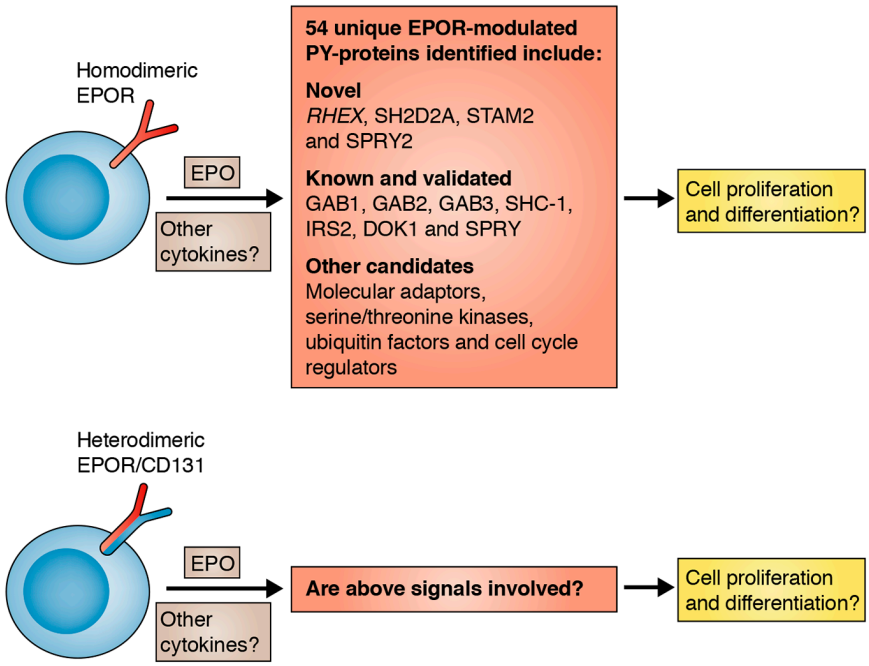
RHEX in the mix of erythropoietin signaling molecules

Insight from Hal Broxmeyer

Erythropoietin (EPO) is of immense practical clinical utility in the treatment of anemia, and insight into EPO-regulated signaling pathways may reveal new targets for the treatment of dysregulated erythropoiesis. While significant headway has been made in understanding the intracellular signals mediating the effects of EPO through the homodimeric EPO receptor (EPOR), we clearly don't know all the intracellular mediators involved. In this issue, Verma et al. report a novel mediator of EPO-dependent expansion of human erythroid progenitor cells and development of erythroblasts, which they have named regulator of human erythroid cell expansion (RHEX).

Applying a global phosphotyrosine (PY) phosphoproteomic approach, the authors identified an uncharacterized 26K open reading frame that was rapidly and highly tyrosine-phosphorylated at two sites after EPO stimulation. RHEX has no significant homology with known proteins and, although well-conserved in humans and other primates, it is not found in the genomes of rats, mice, or lower vertebrates. RHEX knockdown in an EPO-dependent human cell line attenuated cell growth and activation of ERK1 and ERK2, with no effect on cell death. In purified populations of human erythroid progenitor cells, RHEX knockdown delayed the formation of maturing erythroblasts. Coimmunoprecipitation experiments indicated that RHEX associates with the versatile adaptor protein GRB2 following EPO stimulation.

This outstanding paper opens up a number of important questions regarding the function of RHEX and the regulation of EPOR signaling. Is RHEX involved in the intracellular signaling of other cytokines? If so, does EPO signaling compete with other cytokine signaling pathways for GRB2 binding to RHEX? In addition to the homodimeric EPOR, a heterodimeric EPOR (EPOR chain and CD131, the common β-chain of GM-CSFR and IL-3R), which is present on some normal and cancer cell types, mediates nonerythropoietic effects of EPO. Is RHEX also an intermediary in signaling through the heterodimeric EPOR? Finally, it is of interest that RHEX was not detected in rats, mice, and lower vertebrates. Why is this? And how does EPO–EPOR signaling differ in different species?



Newly identified EPO–EPOR signaling molecules

Although the investigators chose to focus on RHEX, their PY-phosphoproteomic analysis identified other EPO-induced candidate targets, including molecular adaptors, serine/threonine kinases, tyrosine phosphatases, ubiquitin factors, and cell cycle regulators, that should provide for continued productive analysis of EPO-induced signaling. The EPO–EPOR signaling gray box is opening up, and with it will come greater insight into EPO's actions and its modulation for clinical advantage.

Verma, R., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20130624.

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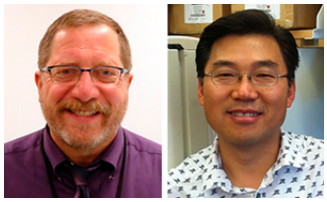
Influenza pathogenesis: Club cells take the "cure"



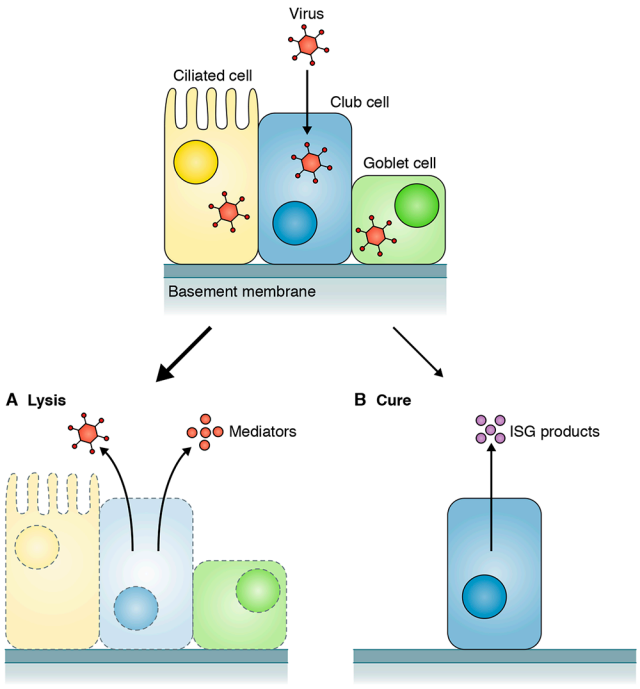
Insight from Thomas Braciale (left) and Taeg Kim

The illness that follows influenza A virus (IAV) infection results from both direct effects of IAV replication in the respiratory tract (RT) and from sequelae of the host immune response. The virus directly induces apoptosis or necrosis of infected RT cells and NK cells, CD8 T cells lyse infected cells, and damaging inflammatory mediators are produced by various infiltrating immune cells. Accordingly, the degree of RT pathology is believed to reflect both the extent of virus replication and the magnitude of the host immune response. In this issue, however, Heaton et al. provide evidence for a novel mechanism of IAV pathogenesis. They find that a small fraction of a particular RT cell type, the club cell, can "cure" itself of IAV but continue to produce inflammatory mediators, which may contribute to sustained RT inflammation and injury following IAV clearance.

Heaton et al. adapted a strategy to indelibly mark IAV-infected cells where any cell infected by the virus will express RFP. Contrary to expectation, they detected RFP$^+$ cells in the RT up to day 21 post-infection (p.i.), long after virus-infected cells are cleared. The authors identify these residual RFP+ cells as club cells—bronchiolar (small airway) exocrine cells (formerly known as Clara cells), which secrete products that protect the small airways.

Remarkably, by day 10 p.i. the residual RFP$^+$ club cells were "cured," that is, they no longer expressed detectable viral RNA. But they retained the type I interferon stimulated gene expression signature of infected cells and production of inflammatory chemokines, suggesting that these cells may contribute to RT pathology. Supporting this idea, selective depletion of the residual RFP$^+$ cells diminished epithelial cell damage in the RT. However, severe RT injury with lethal outcome is associated with infection of alveolar epithelial cells, which are not "cured" in this model, so the contribution of club cells in IAV pathogenesis remains to be determined.

These provocative findings raise many questions, notably, how do infected club cells escape destruction by IAV and recognition by NK cells or CD8 CTL? Also, what sustains the inflammatory signature of club cells after viral RNA elimination? Nevertheless, these results provide a potential explanation for persistent RT inflammation following virus clearance and may presage the development of new strategies to treat the sequelae of IAV infection.



IAV replicates primarily in the respiratory tract epithelium, resulting in cell death via direct- or immune-mediated lysis (A). A small fraction of club cells can "cure" themselves of IAV but continue to produce interferon-stimulated gene (ISG) products (B).

Heaton, N.S., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20140488.

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