unique feature of *C. elegans*. A paper by Nicolay *et al.* [14] published earlier this year does, however, suggest that a function for pRb in the starvation response may be conserved. *Drosophila* cells that are mutant for *rbf1*, one of the two *Rb* homologs in flies, are hypersensitive to fasting conditions, as are intact fly larvae in which *rbf1* activity has been partially inactivated. Furthermore, RBF1 regulates genes involved in glutamine and nucleotide metabolism, a finding supported by metabolic profiling of fly larvae depleted for *rbf1*. The increased demand for glutamine in *rbf1*-depleted larvae is responsible for their hypersensitivity to fasting conditions, and *rbf1*-depleted tissues are also vulnerable to oxidative stress. In addition, Nicolay *et al.* [14] demonstrated that in multiple human cancer cell lines loss of *Rb* altered the metabolism of glutamine, leading to alterations in energy production, redox balance, and nucleotide pools.

Additional recent reports have also implicated pRb family members in the control of metabolism. The simultaneous loss of both *rbf1* and *gig/TSC2*, a negative regulator of TOR signaling, results in the induction of cell death in fly tissues and leads to a synergistic increase in oxidative stress in mammalian cells [15]. Moreover, pRb, along with its binding partner E2F1, has also been implicated in the control of oxidative metabolism in fat tissue, and targeted deletion of *Rb* and associated factors in mice can lead to severe metabolic alterations [2,16–19]. Still other reports have linked the pRb pathway to lipid and oxidative metabolism and to mitochondrial biogenesis [16,19,20]. Finally, pRb is regulated in part through phosphorylation by AMP kinase, a key regulator of cellular energy homeostasis [19].

Although the relative impact of the pRb pathway on cancer cell metabolism still remains to be determined, the findings by Cui *et al.* [4], together with other studies, are starting to build a compelling case (Figure 1). Nevertheless, the effects of pRb family members on metabolism appear to be quite complex and, in some instances, contradictory. Interestingly, loss of *Rb* often appears to render cells less fit to cope with the metabolic requirements and physiological stresses that are encountered by tumor cells. This may point to therapies based on

the metabolic vulnerabilities of *Rb*-deficient tumors but also suggests that cancer cells may be capable of evolving the means to counter these deficiencies.

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Development: Hippo Signalling Turns the Embryo Inside Out

Lineage decisions in development are thought to be primarily due to differential activation of transcription factors. However, cell position and subcellular organization of signalling also play a role. New studies of the Hippo pathway in the early mouse embryo show how.

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In the early mammalian embryo, the first cell-fate decision leads to the formation of the trophectoderm, which will form the placenta, and the inner cell mass, which will give rise to the embryo proper and yolk sac. It has long been proposed that the position in the embryo of the cells that will form the trophectoderm or the inner cell mass is key to this specification event. The reason behind this suggestion is that when the trophectoderm and the inner cell mass segregate, between the 8-cell and 16-cell stage, the embryo resembles a compacted ball of cells with some cells positioned on the outside surface of this ball and others embedded inside it. Those cells that lie on the outside will form the trophectoderm and are polarised with an apical domain enriched in proteins such as the atypical protein kinase C (aPKC) and the polarity protein Par3. By contrast, the cells that lie inside are apolar and will go on to form the inner cell mass (Figure 1) [1,2]. But how is this difference translated into the activation of trophectoderm and inner cell mass specific gene expression? Two new studies in this issue of *Current Biology* by the groups of Hiroshi Sasaki [3] and Janet Rossant [4] shed important new light on this question.

The first clue for an involvement of the Hippo pathway in mammalian pre-implantation development came from analysis of mice lacking the transcription factor *Tead4* [5,6]. Tead is a member of the Hippo pathway, a signalling system that is evolutionarily conserved from *Drosophila melanogaster* to mammals and controls organ size through cell proliferation [7,8]. When the pathway is activated, the Tead co-factors Yap and Taz (homologues of *Drosophila* Yorkie) are phosphorylated and excluded from the nucleus, therefore preventing transcription of target genes. In the blastocyst, the Tead4 protein is present in all nuclei; however, Yap is only localised to the nucleus of outside trophectoderm cells. Consequently, *Tead4* mutants specify an inner cell mass but do not form a trophectoderm and lack proper expression of key regulators of the trophectoderm lineage, such as *Cdx2*. Therefore, activation of the Hippo pathway represses the trophectoderm fate. The protein kinases Lats1/2 (homologues of *Drosophila* Warts), which phosphorylate Yap/Taz, are crucial for this process, but again show no differential expression between inner and outer cells [9].

These studies provided evidence for an involvement of the Hippo pathway in repressing trophectoderm fate in inside cells [9], but several important questions remained unanswered: first of all, it had not been established if Hippo signalling plays any part in the specification of the inner cell mass. An unequivocal answer to this question is provided by the Rossant and Sasaki groups, who analysed the effects of loss of function of two different Hippo pathway components that had not previously been studied during pre-implantation development, Nf2 (the homologue of *Drosophila* Merlin)