Discovery of a Secreted Tumor Suppressor Provides a Promising Therapeutic Strategy for Follicular Lymphoma

Marc R. Mansour[1,2] and A. Thomas Look[1,*]
[1]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Children's Hospital, Boston and Harvard Medical School, 450 Brookline Avenue, Boston, MA 02115, USA
[2]Department of Haematology, UCL Cancer Institute, London WC1E 6BT, United Kingdom
*Correspondence: thomas_look@dfci.harvard.edu
DOI 10.1016/j.ccr.2011.10.027

In a recent paper in *Cell*, Oricchio et al. identify *EPHA7* as a tumor suppressor gene residing in the 6q-deleted region in follicular lymphoma. A truncated EPHA7^{TR} isoform is secreted by normal B cells, inhibits EPHA2 signaling, and, as a secreted tumor suppressor protein, has potential as a targeted therapeutic polypeptide.

Overexpression of *BCL2* resulting from its juxtaposition to the immunoglobulin heavy-chain enhancer due to the t(14;18)(q32;q21) chromosomal translocation occurs in nearly 90% of follicular lymphomas (FLs) and is generally regarded as the primary event in the pathogenesis of this disease (Yunis et al., 1987). However, several lines of evidence support the notion that t(14;18) is not sufficient to initiate tumorigenesis in its own right. Importantly, *Bcl2* transgenic mice have a low penetrance and long latency of FL development, suggesting that additional molecular events are required for transformation (Egle et al., 2004). Rather alarmingly, minor B cell clones harboring t(14;18) are detectable in the peripheral blood of over 50% of healthy individuals. When these clones are followed over time, some are found to persist and even expand during the ensuing years, yet the incidence of FL remains comparatively low with only two to three cases per 100,000 persons diagnosed each year (Roulland et al., 2006).

Relatively little is known about the precise molecular events that lead to the malignant transformation of *BCL2*-overexpressing B cell clones to FL. Some clues have been provided by array comparative genomic hybridization (aCGH), which has demonstrated that both chromosomal deletions and gains are frequent in FL (Cheung et al., 2009). The two most common chromosomal lesions found in FL are ~11 Mb deletions of chromosome 1p36 (25% of cases) and large deletions of chromosome 6q (10%–15% of cases), both of which are associated with inferior outcomes and progression to high-grade lymphoma (Cheung et al., 2009). Among the many hundreds of genes that reside in these genetic loci, the most compelling case for a gene with tumor-suppressor function has been made for *TNFAIP3* at 6q23.3. *TNFAIP3* encodes a zinc finger protein that negatively regulates NF-kB and whose deletion constitutively activates this pathway (Wertz et al., 2004). However, a significant proportion of patients with FL have 6q deletions that do not include the *TNFAIP3* locus, suggesting the presence of other tumor-suppressor genes in this region.

In an elegant paper recently published in *Cell*, Oricchio et al. (2011) find that Ephrin receptor A7 (*EPHA7*) is a novel tumor-suppressor located in a common region of deletion (CRD) of chromosomal band 6q16 in FL (Oricchio et al., 2011). By performing and analyzing aCGH data from FL samples, the authors identified highly heterogeneous and complex 6q11-27 deletions in 23% of cases. They mapped 11 hemizygous CRDs, which varied in size from 5 kb to 27 Mb, encompassing up to 78 genes each.

To search for tumor suppressors, Oricchio and colleagues used a pooled shRNA library specific for genes within the different 6q CRDs and tested them for their ability to rescue *Bcl2*-overexpressing pro-B lymphocytes from growth factor withdrawal in vitro. Two of their top hits were *Tnfaip3*, supporting previous work that implicated this gene as a bona fide tumor suppressor in B cell non-Hodgkin lymphoma, and *EphA7*, a novel candidate tumor suppressor gene. EPHA7 protein was readily detectable in normal germinal center B cells by immunohistochemistry, but was absent in nearly three quarters of a large series of primary FL cases, which was often found to be due to the deletion of one allele and suppression of the other by aberrant methylation of the *EPHA7* promoter.

The pathobiological significance of EPHA7 became apparent when Oricchio et al. tested the in vivo consequence of *EphA7* knockdown on the development of FL in a *Bcl2* transgenic mouse model. Transduction of hematopoietic stem cells from these animals with *EphA7* shRNAs, followed by their transplantation into irradiated recipients, led to marked acceleration of lymphoma onset as well as an increase in disease penetrance. Interestingly, lymphomas in the EphA7 knockdown animals maintained the typical features of FL but expressed higher levels of the proliferation marker Ki67, which might explain the clinical association between 6q deletion and transformation to a more aggressive phenotype seen in patients.

With 14 members, Ephrin receptors form the largest and possibly most complex family of signaling receptor tyrosine kinases in mammals and have diverse functions in embryonic development and angiogenesis (Pasquale, 2010). They have both oncogenic and tumor suppressor roles, and somatic mutations have been described in EPHA7 in lung cancer (Ding et al., 2008). The Ephrin

receptor kinases are activated primarily through cell-to-cell contact with their membrane-bound ligands, which not only drive a "forward" kinase signal, but also a "reverse" SRC kinase signal in the Ephrin-expressing cell (Pasquale, 2010). The complexity of the signaling cascade comes not only from this bidirectional signaling, but also from the promiscuity of ligand-receptor interactions and the impact of secreted forms of ligand that can be generated by alternative splicing. As an example of the latter, a truncated form of the EPHA7 receptor (EPHA7^{TR}) that lacks the intracellular kinase domain has been identified in neuronal development where it binds to and inhibits the full-length EPHA7 isoform, leading to closure of the neural tube (Holmberg et al., 2000).

Oricchio and colleagues have uncovered an intriguing tumor suppressor function for the EPHA7^{TR} isoform in lymphoma. Normally, germinal center B cells express only the EPHA7^{TR} isoform, which is secreted, and in the absence of a full-length EPHA7 receptor, heterodimerizes with the EPHA2 receptor and inhibits its homodimerization in a dominant-negative fashion (Figure 1A). This maintains the EPHA2 receptor in the unphosphorylated and thus inactive state. In FL, when EPHA7^{TR} is epigenetically silenced or deleted, EPHA2 is able to homodimerize and activate oncogenic signaling through ERK, STAT3, and SRC (Figure 1B).

The unique role of the EPHA7^{TR} isoform as a secreted tumor suppressor renders it potentially exploitable therapeutically. With this in mind, the authors show that a recombinant EPHA7 ecto-

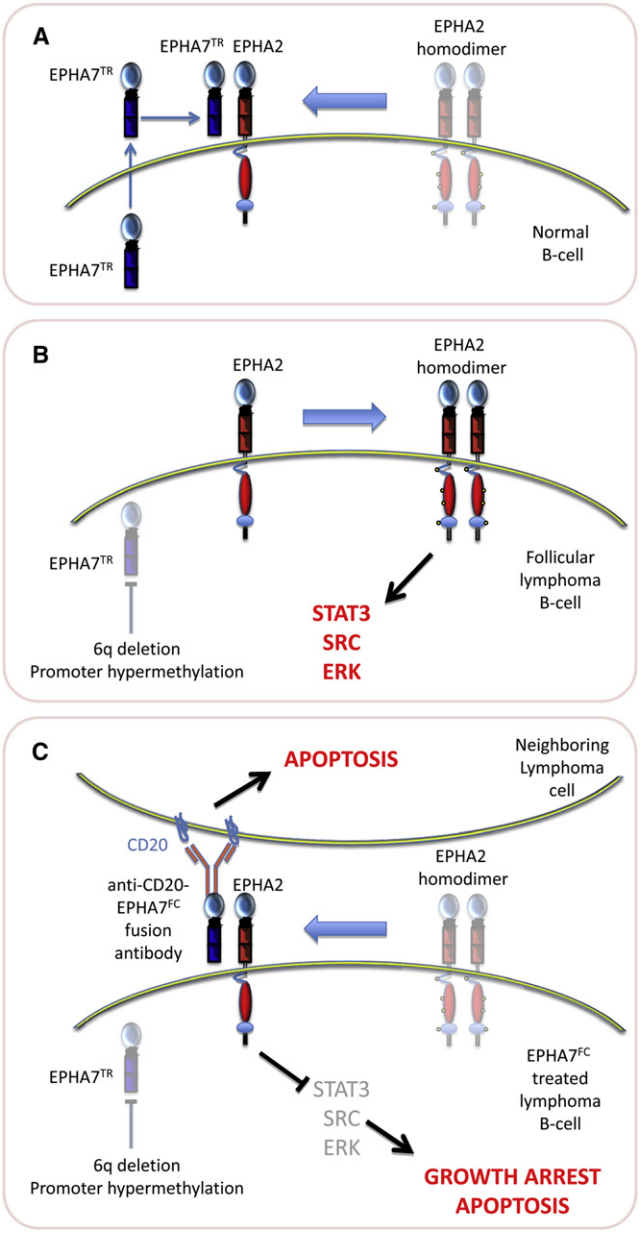

Figure 1. A Proposed Mechanism of EPHA7/EPHA2 Signaling in Normal and Malignant B Cells

(A) Normal B cells express only a truncated form of EPHA7, termed EPHA7^{TR}, that lacks the transmembrane domain and all intracellular domains and is secreted from B cells. EPHA7^{TR} binds the homologous EPHA2 receptor and acts as a dominant-negative inhibitor of EPHA2 receptor dimerization and activation.

(B) In follicular lymphoma, loss of EPHA7^{TR} expression through genetic deletion and/or promoter hypermethylation allows EPHA2 to undergo homodimerization, presumably after binding one or more of the Ephrin ligands. This leads to phosphorylation of several intracellular sites (yellow circles), activating downstream signaling proteins, including STAT3, SRC, and ERK. Lymphoma cells become dependent on these oncogenic signals.

(C) An anti-CD20-EPHA7^{FC} fusion antibody is able to bind both CD20 and EPHA2. Apoptosis is initiated by both direct CD20 binding and via the inhibition of ERK and SRC signaling as a consequence of the EPHA7^{FC}/EPHA2 interaction. The figure depicts that the fusion antibody bridges neighboring cells; however, the steric orientation of binding of this antibody to both receptors is unknown. Therefore, the antibody may also bind to both receptors on the same cell.

domain protein (EPHA7^{FC}) inhibits EPHA2 phosphorylation, reduces ERK signaling and inhibits cell growth in vitro and can induce striking responses after intratumoral injection into human lymphoma xenografts in mice. Furthermore, an anti-CD20-EPHA7^{FC} fusion protein generated to target tumor cells was able to bind CD20 on the surface of B cells, inhibit EPHA2 signaling and induce apoptosis of lymphoma cells after intravenous administration (Figure 1C). Not only was the fusion protein superior to anti-CD20 antibody treatment alone, with the ability to induce complete responses in xenografts, but there was also no significant short-term toxicity.

This important study raises some questions that will require further investigation. First, does EPHA7^{TR} act exclusively through EPHA2 or can it also bind to other receptors? Second, are there other tumor suppressor genes in the 6q region besides *TNFAIP3* and *EPHA7*, and will combined silencing of TNFAIP3 and possibly other proteins encoded by genes in this region cooperate with the loss of EPHA7^{TR} expression? Given that 6q deletions occur in other B cell malignancies, such as B cell acute lymphoblastic leukemia and diffuse large B cell lymphoma, could this therapeutic approach be applicable to other B lymphoid malignancies? Furthermore, might these findings extend to colon, prostate, and gastric cancer, where EPHA7 is also known to be silenced, or to lung cancers harboring *EPHA7* mutations or to breast and ovarian cancers that have genomic gains of *EPHA2* (Pasquale, 2010)? While most tumor

suppressors in cancer are not readily amenable to therapeutic intervention, the intriguing role of EPHA7^{TR} as a secreted tumor suppressor opens many new avenues of investigation, including routes with promising therapeutic applications.

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