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Edging toward New Therapeutics with Cyclin D1 Egl'ng on Cancer

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In this issue of *Cancer Cell*, Zhang et al. reports that the iron-dependent 2-oxoglutarate dioxygenase or prolyl hydroxylase EglN2 induces Cyclin D1 levels, egging on breast tumorigenesis. Their observations through loss of function studies suggest the potential for drug-like molecules inhibiting EglN to serve as new cancer therapeutics.

EglN2 (also known as PHD1) is among three known EglNs, with EglN1 (PHD2) being key to the hydroxylation and degradation of the hypoxia inducible factor 1α (HIF-1α) (Berra et al., 2006; Epstein et al., 2001; Kaelin and Ratcliffe, 2008). Although the direct substrates for hydroxylation by EglN2 and EglN3 are not well understood, Zhang et al. document that EglN2 activity positively influences the level of Cyclin D1, independent of HIF, in a series of in vitro experiments and in genetically engineered mice (Zhang et al., 2009). The authors linked EglN2 to Cyclin D1 in breast tumorigenesis, partly on the basis of their genetic connection in *Drosophila* and the fact the EglN2 is estrogen inducible, such that loss of EglN2 activity diminishes breast tumorigenesis that could be rescued by ectopic Cyclin D1 expression.

Prolyl hydroxylases belong to the superfamily of iron-dependent, 2-oxoglutarate dioxygenases that use molecular oxygen to hydroxylate specific prolyl residues of substrates whose functions may be altered, or the hydroxylated substrates are destined for degradation through the proteasome (Kaelin and Ratcliffe, 2008). EglNs have Km values for oxygen that are higher than oxygen concentrations in mammalian tissues, causing these enzymes to be highly sensitive of oxygen levels, which are naturally diminished in areas of tissues distal from a blood vessel or abruptly decreased by blockage of the blood supply. With decreased oxygen concentration, the EglNs are thought to not only be inactivated by the lack of its substrate oxygen but may also be disabled by increased reactive oxygen species (ROS) resulting from inefficient mitochondrial respiration. ROS oxidizes and inactivates the catalytic EglN ferrous ion, resulting in loss of prolyl hydroxylation and stabilization of the EglNs' substrates. Chief among the EglNs' substrates is HIF-1α, which is a ubiquitous transcription factor mediating cellular genomic responses to hypoxia and a substrate of EglN1 (Figure 1) (Semenza, 2007).

Zhang et al. noted on the basis of earlier work by Frei and Edgar that the sole EglN, Egl9, in *Drosophila* interacts genetically with Cyclin D1 (Figure 1) (Frei and Edgar, 2004). Specifically, Frei and Edgar observed that Cyclin D1 overexpression in flies resulted in eye overgrowth, a phenotype that could be extinguished by the concurrent loss of Egl9 function. By examining *EglN2*$^{-/-}$ mice, which are grossly normal, Zhang et al. found that Cyclin D1 mRNA and protein levels are diminished in the mutant murine embryonic fibroblasts. They also document hypoproliferation of mammary glands in older EglN2 null mice reminiscent of the more severe phenotype reported for the *Cyclin D1*$^{-/-}$ mice. These observations in genetically engineered mice suggest that loss of EglN2 function phenocopies decreased Cyclin D1 activity in mammary tissue. Intriguingly, although it is documented that hypoxia inhibits cell proliferation partly through the induction of p27 putatively downstream of HIF-1 in mammalian cells (Gardner et al., 2001), the connection between EglN and Cyclin D1 in *Drosophila* and mice suggests a possible alternative pathway to inhibit cell proliferation when oxygen is lacking. In this regard, EglN could play a direct role in regulating the cell cycle by suppressing a putative suppressor of Cyclin D1 (Figure 1).

Cyclin D1 promotes the phosphorylation of the retinoblastoma protein (pRB),

by releasing E2F transcription factors to stimulate cell proliferation. The Cyclin D1 gene is frequently amplified in breast cancer and induced by estrogen, and its loss of function resulted in delayed mammary tumorigenesis in the mouse. EglN2, similar to Cyclin D1, is induced by estrogen, and EglN2 overexpression stimulates breast cancer cell proliferation, suggesting that estrogen could stimulate breast cancer cell proliferation through EglN2 and its downstream effectors (Seth et al., 2002). The observation in *Drosophila* linking Egl9 to Cyclin D1 suggests that Cyclin D1 could be an effector of estrogen-stimulated breast cancer cell proliferation downstream of EglN2. In this regard, Zhang et al. document that loss of EglN2 function through the use of interference RNAs resulted in diminished Cyclin D1 levels not only in breast cancer cells but also in several other cancer cell types, linking EglN to Cyclin D1 in mammalian cells (Figure 1). Although the work of Zhang et al. did not delineate the EglN2 substrate that in turn regulates Cyclin D1 levels, their experiments document that EglN2 is likely to affect Cyclin D1 transcription, suggesting that a substrate of EglN2 could be a transcriptional repressor. It is notable, however, that ectopically expressed Cyclin D1 levels were also affected by shRNA-mediated decreased in EglN2 levels, leaving open the possibility of an additional posttranscriptional level of regulation.

The correlation and mechanistic connection between EglN2 and Cyclin D1 breast tumorigenesis was delineated by Zhang et al. through a series of experiments that documents the correlation of EglN2 mRNA levels with the estrogen receptor (ER) status in human breast cancers, such that EglN2 levels are highest in ER-positive tumors that contrast with EglN1 levels, which are highest in ER-negative, HER2-positive tumors. Gain of EglN2 function via overexpression in an estrogen-dependent breast cancer cell line rendered it estrogen independent, and conversely shRNA-mediated loss of EglN2 function in this cell line resulted in decreased Cyclin D1 expression and diminished estrogen-stimulated proliferation. Furthermore, diminished EglN2 expression via inducible shRNA expression resulted in decreased in vivo tumor xenograft growth, which was rescued by ectopic, constitutive expression of Cyclin D1. These observations collectively link EglN2 to the regulation of Cyclin D1 in estrogen-mediated breast tumorigenesis (Figure 1).

The findings of Zhang et al. are intriguing not only because they suggest a link between the prolyl hydroxylase EglN2, through a yet unknown EglN2 substrate, and Cyclin D1 in breast tumorgenesis, but they also suggest that small molecule inhibitors of EglNs could serve as a new class of anticancer drugs (Fraisl et al., 2009). Until better structural work becomes available on EglNs and the specificities of drug-like EglN inhibitors are better established, the inhibition of EglNs could affect the HIFs, particularly because HIF-2α is suggested to be downstream of EglN2 (Aragones et al., 2008). On the one hand, inhibition of EglN2 should diminish Cyclin D1 levels, leading to inhibition of tumorigenesis; on the other hand, cross-inhibition of EglN1 by these drugs could lead to the elevation of HIFs that can potentiate tumorigenesis (Figure 1). Furthermore, little is known about the chemistry and biology of EglN3, prompting a cautionary note about the use of EglN inhibitors in human subjects, particularly given that our understanding of oxygen homeostasis in the physiological setting of normal tissue turnover and repair is at best rudimentary at this time.

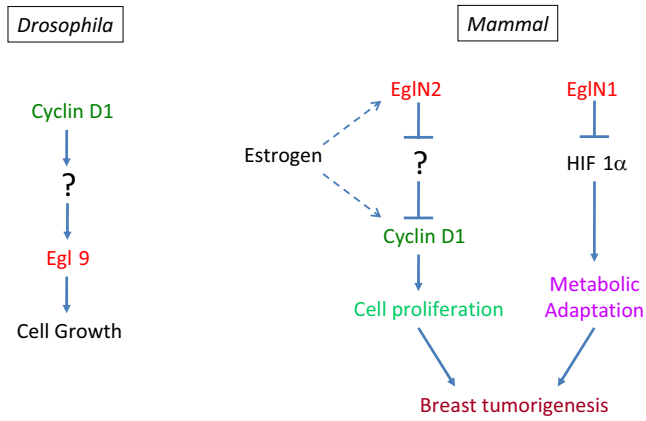

Figure 1. Schematic Diagrams Depicting the Links between EglNs and Cyclin D1 in *Drosophila* and Mammals

As shown on the left, the diagram shows the genetic connection between Cyclin D1, which is placed by epistasis upstream, and Egl 9, which is involved in cell growth but not cell proliferation of the fly eye. As shown on the right, in mammary tissue, EglN2 and Cyclin D1 are both responsive to estrogen with EglN2 being placed upstream of an unknown prolyl hydroxylase substrate (?) that regulates cell proliferation through Cyclin D1. The EglN1 substrate HIF 1α is documented to play a pivotal role in tumor metabolic adaptation to hypoxia and contributes to breast tumorigenesis.

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