Gene-expression analysis identifies novel RBL2/p130 target genes in endemic Burkitt lymphoma cell lines and primary tumors

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Burkitt lymphoma (BL) is a B-cell tumor whose characteristic gene aberration is the translocation t(8;14), which determines c-myc overexpression. Several genetic and epigenetic alterations other than c-myc overexpression have also been described in BL. It has been demonstrated that the RBL2/p130 gene, a member of the retinoblastoma family (Rbfs), is mutated in BL cell lines and primary tumors. The aim of this study was to investigate the biologic effect of RBL2/p130 in BL cells and its possible role in lymphogenesis. Therefore, we reintroduced a functional RBL2/p130 in BL cell lines where this gene was mutated. Our results demonstrated that RBL2/p130-transfected cells regained growth control. This suggests that RBL2/p130 may control the expression of several genes, which may be important for cell growth and viability. Gene-expression analysis revealed a modulation of several genes, including CGRF1, RGS1, BGT1, T1A1, and PCDHA2, upon RBL2/p130 reintroduction. We then monitored their expression in primary tumors of endemic BL as well, demonstrating that their expression resembled those of the BL cell lines. In conclusion, these data suggest that, as RBL2/p130 modulates the expression of target genes, which are important for cell growth and viability, its inactivation may be relevant for the occurrence of BL. (Blood. 2007;110:1301-1307)

Introduction

Burkitt lymphoma is an aggressive B-cell tumor that occurs in several clinical forms, with a high growth rate and a large fraction of cycling cells.1 The World Health Organization (WHO) classification recognizes 3 subsets of BL: endemic, sporadic, and immunodeficiency associated. Each affects different populations and can present in different forms.2 All subtypes of BL are characterized by translocation of genetic material between the long arm of chromosome 14 and the immunoglobulin heavy chain region on chromosome 8 (t(8;14)) and the immunoglobulin heavy chain region on chromosome 14, resulting in t(8;14). The translocation may infrequently occur between 8q24 and the kappa light chain locus on chromosome 2 (t(2,8)) or between 8q24 and the lambda light chain locus on chromosome 22 (t(8;22)). Regardless of which of these translocations occurs, the result is deregulation of the oncogene c-myc. The mechanism by which c-myc deregulation results in lymphogenesis is still unclear. In experimental models, deregulation of c-myc results in slowing of differentiation, impaired cell cycle exit, and increased tumor angiogenesis.3 However, genetic and epigenetic alterations other than c-myc have been described in different forms of BL, suggesting that c-myc translocation is not the only genetic alteration implicated in BL molecular pathogenesis and that different mechanisms may be involved in the different subtypes of BL.4 Subsequent tumor progression involves selection for additional genetic and epigenetic changes, including p53 point mutation and p16INK4a gene silencing by promoter methylation.5 Genetic alteration occurring in a subset of BL, including mutations in p73, Bax, and Bcl6, may promote cell growth and/or antagonize apoptosis.6,7 These genes belong to the pathways of pRb/p105 and p53 and may confer a growth advantage or resistance to apoptosis, resulting in the enhancement of cell growth rate typical of BL. pRb/p105 is frequently targeted in many types of cancer but appears functional, normally expressed, and phosphorylated in BL.3 The retinoblastoma family is composed of 3 members, pRb/p105, p107, and pRb2/p130.9 Many of the sequence similarities among these genes reside in a homologous functional domain known as the pocket region. This particular region mediates the interaction with E2F/DP members and viral oncoproteins. There are fundamental differences in the specific mechanisms of growth inhibition employed by the proteins,10 because they exert growth arrest properties in different cell lines. It has been previously demonstrated that the pRb-related gene RBL2/p130 is mutated in its nuclear localization signal in BL cell lines and primary tumors.11,12 This raises the possibility that inactivation of this member of the pRb family renders BL cells more susceptible to transformation by activated c-myc. However, it has been shown that the RBL2/p130 gene is mutated in most of the cases of endemic BL and to a lesser extent in sporadic BL.11,12 In contrast, in AIDS-related BL, the wild-type RBL2/p130 gene is highly expressed.11,13 This finding already suggests that different pathogenetic mechanisms are involved in different BL subtypes.