

Acute promyelocytic leukemia: STATs, HATs, and HDACs

In this issue, both Dong and Twardy (page 2637) and Maurer et al (page 2647) offer a detailed functional and biochemical characterization of the *Stat5b-RAR α* fusion protein, which was originally described in a patient with acute promyelocytic leukemia (APL) several years ago (Arnould et al, *Hum Mol Genetics*. 1999;8:1741-1749). *Stat5b* is one of 5 genes that have now been identified as fusion partners of the retinoic acid receptor (RAR α) in human APL, the others being *PML* (by far the most common), *PLZF*, *NPM*, and *NuMA*.

Although alternative technical approaches likely account for some differences in the observations of these investigators, a number of take-home messages are clear and consistent. The *Stat5b-RAR α* fusion protein blocks myeloid differentiation by inhibiting the transcriptional activity of the normal RAR α . Moreover, it does so because, compared with the normal RAR α , it is more efficient at recruiting transcriptional repressor complexes harboring histone deacetylases (HDACs) and less efficient at recruiting transcriptional coactivator complexes harboring histone acetylases (HATs). Both laboratories identified the coiled-coil domain within the *Stat5b* partner as the critical mediator of the *Stat5b-RAR α* recruitment of the HDAC repressor complexes through its interaction with the SMRT corepressor. Although there is strong genetic evidence that the inhibition of normal *PML* activity by the *PML-RAR α* fusion protein also contributes to the leukemic phenotype (Salomoni and Pandolfi, *Cell*. 2002;108:165-170), the current investigators did not demonstrate any inhibition of *Stat5* transcriptional activity by *Stat5b-RAR α* .

The generation of *Stat5b-RAR α* is a rare event resulting from an interstitial chromosome 17 deletion rather than a chromosome translocation, which generates the other

APL fusion proteins. Nevertheless, both these studies characterizing *Stat5b-RAR α* fit the current paradigm that transcriptional repression is critical to the pathogenesis of certain types of human leukemia and offer further incentive for the development of rational drug therapy that can relieve this transcriptional repression by targeting HDACs or other members of the repressor complex.

—Steven J. Collins

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Reconstitution of active NADPH oxidase in nonhematopoietic cells: a milestone in the study of a complex system

Price and colleagues (page 2653) have succeeded in reconstituting NADPH oxidase, the enzyme responsible for respiratory burst of phagocytes, by expressing its 4 main components in COS-7 cells, a cell line that is readily and efficiently transfected by a variety of vectors. In activated phagocytes, membrane-associated NADPH oxidase (also referred to as phagocyte oxidase, or *phox*) reduces molecular oxygen (O₂) to superoxide (O₂⁻), which is then converted into a variety of potent microbicides. Although many cell types can divert trace amounts of their metabolic oxygen to superoxide, respiratory burst is on a different scale: the amounts of oxygen reduced to superoxide during phagocytosis greatly exceed the normal metabolic oxygen consumption.

When the engineered COS cells were stimulated by appropriate agonists, the reconstituted components of the NADPH oxidase assembled in the membrane and produced superoxide at rates comparable to professional phagocytes. The importance of this milestone is best appreciated in its historical context. Since the 1950s, the molecular analysis of the oxidase was propelled by studies of phagocytes from patients with chronic granulomatous disease, a severe

defect in the phagocytic production of superoxide and its products. In a tour de force of biochemistry, analyses of several broken-cell and cell-free systems were eventually combined with the genetic information to identify 5 proteins that were essential for the normal function and activation of the oxidase. But the molecular dissection of the assembly and regulation of the NADPH oxidase has been hampered by the lack of a system that could be manipulated by such powerful techniques as site-directed mutagenesis and the expression of modified components and regulators of the oxidase.

Price and colleagues have provided initial evidence that the reconstituted COS-*phox* system will be useful. They identified specific molecular features of the 2 cytoplasmic components p47^{phox} and p67^{phox} that are required for enzyme assembly and activation, complementing previous work done in the cell-free NADPH oxidase systems. By demonstrating the inhibitory effects of the transfection of dominant-negative Rac mutants and the Rac-antagonist RhoGDI, they confirmed the requirement of the oxidase for a small G-protein Rac. (Rac1 is naturally present in COS-7 cells.) Looking ahead and linking to ongoing studies of reconstituted Fc receptors that normally mediate phagocytosis of antibody-opsonized particles, we may soon see the full reconstitution of the phagocytic response from the afferent ligation of antibody-coated targets to the respiratory burst.

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Gene therapy for hemophilia B in dogs: finally prevention of bleeding, but concerns about inhibitors remain

High and colleagues (page 2670) report complete prevention of spontaneous bleeding in 3 dogs with hemophilia B that were treated with liver-directed gene

therapy with an adenovirus-associated virus (AAV) vector. The utilization of a strong liver-specific promoter was what allowed them to achieve 5% to 12% of normal plasma factor IX with a relatively low dose of AAV. Previous studies in dogs and in human patients have resulted in lower than 5% of normal levels, which was still associated with spontaneous bleeding. These results in dogs are very exciting, as effects in large animals are probably most predictive of success in human patients. A clinical trial of gene therapy in humans with hemophilia B that uses a similar AAV vector and a liver-directed approach has just been initi-

ated. This study raises hopes that these patients may become completely independent of factor except during surgery or with trauma.

Although 3 dogs did well, 1 dog that derived from the Auburn University colony with an early truncation mutation developed high titers of an inhibitory antibody in response to the gene therapy and died of a bleeding episode that did not respond to plasma. This result raises the sobering concern that some gene therapy approaches might result in an antibody response that would leave the patient unable to respond to factor during bleeding episodes. Although

the inhibitor formation may have been due to other clinical features in this dog and the incidence was less frequent with the liver-directed approach than with a muscle-directed approach in a previous study, the fact remains that one dog had a catastrophic response to the gene therapy. Studies to further define the risk of inhibitors and ways to prevent them, as pioneered by the High laboratory, will be essential prior to applying these treatments to patients that are at high risk for inhibitor development.

—**Katherine Ponder**

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