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LYMPHOMA

Suppressive EPH-ect

> “EPHA7^{TR} could bind to EPHA2 and inhibit downstream signalling”

Follicular lymphoma, one of the most common non-Hodgkin's lymphomas, is characterized by a chromosomal translocation that increases the expression of the anti-apoptotic protein BCL-2, but additional events are required for lymphomagenesis. Hans-Guido Wendel and colleagues have further investigated the molecular pathogenesis of follicular lymphoma using functional genomics.

Array-comparative genomic hybridization analysis of 64 primary follicular lymphomas showed that the most common deletions (occurring in 23% of these follicular lymphomas) were those affecting chromosome 6q11-27; this confirmed previous cytogenetic data showing 6q losses in follicular lymphoma. The deletions observed in this study were large and hemizygous, suggesting the presence of several tumour suppressor genes. To determine which of these tumour suppressors might have an important role in follicular lymphoma, the authors constructed a library of short hairpin RNAs (shRNAs) against 84 genes in the 6q deletion. Screening this library for shRNAs that permitted the proliferation of BCL-2-expressing pro-B lymphocytes during interleukin-3 depletion identified tumour necrosis factor-α-induced protein 3 (*Tnfaip3*) and ephrin receptor A7 (*Epha7*), and these genes were affected by common regions of deletion in follicular lymphoma. TNFAIP3 has previously been shown to be a tumour suppressor in lymphoma, so the authors focused their attention on EPHA7.

The authors expressed shRNAs against *Epha7* in haematopoietic progenitors from *VavP-Bcl2* mice (which develop follicular lymphoma that is similar to the human disease) and transplanted these cells into the bone marrow of irradiated recipient mice to create a mosaic model. Knockdown of EPHA7 enhanced lymphomagenesis in this model to a similar degree as loss of p53, which has an established role in follicular lymphoma. Pathological analyses confirmed that *VavP-Bcl2* tumours with EPHA7 loss were similar to human follicular lymphoma. Going back to human follicular lymphoma cells, the authors showed that *EPHA7* mRNA and protein levels were reduced compared with normal B lymphocytes. Quantitative analysis of DNA methylation indicated silencing of *EPHA7* in some primary follicular lymphomas and lymphoma cell lines (including diffuse-large B cell lymphoma and Burkitt's lymphoma lines), and expression of *EPHA7* was restored by treatment with a demethylating agent. Therefore, deletion may not be the only way by which this tumour suppressor is inactivated, and EPHA7 may be involved in other lymphocyte malignancies.

How does EPHA7 suppress lymphomagenesis? Normal B lymphocytes express an alternatively spliced truncated form of this receptor (EPHA7^{TR}) but not the full-length receptor, and this was detected not only in cell lysates but also in conditioned media from normal cells, suggesting that this protein is shed from the cell surface. *In vitro* experiments showed that the follicular lymphoma-derived cell line DoHH2 and the Burkitt's lymphoma cell line Raji expressed the EPHA2 receptor tyrosine kinase, and that EPHA7^{TR} could bind to EPHA2 and inhibit downstream signalling to oncogenic kinases (such as ERK and SRC).

Can EPHA7^{TR} be exploited therapeutically? A purified FC-tagged EPHA7^{TR} inhibited proliferation of several lymphoma cell lines *in vitro*. Intratumoural injection of FC-tagged EPHA7^{TR} caused significant regression of Raji xenograft tumours, and systemic administration significantly increased tumour latency. The authors attempted to enhance the efficacy of EPHA7^{TR} by fusing it to the FC-terminus of rituximab, an antibody against CD20 that is approved for treatment of follicular lymphoma; indeed this fusion antibody was more effective than rituximab alone when used to treat Raji or DoHH2 cells *in vitro* and Raji xenografts.

The rituximab–EPHA7^{TR} fusion antibody had minimal toxicities in mice and may have therapeutic potential in human follicular lymphoma, as well as in other lymphomas in which EPHA7 functions as a tumour suppressor.

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ORIGINAL RESEARCH PAPER Oricchio, E. *et al.* The Eph-receptor A7 is a soluble tumor suppressor for follicular lymphoma. *Cell* **147**, 554–564 (2011)

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