The nuclear phosphoprotein DEK has been shown to be involved in different human diseases. The protein was first identified as one of the parts of the fusion protein DEK-CAN, resulting from the t(6;9) chromosomal translocation occurring in acute myeloid leukemia.1,2 A higher expression of DEK mRNA in many different types of malignant cells, as compared to in their normal counterparts,3,4 suggests a regulation of DEK expression in response to cellular proliferation. The association between tumorigenesis and dysregulated proliferation is well established. The involvement of proliferation-related factors in the regulation of DEK expression is intriguing since the fusion gene DEK-CAN is under transcriptional control of the DEK promoter. In this study, we evaluated the expression of DEK in normal, immature and mature cells from bone marrow and peripheral blood and its correlation with cellular proliferation.

DEK expression was investigated, using real-time reverse transcription polymerase chain reaction (PCR), in immature and mature cells from bone marrow and peripheral blood, which was donated by healthy volunteers after informed consent. RNA extraction, cDNA synthesis, standard preparation of PCR samples, and the composition and condition of the PCR reaction mixture have been described previously.5 We used the assay-on-Demand™ Hs180127 Primer/Probe mix from Applied Biosystems.

We found a high level of DEK mRNA in immature CD34-positive cells from bone marrow, while an almost 10-fold lower DEK expression was seen in more mature cells from peripheral blood (Figure 1). These findings suggest a correlation between DEK expression and cellular maturation and are in line with our unpublished data indicating downregulation of DEK expression during differentiation of hematopoietic cell lines, although the downregulation of DEK seen in cells induced to differentiate could be a result of a proliferation arrest following differentiation and not the differentiation per se as most CD34-positive cells from bone marrow are in a proliferating state, whereas most mature leukocytes from peripheral blood are quiescent non-proliferating cells. It is known that the transcriptional activation of the proximal promoter of DEK is mediated by the transcription factors nuclear factor-Y (NF-Y) and yin yang-1 (YY1),6 which are both implicated in cellular proliferation and cancer.7 In order to investigate the role of proliferation in the variation of DEK mRNA level between mature and immature cells, lymphocytes were activated and induced to proliferate with phytohemagglutinin and interleukin-2. Subsequently, a 3H-thymidine incorporation assay was performed to determine the proliferation rate of the cells. As shown in Figure 2A, the proliferation rate of lymphocytes stimulated with interleukin-2 was higher than that of control cells. A dose-response effect could be observed for both agents, although it was far more pronounced for phytohemagglutinin-stimulated cells (Figure 2A). The DEK expression of stimulated and unstimulated cells was also analyzed, showing a 2- to 3-fold upregulation of the DEK expression in cells stimulated with interleukin-2 (Figure 2B). The greatest increase of DEK expression was found in phytohemagglutinin-stimulated lymphocytes in which DEK expression was upregulated 5 to 6-fold (Figure 2B). Intriguingly, phytohemagglutinin-stimulated cells also showed the highest proliferation rate, as evaluated by the 3H-thymidine incorporation assay (Figure 2A).

As DEK expression is increased in several tumor cells,6,8 dysregulation of DEK might have a role in the maturation block seen in leukemia. As mentioned above, transcription factors responsive to cellular proliferation, have been reported to be involved in the activation of the DEK promoter, supporting a proliferation-dependent expression of the DEK gene. The present study strongly supports the correlation between proliferation and DEK expression, given the fact that the expression of DEK was powerfully upregulated when normal lymphocytes from peripheral blood were stimulated to proliferate by mitogens.

In conclusion, the high level of DEK mRNA found in immature cells, compared to in mature cells, clearly indicates that the regulation of DEK expression in hematopoietic cells is affected by cellular maturation and the proliferation status of the cells. The observed correlation between DEK expression and proliferation is further supported by the assumed responsiveness of the DEK promoter to changes in cellular proliferation. If the regula-
tion of proliferation is in some way altered in the cell, this would probably result in disturbed regulation of DEK expression by the transcription factors NF-Y and YY1, known to be responsive to proliferation. The DEK-CAN fusion gene, like the normal DEK gene, is regulated by the DEK promoter and would also be affected by cell growth alterations. If the fusion protein obtains novel functions compared to the normal DEK and CAN proteins, possibly including dominant negative effects such as an influence on transcriptional regulation of genes involved in differentiation, this could explain the occurrence of maturation arrest in cells expressing the DEK-CAN fusion gene. The fact that the expression of the fusion gene is regulated by a promoter sensitive to proliferation signals provides a potential feed-back loop driving the expansion of cells containing the fusion gene.

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