Introduction

Haemoglobin (Hb) is composed of two α-like and two β-like globin polypeptides. Around birth, a shift from γ- to β-globin gene expression causes a switch from foetal haemoglobin (Hbf) to adult haemoglobin (HbA). Residual amounts of Hbf are synthesized throughout life, in a subpopulation of erythrocytes termed F-cells. Increased Hbf levels ameliorate symptoms of β-haemoglobinopathies (β-thalassaemia, sickle cell anaemia); molecules that could maintain or re-induce high Hbf levels in these patients are thus being sought.

Carriers of mutations for hereditary persistence of foetal haemoglobin (HPFH) show variably elevated (10-40%) Hbf levels; they are otherwise normal. Studying families of individuals with this condition has allowed the discovery of multiple factors controlling globin gene switching. Krüppel-like factor 1 (KLF1) plays a central role in the developmental globin gene switching mechanism; KLF1 haploinsufficiency is one cause of HPFH. The exact cellular mechanism of HPFH and the variation in Hbf levels expressed are as yet unexplained; the latter may be due to differential expression of modifier genes (friends or foes) acting in concert with KLF1 to regulate the switch (Figure 1). The KLF1 interactome can be further defined by identifying potential molecular targets and observing their expression at the cellular level in comparison with Hbf expression and distribution in F-cells.

Methodology

A direct intracellular antibody-labelling technique normally used for flow cytometry was optimized for use with the Nikon Eclipse Ti inverted fluorescence microscope. The technique employs fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies to Hbf (Figure 2). The NIS-Elements AR software was then used to automatically count F-cells and quantitatively observe them individually for the presence of Hbf in order to assess its distribution.

Results

The number of F-cells visible on microscopy was comparable to that obtained by flow cytometry (Figure 3).

Conclusion

This protocol allows the direct quantitation of Hbf in each F-cell. It will be used for the association of Hbf distribution in F-cells with the expression of potential molecular targets by progenitors at the cellular level, in adults with normal (low) and high Hbf levels. Direct quantitation by imaging cytometry will also be used with molecular probes to observe changes in the expression of KLF1 and other target molecules during erythroid development. This approach should enable the identification of new modifier genes acting with KLF1 to regulate Hbf expression, which could be targeted in the development of new therapies for β-haemoglobinopathies.

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References