Deregulated over expression of FOXP1 protein in diffuse large B-cell lymphoma does not occur as a result of gene rearrangement

Strong uniform expression of FOXP1 protein occurs in a subgroup of non-germinal centre (GC) diffuse large B-cell lymphomas (DLBCL). We have investigated gene rearrangement as a potential mechanism for deregulated expression of FOXP1; however, using FISH FOXP1 translocations were not found in any case with over-expression of the protein.

FOXP1 protein is expressed in a significant number of predominately non-GC phenotype DLBCL, with strong uniform expression identifying a subgroup of patients with notably poor outcome and suggesting a role for FOXP1 in the pathogenesis of this sub-group of tumours. The mechanism by which FOXP1 expression is deregulated is presently unclear, but characterization of t(3;14)(p14;q32) involving the IgH and FOXP1 loci in DLBCL gives one possible mechanism, placing FOXP1 under the influence of the IgH enhancers.

We have examined FOXP1 protein expression in an extended series of 499 presentation DLBCL and have used FISH analysis to specifically investigate cases showing strong uniform expression of FOXP1 protein to determine whether FOXP1 deregulation occurs as a result of gene rearrangement. Presentation biopsies were lymph node (n=321, 64% of patients), extranodal (n=150, 30% of patients), or unknown (n=28, 6% of patients). FOXP1 expression was scored as negative; weak expression in a variable proportion of cells; or uniform, strong expression in all tumour cells, as previously described. This classification of FOXP1 expression was highly reproducible, with 100% concordance between observers. Uniform, strong FOXP1 expression was demonstrated in 121/499 (24%) cases and was significantly associated with a non-GC phenotype, BCL2 expression, and an adverse outcome that was independent of IPI, BCL2 and GC status (24%) cases and was significantly associated with a non-GC phenotype, BCL2 expression, and an adverse outcome.

FISH for FOXP1 gene rearrangement (Figure 1A) was investigated in 58 cases with uniform, strong FOXP1 protein expression. An index case of gastric DLBCL, previously characterised as a t(3;14)(p14;q32) using FIBRE-FISH and inverse PCR, provided a positive control for the FISH assay. No rearrangements were found in any case of DLBCL in conjunction with high expression of FOXP1 protein, with the exception of the control case that showed rearrangement of FOXP1 as demonstrated by a split FISH signal pattern (1F1R1G) (Figure 1B(ii)). Extra copies of the gene were frequently observed in 39/58 (67%) cases (Figure 2B and D). This is probably due to extra copies of chromosome 3, a common feature of DLBCL.

FOXP1 rearrangements have been demonstrated at a higher frequency in extranodal DLBCL, in particular gastrointestinal presentation. Due to availability of material for FISH, 55/58 cases investigated by FISH for FOXP1 rearrangement were nodal, which also reflects the overall bias towards nodal DLBCL in the series as a whole. Given the results of other studies, this may explain why no rearrangements were demonstrated in the current series. However, in the present study, cases that showed strong uniform expression of the FOXP1 protein were specifically targeted for FISH analysis in order to attempt to determine whether gene rearrangement was the primary mechanism for deregulation. It is also of interest that there was no association between strong expression of FOXP1 protein and site of presentation in this study (data not shown). Overall, the data presented both here and in other studies suggest that the incidence of FOXP1 gene rearrangement in DLBCL is rare, and that alternative mechanisms must be responsible for gene deregulation. One possible mechanism is gain of genomic material at
the FOXP1 locus.\textsuperscript{4,6,7} Extra copies of FOXP1 were demonstrated in 67\% of cases investigated, which supports this as a mechanism of over-expression of the protein; however a significant proportion of cases have strong expression of the protein in the absence of any increase in copy number (7 and present study). Given that hypermutation of multiple loci\textsuperscript{9} is frequently demonstrated in DLBCL, it is conceivable that mutational activation of FOXP1 may be a mechanism of deregulation. An alternative explanation is that epigenetic effects are responsible. It is also conceivable that strong expression of FOXP1 is the normal level of expression for B-cells at a very specific stage of differentiation from which these tumours may be derived, which is also supported by the observation that FOXP1 mRNA expression is an excellent marker to classify DLBCL as ABC-type.\textsuperscript{10}

In summary, strong uniform expression of FOXP1 occurs in a subgroup of non-GC DLBCL. Although gene rearrangement is a potential mechanism that may cause deregulated expression of FOXP1, this does not appear to be the primary mechanism linked to expression of the protein in poor prognosis DLBCL.

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