Reconstructing a disease: What essential features of the retinoic acid receptor fusion oncoproteins generate acute promyelocytic leukemia?

Acute promyelocytic leukemia (APL) is associated with rearrangement of the retinoic acid receptor α (RARα) gene leading to the formation of chimeric receptor proteins. In this issue of *Cancer Cell*, studies by Kwok et al. and Sternsdorf et al. indicate that the ability of the RARα oncoproteins to dimerize/multimerize is an essential feature required for the development of disease. Homodimerization allows RARα to bind to corepressors with increased affinity and the ability to bind to novel DNA sequences. However, artificial RARα dimers were weak oncogenes in vivo, indicating that the fusion partners confer additional properties to RARα to efficiently generate disease.

The clinical observation that acute promyelocytic leukemia (APL) was uniquely sensitive to all-*trans*-retinoic acid (ATRA) led to the finding that the retinoic acid receptor α (*RARα*) gene was rearranged and fused to the *PML* gene in APL associated with t(15;17). Subsequently, four other proteins, including PLZF, NPM, NuMA and Stat5b, were found as much rarer translocation partners. These diverse proteins have at least one property in common, the ability to self-associate. Two papers in this issue of *Cancer Cell* (Kwok et al., 2006; Sternsdorf et al., 2006) represent the latest attempts to deconstruct the mode of action of these leukemogenic proteins. While dimerization indeed is a critical property of the RARα fusion oncoproteins, there are a number of other characteristics of these proteins that collaborate to generate disease.

Only RARα is rearranged in APL

Knockout studies do not indicate an essential role for any RAR in myeloid development. However, retinoid-deficient rodents develop leukopenia, and treatment of their bone marrow ex vivo with ATRA increases myeloid colony growth, indicating that RAR can modulate myeloid development, by regulation of an incompletely identified set of target genes. Why is RARα in particular rearranged in every case of APL? The answer may lie in the inherent repression activity of RARα. RARα in the unliganded state strongly binds corepressors and binds coactivators upon addition of ATRA. By contrast, RARβ and RARγ bind corepressors weakly and can activate genes even in the absence of ATRA (Hauksdottir et al., 2003). Overexpression of RARα itself in murine marrow yields promyelocytes. The overexpressed protein might outstrip available retinoids in the cell, assume the repressor conformation, and block differentiation. Nevertheless, enforced expression of RARα itself has not yielded leukemia in mice. Clearly the APL fusion proteins must have acquired additional properties in order to cause disease.

RARα fusion proteins are multimeric

PML and all of the RARα partner proteins in APL contain self-association domains, which allow the RAR chimeras to bind to retinoic acid response elements as a dimer in the absence of retinoid X receptor (RXR), as a 1:1 heteromer with RXR, and as multimeric complexes. RARα/RXR heterodimers bind to direct repeats separated by two or five nucleotides, and PML-RAR homodimers have somewhat altered affinity for such sites. Recently, de Thé's group found that PML-RARα/RXR multimers could bind to direct repeats separated by as many as 13 nucleotides (Kamashev et al., 2004), allowing the oncoprotein to bind and potentially regulate an expanded repertoire of genes normally not affected by RARα (Meani et al., 2005). PML-RARα as well as PLZF-RARα can be purified as high-molecular weight multimers (Sternsdorf et al., 2006 and references therein) and display decreased ability to move within the nucleus, suggesting that they might firmly bind to novel or natural target genes, potentially occluding binding of wild-type RARα. The FKBP-RARα chimera generated by Kwok et al. (2006) formed such high-molecular weight complexes, and disruption of these multimers reversed the ability of the fusion protein to block differentiation and stimulate self-renewal of hematopoietic progenitors.

RARα fusion proteins robustly recruit corepressors

While RARα/RXR heteromers bind one molecule of the SMRT corepressor, PML-RARα homodimers as well as artificial homodimers can bind two SMRT molecules (Lin and Evans, 2000), in part explaining why it takes pharmacological doses of ATRA to dislodge corepressors from PML-RARα. Despite the enhanced ability of artificial RARα dimers to bind corepressors and immortalize a hematopoietic cell line, Sternsdorf et al. (2006) showed that such proteins are very weakly oncogenic. Only 1.5%–3% of a set of transgenic mice harboring artificial RARα dimers developed myeloproliferation, and few of those mice showed a differentiation block. Thus, bona fide APL fusion proteins must have additional functions. This was first evident in PLZF, which complexes with several corepressors including SMRT, NCoR, and ETO/MTG8 (Kwok et al., 2006 and references therein). Similarly, the PML moiety of PML-RARα interacts with the Daxx corepressor. A mutation that precluded Daxx recruitment but preserved dimerization drastically reduced the ability of PML-RARα to block hematopoietic differentiation and immortalize cells. Fusion of Daxx or a PLZF repression domain to such a mutant form of PML-RARα rescued its immortalization function (Zhu et al., 2005). Kwok et al. (2006) take this a step further: a mutation in PLZF-RARα that preserved corepressor binding but prohibited multimerization could not immortalize hematopoietic cells. Immortalization activity was rescued by artificial reconstitution of multimerization. One interpretation of these results is that multimerization of RARα allows firm binding to novel target genes while the partner protein alters the nature repression complex brought to the promoter. Accordingly, PML-RARα aberrantly recruits DNA methyl transferase and methyl DNA binding proteins to target loci, leading to long-lasting gene repression (Di Croce et al., 2002; Villa et al., 2006). Lacking partner protein sequences, artificial RAR homodimers might fail to transform cells because of an inability to recruit such a robust repression complex to target genes.

RARα is fused to specific partner proteins

The RARα fusion partners have important properties aside from their ability to dimerize. PML functions in the nucleus to organize multiple proteins into large

nuclear bodies, modifying proteins such as p53 (de Stanchina et al., 2004), and in the cytoplasm to affect the TGFβ pathway (Lin et al., 2004). PML has tumor growth suppressor properties and can be downregulated in human cancer. Furthermore, *Pml* null mice have an increased tendency to form tumors upon carcinogenic challenge. Sternsdorf et al. (2006) found that, unlike PML-RARα the artificial RARα dimers did not disrupt PML nuclear bodies. Furthermore, engineered RARα dimers were no more likely to induce myeloid disease in *Pml* null mice than in *Pml* replete mice. By contrast, when PML-RARα is expressed in PML null marrow, leukemogenesis is accelerated (Rego et al., 2001). This suggests that another gain of function of the PML-RARα chimera compared to artificial dimeric RARα is its ability to be affected by PML-mediated processes. The reverse is true as well; PML-RARα can affect growth regulation by PML. For example, PML facilitates the acetylation and stabilization of p53, while PML-RARα through its interaction with wild-type PML recruits deacetylases to p53, leading to its degradation (Insinga et al., 2004). The other APL partners also have roles in the control of cell proliferation. PLZF is a growth suppressor that can repress *cyclin A2* and c-*myc*. NPM is essential for genomic stability, NuMA is an essential component of the nuclear matrix, and Stat5b is a critical gene regulator in cytokine signaling. The ability of the RARα proteins to heterodimerize and potentially block the activity of their normal counterparts may play a role in the increased oncogenicity of bona fide APL fusion proteins compared to artificial dimers.

RARα fusion proteins activate as well as repress transcription

Leukemogenic chimeric repressor proteins can also activate target genes. Comparison of the gene expression patterns set by PML-RARα and PLZF-RARα in the absence of ATRA revealed the repression of a number of myeloid transcriptional regulators, inhibition of genes involved in DNA repair and activation of the Wnt/Catenin and Jagged/Notch pathways, which promote self-renewal of the leukemic cell (Alcalay et al., 2003; Muller-Tidow et al., 2004). Gene activation appears to be indirect, perhaps due to sequestration of corepressors by the fusion proteins. If engineered RARα dimers did not have sufficient ability to sequester corepressors they might fail to fully activate such growth-promoting genes. This could explain the reduced ability of an artificial FKBP-RARα fusion to stimulate self-renewal of hematopoietic progenitors relative to PLZF-RARα (Kwok et al., 2006) and the limited oncogenicity of artificial dimers in mice (Sternsdorf et al., 2006).

RARα fusion proteins collaborate with second oncogenic events to generate APL

Although the RARα fusion proteins block differentiation and promote self-renewal in cell lines and cultured murine marrow, they cause leukemia in mice only after a long latent period, proceeded in one model by subtle changes in myeloid differentiation and in another by myeloid expansion and no differentiation block. Expression profiling of promyelocytes harboring PML-RARα from premalignant mice showed that <2% of genes had significant changes in expression compared with normal promyelocytes. Only after a latent period of 3–9 months, when overt leukemia develops, is a major change in gene expression noted (Walter et al., 2004). Changes in expression of relatively few genes, conceivably ones involved in self-renewal and DNA repair, might be required to initiate the leukemic process, setting up the myeloid progenitor for additional mutations that convert the disease to frank leukemia. It is clear that one such additional hit can be an activating mutation of a receptor tyrosine kinase (RTK) (Sternsdorf et al., 2006 and references therein). How does the full differentiation block occur? Given the ability of an activated tyrosine kinase to collaborate with the artificial RARα dimer, it is possible that aberrant signaling can alter the nature of the transcription complex recruited to RARα. Alternatively, by repressing the expression or function of myeloid transcription factors, the activated RTK pathways may converge with the RARα pathways to fully block differentiation and/or activate self-renewal.

The papers under consideration show that dimerization is an essential quality of the APL fusion proteins. Drugs that block dimerization, as in the case of the FKBP-RARα fusion (Kwok et al., 2006), represent another potential therapeutic approach for the treatment of APL, particularly in ATRA-resistant forms associated with mutations in the ligand binding domain of RARα. However, collectively the data indicate that the intrinsic ability of the RARα partner proteins to recruit repressors and alter gene expression play an equally critical role in leukemogenesis.

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Selected reading

Alcalay, M., Meani, N., Gelmetti, V., Fantozzi, A., Fagioli, M., Orleth, A., Riganelli, D., Sebastiani, C., Cappelli, E., Casciari, C., et al. (2003). J. Clin. Invest. *112*, 1751–1761.

de Stanchina, E., Querido, E., Narita, M., Davuluri, R.V., Pandolfi, P.P., Ferbeyre, G., and Lowe, S.W. (2004). Mol. Cell *13*, 523–535.

Di Croce, L., Raker, V.A., Corsaro, M., Fazi, F., Fanelli, M., Faretta, M., Fuks, F., Lo Coco, F., Kouzarides, T., Nervi, C., et al. (2002). Science *295*, 1079–1082.

Hauksdottir, H., Farboud, B., and Privalsky, M.L. (2003). Mol. Endocrinol. *17*, 373–385.

Insinga, A., Monestiroli, S., Ronzoni, S., Carbone, R., Pearson, M., Pruneri, G., Viale, G., Appella, E., Pelicci, P., and Minucci, S. (2004). EMBO J. *23*, 1144–1154.

Kamashev, D., Vitoux, D., and de Thé, H. (2004). J. Exp. Med. *199*, 1163–1174.

Kwok, C., Zeisig, B.B., Dong, S., and So, C.W.E. (2006). Cancer Cell, this issue.

Lin, R.J., and Evans, R.M. (2000). Mol. Cell *5*, 821–830.

Lin, H.K., Bergmann, S., and Pandolfi, P.P. (2004). Nature *431*, 205–211.

Meani, N., Minardi, S., Licciulli, S., Gelmetti, V., Coco, F.L., Nervi, C., Pelicci, P.G., Muller, H., and Alcalay, M. (2005). Oncogene *24*, 3358–3368.

Muller-Tidow, C., Steffen, B., Cauvet, T., Tickenbrock, L., Ji, P., Diederichs, S., Sargin, B., Kohler, G., Stelljes, M., Puccetti, E., et al. (2004). Mol. Cell. Biol. *24*, 2890–2904.

Rego, E.M., Wang, Z.G., Peruzzi, D., He, L.Z., Cordon-Cardo, C., and Pandolfi, P.P. (2001). J. Exp. Med. *193*, 521–529.

Sternsdorf, T., Phan, V.T., Maunakea, M.L., Ocampo, C.B., Sohal, J., Silletto, A., Galimi, F., Le Beau, M.M., Evans, R.M., and Kogan, S.C. (2006). Cancer Cell, this issue.

Villa, R., Morey, L., Raker, V.A., Buschbeck, M., Gutierrez, A., De Santis, F., Corsaro, M., Varas, F., Bossi, D., Minucci, S., et al. (2006). Proc. Natl. Acad. Sci. USA, in press. Published online January 23, 2006. 10.1073/pnas.0509343103.

Walter, M.J., Park, J.S., Lau, S.K., Li, X., Lane, A.A., Nagarajan, R., Shannon, W.D., and Ley, T.J. (2004). Mol. Cell. Biol. *24*, 10882–10893.

Zhu, J., Zhou, J., Peres, L., Riaucoux, F., Honore, N., Kogan, S., and de Thé, H. (2005). Cancer Cell *7*, 143–153.

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