Bcl6 gene-silencing facilitates PMA-induced megakaryocyte differentiation in K562 cells

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Abstract Targeted therapy via imatinib appears to be a promising approach for chronic myeloid leukemia (CML) therapy. However, refractory and resistance to imatinib therapy has encouraged many investigators to get involved in development of new therapeutic agents such as Phorbol 12-myristrate 13-acetate (PMA) for patients with CML. In that line, we attempted to investigate the chemosensitizing effect of PMA on the imatinib-resistant cells. Based on our western blot analyses, resistant K562 cells (K562R) showed high levels of FoxO3a and Bcl6 expressions which were not modulated by imatinib treatment. However, upon PMA treatment, the levels of both FoxO3a and Bcl6 were up-regulated among both the sensitive and the resistant cells and this treatment was associated with initiation of megakaryocytic differentiation of the cells. siRNA-silencing of FoxO3a led to augmentation of megakaryocytic differentiation of the cells. Similarly, siRNA gene silencing of Bcl6 enhanced the differentiation and induced cell apoptosis among both types of cells. Regarding these results, it might be concluded that Bcl6 knockdown combined with PMA therapy could present a new therapeutic strategy for refractory CML patients to imatinib.

Keywords Bcl6 · Differentiation · FoxO3a · Imatinib · K562

Abbreviations
CML chronic myeloid leukemia
PMA phorbol 12-myristrate 13-acetate
Ph Philadelphia
TKIs tyrosine kinase inhibitors
DLCL diffuse large cell lymphoma
PKC protein kinase C
LICs leukemia-initiating cells
Th facilitates follicular helper T
ALL acute lymphoblastic leukemia

Introduction
Many patients with chronic myeloid leukemia (CML) have a characteristic and specific chromosomal abnormality known as Philadelphia (Ph) chromosome that results from reciprocal translocation between the Ab1 gene on chromosome 9 and the Bcr gene on chromosome 22 in pluripotent hematopoietic stem cells (Nowell 1962; Rowley 1973). The resulting chimeric Bcr-Abl oncoprotein encodes p210bc-Abl protein with constitutive tyrosine kinase activity which is involved in growth factor independent cell proliferation, resistance to apoptosis and altered adhesion of cells in CML patients.

CML starts with an indolent chronic phase in which leukemia myeloid progenitors possess normal function and differentiation program accompanied by enhanced proliferation. By applying an effective therapeutic strategy in this phase, CML can be remedied for many years. Otherwise, CML progresses to irreversible blastic phase that is associated with accumulation of undifferentiated CML cells in bone marrow and peripheral blood which are invariably resistant to many therapeutic options (Druker et al. 2001; Klimm et al. 2009; Calabretta and Ferrarotti 2004; Quintas-Cardama and Cortes 2006). Among CML therapies, targeting Bcr-Abl protein by small molecule tyrosine kinase inhibitors (TKIs) such as imatinib has substantially improved CML prognosis (Deininger et al. 2005; Druker et al. 2006). However, despite the


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