Hematopoietic cells

The involvement of cellular proliferation status in the expression of the human proto-oncogene *DEK*

The role of the DEK protein, involved in the leukemia-associated fusion protein DEK-CAN, is not yet known. In this study, we show a higher expression of *DEK* mRNA in immature cells than in mature cells. Furthermore, a correlation between *DEK* expression and cell proliferation was demonstrated, suggesting that DEK plays a role in the proliferation of hematopoietic cells and raising the question of whether the DEK-CAN fusion protein might perturb regulation of proliferation in leukemic cells.

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The nuclear phospoprotein 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,[3-5] 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.[6] 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 These findings suggest a correlation between DEK expression and cellular maturation and are in line with our unpublished data indicating downregulation of DEK expression during differentiation of hematopoietic cell lines, although the downregulation of DEK seen in cells induced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),[7] 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

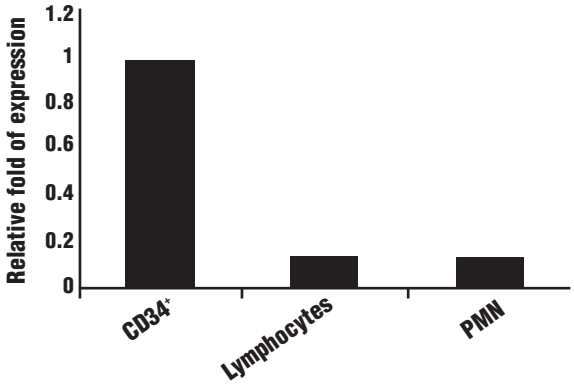

Figure 1. Real-time PCR analysis of *DEK* gene expression in CD34+ cells from bone marrow and lymphocytes and polymorphonuclear cells polymorphonuclear cells (PMN) from peripheral blood. Total RNA was extracted from cells and the reverse transcription reaction was performed using TaqMan RT Reagents (Applied Biosystems) according to the manufacturers instructions. cDNA amplification was performed in dupliate with cDNA corresponding to 10 ng of total RNA together with 1X TaqMan Universal PCR Master Mix and 1X Assays-on-Demand (Hs180127, Applied Biosystems). Data presented are the relative fold difference in DEK expression, normalized for β-actin.

cells, lymphocytes were activated and induced to proliferate with phytohemagglutinin and interleukin-2. Subsequently, a ^{3}H-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 or phytohemagglutinin 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 ^{3}H-thymidine incorporation assay (Figure 2A).

As DEK expression is increased in several tumor cells,[3,4,8] 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-

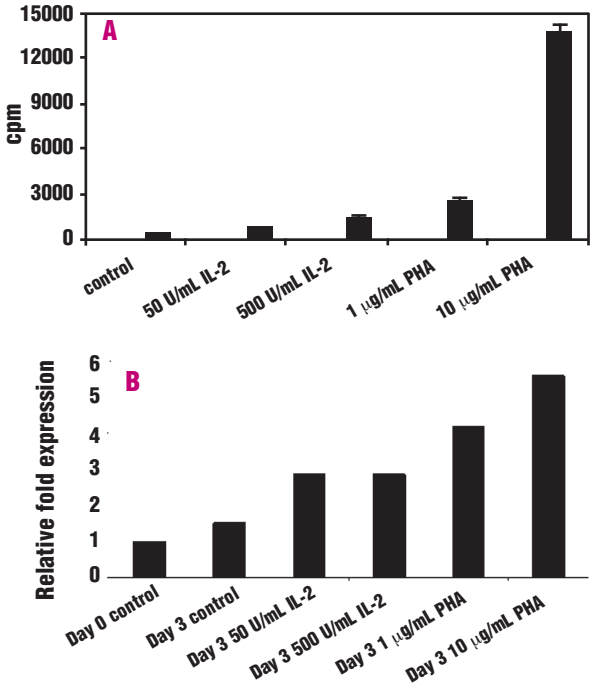

Figure 2. Proliferation and DEK expression in stimulated lymphocytes. A. The lymphocyte activation and proliferation were measured with a ^{3}H-thymidine incorporation assay. Lymphocytes were isolated from peripheral blood and activated with 50 and 500 U/mL of IL-2 or 1 and 10 μg/mL of PHA , respectively, and incubated for 72 hours. After 6 hours of incubation with 0,5 μCi/mL of ^{3}H-thymidine, the cells were lysed and the incorporated radioactivity was measured in a beta counter. The analysis of each sample was performed in six replicates. Error bars represent S.E.M. B. Lymphocytes from peripheral blood were isolated and stimulated with IL-2 or PHA as above. After 72 hours, cDNA amplification was performed in tripilicate with cDNA corresponding to 10 ng of total RNA. Data presented are the relative fold difference in DEK expression normalized for 18S rRNA and compared to control cells.

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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