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.

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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[1,2] 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[3,5,6,7] and marginal zone (MALT) lymphomas[4,8] 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.[1] 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 as previously demonstrated,[1] extended analysis (*not shown*).

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,[3] 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.[4]

FOXP1 rearrangements have been demonstrated at a higher frequency in extranodal DLBCL, in particular gastric presentation.[7] 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

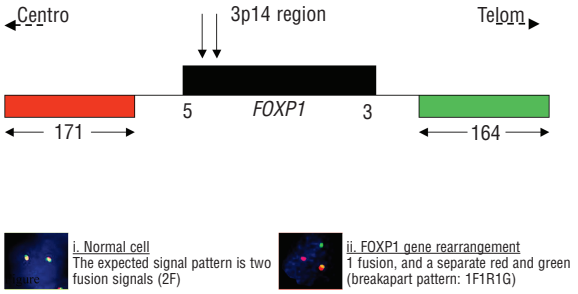

Figure 1. A. *FOXP1* FISH Strategy. A dual color, break-apart assay was used. FISH was performed on either fresh lymph node touch preparations or thin paraffin sections using established methods. A dual color, Break-Apart FISH assay was devised using a mixture of a 5' *FOXP1* Digoxigenin-labelled probe (detected using Anti-digoxigenein-Rhodamine) and a 3' *FOXP1* Biotin-labelled probe (detected using Avidin-FITC) that flank the *FOXP1* gene, including the reported breakpoint region (4,8). Reported breakpoints indicated by arrows. A normal result is defined by 2 red/green fusion signals, indicating an intact gene; and a rearrangement is defined by 1 fusion and a separate red and green signal, indicative of a break in the gene. B. Expected FISH patterns. i) Normal cell. The expected normal signal pattern is two fusion signals (2F); ii) FOXP1 gene rearrangement. FISH pattern of 1 fusion, and a separate red and green (breakapart pattern: 1F1R1G), demonstrating a rearrangement of the *FOXP1* gene. Index case of gastric DLBCL with t(3;14).

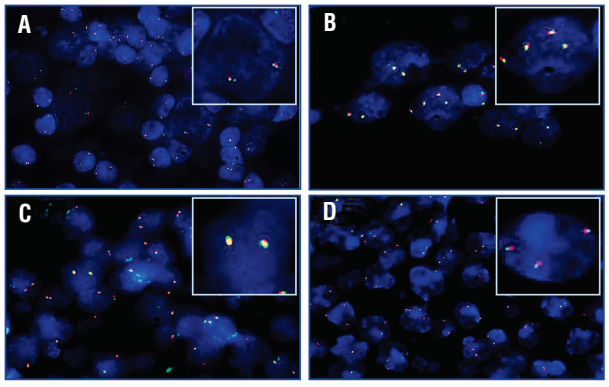

Figure 2. Representative *FOXP1* interphase FISH images. A. FISH on a fresh touch preparation showing normal FISH pattern (2F). B. Touch preparation showing extra copies of *FOXP1* (not rearranged) (4F). C. FISH on a thin paraffin section, showing a normal FISH pattern (2F). D. Thin paraffin section showing extra copies of *FOXP1* (not rearranged) (3F).

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.[4,6,7,8] 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[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.[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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