

Subcellular Localisation of KLF2, RBPJ and NOTCH2

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PURPOSE: Splenic marginal zone lymphoma (SMZL) is a low-grade B-cell lymphoma, originating from marginal zone B-cells of the spleen. Recent studies by whole-exome sequencing have identified KLF2 mutation as the most frequent genetic change in SMZL, with the majority being frameshift indels and nonsense mutations. The mutations inactivate the ability of KLF2 to suppress NF- κ B activation by TLR, BCR and BAFFR signalling and also its ability to repress RBPJ activation by the NOTCH2 intracellular domain (N2ICD); these two pathways are critical for marginal zone B-cell development. To investigate whether KLF2 directly interacts with these transcription factors, we have studied the subcellular localisation of KLF2, N2ICD and RBPJ.

METHODS: HEK293 cells were transfected with HA-tagged KLF2, flag-tagged RBPJ and N2ICD, grown on poly-L-lysine-coated coverslips and subjected to immunofluorescence staining with specific primary antibodies, followed by appropriate, fluorophore-conjugated secondary antibodies. Confocal microscopy was carried out to examine the subcellular localisation of the three proteins 48 hours after transfection.

SUMMARY OF RESULTS: Single transient transfection showed expression of each construct in up to 20% of transfected cells, with each of the three proteins, namely KLF2, RBPJ and N2ICD, localised to the nucleus. Co-transfected cells with all three expression constructs showed evidence of co-localisation of these three proteins in the nucleus.

CONCLUSION: KLF2 co-localises with RBPJ and N2ICD to the nucleus and this may enable KLF2 to suppress the transcriptional activity of RBPJ and N2ICD. We are in the process of investigating whether KLF2 mutants show altered subcellular localisation and thus potential altered interaction with RBPJ and N2ICD.

This work was made possible by the kind support of the Pathological Society Undergraduate Bursary.