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The *MYB* oncogene can suppress apoptosis in acute myeloid leukemia cells by transcriptional repression of *DRAK2* expression

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ABSTRACT

RNA interference-mediated suppression of *MYB* expression promoted apoptosis in the AML cell line U937, without affecting expression of the anti-apoptotic MYB target *BCL2*. This was accompanied by up-regulation of the pro-apoptotic gene *DRAK2* and stimulation of caspase-9 activity. Moreover, RNA interference-mediated suppression of *DRAK2* in U937 cells alleviated apoptosis induced by *MYB* down-regulation. Finally ChIP assays showed that in U937 cells MYB binds to a conserved element upstream of the *DRAK2* transcription start site. Together, these findings identify a novel mechanism by which *MYB* suppresses apoptosis in an AML model cell line.

1. Introduction

The oncogene *MYB* encodes a transcription factor which is an essential regulator of hematopoiesis [1–3]. *MYB* is highly expressed in hematopoietic stem and progenitor cells, while hematopoietic cell differentiation is accompanied by a decrease of *MYB* expression. Moreover, *MYB* is frequently deregulated in hematological diseases such as lymphoma and leukemia.

The *MYB* oncogene was first identified as the transforming gene of avian myeloblastosis virus (AMV) and the avian erythroblastosis virus E26 [4,5]. Both viruses induce acute myeloid leukaemias (AML) in birds and transform immature myeloid cells in tissue culture (reviewed in [6]). In birds and mice, the *Myb* gene is a common target for retroviral insertions leading to myeloid and lymphoid tumors [7–9]. Genetic lesions affecting *MYB*, such as chromosomal translocation [10], duplication [10–12], and structural alteration [13] have been found in some types of human leukemia. *MYB* has also been shown to be essential for the leukemic transformation by several other oncogenes such as MLL-ENL and p210BCR/ABL [14–17]. Moreover, *MYB* is expressed at high levels in most human myeloid and acute lymphoid leukemias [18] even in the absence of obvious structural alterations and is essential for the continued proliferation and/or survival of leukemic cells [16,19,20]. Importantly, these leukaemic cells appear more sensitive to the inhibition of *MYB* expression than normal hemopoietic progenitors [16,21]. Therefore, *MYB* is regarded as a potential therapeutic target in the treatment of leukemia [22].

Enforced *MYB* expression can suppress differentiation and promote continued proliferation; this activity is clearly a major contributor to *MYB*'s oncogenic potential (reviewed in [23,24]). In addition, *MYB* and/or its oncogenic variants are also known to suppress apoptosis, predominantly by enhancing the expression of its direct target, the anti-apoptotic gene *BCL2*. This has been shown in several different cell types including lymphoid and myeloid hematopoietic cells [25–27] as well as breast cancer [28] and colonic epithelium [29]. Studies conducted in our laboratory using inducible shRNA to knock down *MYB* revealed that *MYB* was essential for the proliferation of breast cancer cells [30]. More recently we showed that *MYB* knockdown promoted differentiation of breast cancer cells and greatly sensitized them to the pro-apoptotic effects of several differentiation-inducing agents [28]. Again, the latter effect was mediated by *BCL2* expression which was directly regulated by MYB.

In our current study, we demonstrate that knocking down *MYB* promoted apoptosis in U937 and K562 cells by different mechanisms. In K562 cells, apoptosis appeared to involve the repression of anti-apoptotic factor BCL2, consistent with its positive regulation by *MYB* as discussed above. In contrast, the pro-apoptotic

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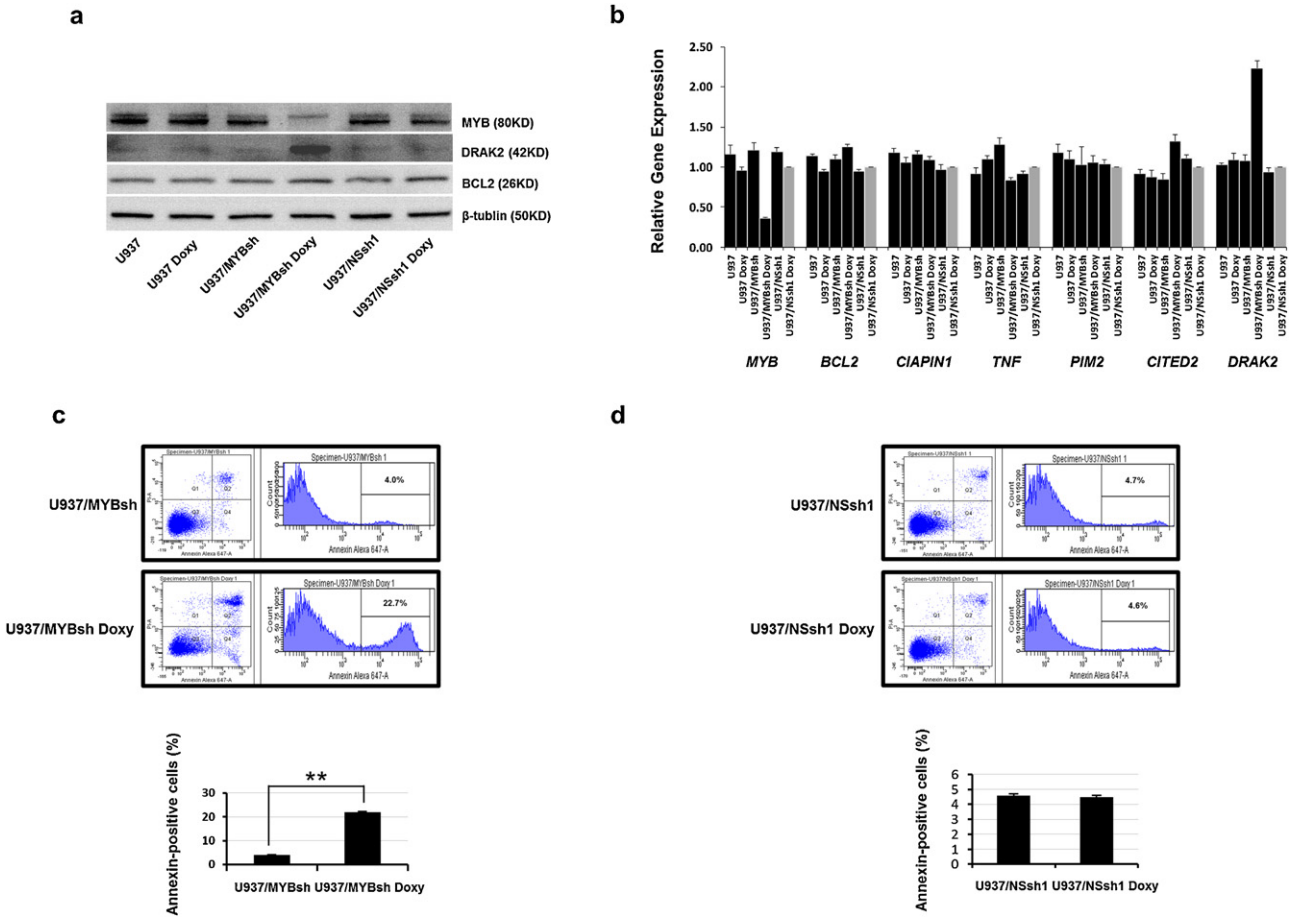

Fig. 1. Knocking down MYB induces apoptosis in U937 cells by stimulating DRAK2 expression and downstream caspase-9 activity. (a) Western blot analysis and (b) qRT-PCR were used to measure protein expression levels of MYB, DRAK2, and BCL2 and mRNA expression levels of *MYB, BCL2, CIAPIN1, TNF, PIM2, CITED2*, and *DRAK2* in U937 cells. The mRNA expression of each gene was normalized to that of the U937/NSsh1 Doxy sample (gray bars); (c), (d) apoptosis assay. The percentage of apoptotic cells is shown below each panel and statistical significance determined using Student's *t*-test ($n = 3$, $^{**}P \leq 0.01$).

effect in U937 cells resulted from the induction of a novel MYB target, the pro-apoptotic death-associated protein-kinase-related 2 (DRAK2/STK17B), and subsequent activation of cysteine-aspartic protease-9 (caspase-9), which is associated with the mitochondrial cell death pathway.

2. Materials and methods

2.1. Cell culture and treatment

U937 and K562 cells were grown in RPMI 1640 (Invitrogen, Mount Waverley, VIC, Australia), 10% FCS, and penicillin–streptomycin–L-glutamine (both from GIBCO/BRL, Grand Island, NY). H293T cells were grown in DMEM (Invitrogen, Mount Waverley, VIC, Australia), 10% FCS (GIBCO/BRL, Grand Island, NY) and as a monolayer. All cell lines were maintained at 37 °C in a humidified 5% carbon dioxide/95% air incubator. Cells were plated in 6-well plates at an initial concentration of 2×10^5 cells per well in 4.0 ml of phenol-red media. Where indicated, cells were treated with 1 μg/ml doxycycline (Sigma, Castle Hill, NSW, Australia) for 72 h for the induction of shRNA before harvested for apoptosis assay, protein and RNA isolation.

2.2. Western blot

Western blot analysis was performed as previously described [28]. Briefly, protein extracts mixed with SDS loading buffer were resolved in SDS/10% PAGE gels and transferred to PVDF membranes. The membranes were then incubated overnight in the presence of antibodies MYB (mouse monoclonal 05-175, 1:500) (Millipore, Billerica, MA), DRAK2 (mouse monoclonal sc-100370, 1:200) (Santa Cruz Biotechnology, Santa Cruz, CA), or BCL2 (mouse monoclonal, sc-7382, 1:200) (Santa Cruz Biotechnology, Santa Cruz, CA) and were developed by using ECL Western blotting substrates (Pierce Biotechnology, Rockford, IL). β-Tubulin was used as protein loading control and detected with mouse monoclonal antibody T0198 (Sigma, Castle Hill, NSW, Australia) at 1:7000.

2.3. Quantitative real-time RT-PCR (qPCR)

Total RNA isolation was carried out by using an RNeasy Mini Kit (QIAGEN, Valencia, CA) and quantitated using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA (2 μg) was reverse transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) and incubated at 25 °C for 10 min and 37 °C for 2 h. The synthesized cDNA was diluted 1:10 and stored at −20 °C. The primer sets for human *MYB*, *18s rRNA*, *BCL2*, *DRAK2*, *CIAPIN1*, *CITED2*, *PIM2* and *TNF* were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA) and purchased from Sigma (Castle Hill, NSW, Australia). The sequences of the primers are listed in Supplementary Material. qPCR was performed using the RotorGene 3000 (Corbett Research, Sydney, NSW, Australia) with a total volume of 20 μl per reaction following the reaction parameters recommended by the manufacturer which includes denaturation at 95 °C for 10 min, followed by amplification for 40 cycles (20 s at 95 °C, 20 s at 56 °C and 30 s at 72 °C, fluorescence measurement). For each reaction, the 20 μl total volume contained 10 μl SsoFast EvaGreen Supermix master mix (Bio-Rad Laboratories, Gladesville, NSW, Australia), 500 nM of each primer and 5 μl of each first-strand cDNA sample. Negative controls contained water instead of first-strand cDNA. Quantitative normalization of cDNA in each tissue-derived sample was performed using expression of *18s rRNA* as an internal control. The cycle threshold (Ct) value of each gene was normalized with respect to the corresponding Ct value for *18s rRNA* (ΔCt). This value was further normalized using the average ΔCt value of the relative U937/NSsh1 Doxy or K562/NSsh1 Doxy sample (ΔΔCt). The final fold expression changes were calculated using the equation $2^{-\Delta\Delta Ct}$ [31].

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2013.01.012.

2.4. MYB knockdown by RNA interference

The tetracycline-inducible lentiviral shRNA vector pLV711G has been previously described [30]. Vectors used encoded a *MYB* shRNA (MYBsh) or a non-silenceing shRNA (NSsh1). Constitutively expressed shRNAs against *DRAK2* (DRAK2sh-A and