The DEK oncoprotein is upregulated by multiple leukemia-associated fusion genes.

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LETTER TO THE EDITOR

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To the Editor

The DEK oncoprotein is ubiquitously expressed with multiple roles in epigenetic and transcriptional regulation. It is an architectural protein that contributes to heterochromatin integrity but also binds to transcriptionally active chromatin and has been shown to both promote and repress gene transcription in different genetic contexts [1, 2]. On a cellular level, DEK has been implicated in multiple cancer-related processes. Overexpression of DEK promotes cellular proliferation whereas depletion reduces proliferation and promotes differentiation. The cellular lifespan of HeLa cells is prolonged by overexpression of DEK and, conversely, treatment with shRNA against DEK leads to cellular senescence [3]. DEK also protects against cell death. Knockdown of DEK increases both p53-dependent and –independent apoptosis as well as the sensitivity to apoptotic agents such as etoposide [4, 5]. The importance of DEK in oncogenesis has been convincingly demonstrated by the finding that overexpression of DEK promotes cellular transformation in both cellular systems and mouse models [6]. Intriguingly, tumor cells appear to be more sensitive to reductions in DEK expression than normal cells, suggesting that DEK may be a potential target for cancer therapy [6].

The *DEK* oncogene is commonly upregulated in many cancers and a partner of the (6;9) chromosomal translocation, which leads to the formation of the fusion gene *DEK-NUP214* in a subset of acute myeloid leukemia [3]. However, the underlying mechanisms of the overexpression are still unknown and the only factors known to regulate DEK expression are

the transcription factors E2F, YY1 and NF-Y, which promote transcription of the *DEK* gene by binding to defined sites in its promoter. The expression of DEK has long been considered upregulated also in AML, based on two studies using quantitative PCR analysis to demonstrate overexpression in cohorts of 15 and 41 patients [7, 8]. However, in a recent study in this journal, Logan *et al* showed similar expression levels in normal and malignant hematopoietic cells in an *in silico* analysis of a large pre-existing dataset, verified by tissue microarray [9].

To functionally address the question of DEK expression during leukemogenesis, we examined if the protein levels are affected by fusion proteins known to cause the disease. Thus, we expressed four of the most common leukemia-associated fusion proteins (AML1-ETO, BCR-ABL1, NUP98-HOXA9 and PML-RARα) in human primary hematopoietic cells by viral transduction and analyzed the DEK expression. Cells were isolated from umbilical cord blood using the Indirect CD34 MicroBead Kit from Miltenyi Biotec, after informed consent from donors. The cells were then transduced with viruses encoding the fusion gene and an IRES-driven marker gene coding for green fluorescent protein. Following sorting of successfully transduced cells by FACS, the cells were grown in StemSpan SFEM medium supplemented with 20% fetal bovine serum and the CC100 cytokine mix, all from Stemcell Technologies. After 6 days, total protein was extracted and analyzed for DEK expression by western blot (anti-DEK, BD Transduction Laboratories).

The results show that several of the fusion proteins cause an increase in DEK protein expression. Both NUP98-HOXA9 and BCR-ABL1 significantly upregulated DEK and the same trend was observed for AML1-ETO but not PML-RARα (Figure 1). Since DEK levels can be affected by cellular proliferation and associated factors such as E2F, we next examined

whether the increased DEK expression was the result of an increase in proliferation rate. Here, we observed the same proliferation patterns that have been previously described for primary $CD34^+$ cells expressing these fusion genes. Namely, that BCR-ABL1 but not AML1-ETO, NUP98-HOXA9 or PML-RARα confers a pro-proliferative effect during 10 days of culture (Figure 2). We thus conclude that the upregulation of DEK is not secondary to an increase in proliferation. However, it should be noted that both of the fusion genes which increase DEK expression promote proliferation when the cells are cultivated for an extended period of time. It is thus possible that upregulation of DEK is linked to proliferation in that it precedes rather than follows a proliferative effect.

Although no studies have demonstrated an effect of NUP98-HOXA9 on either of the known DEK regulators E2F, YY1 and NF-Y, the possibility that the regulation is mediated by one of these factors cannot be excluded. However, it is also possible that the upregulation occurs through a pathway that is yet to be defined. Regardless, our findings represent a novel effect of BCR-ABL1 and NUP98-HOXA9 on DEK expression and suggest that DEK could mediate leukemogenic functions of these fusion genes. These results also suggest that upregulation of DEK is not an initiating event during leukemogenesis but rather secondary to the expression of oncogenes such as BCR-ABL1 and NUP98-HOXA9.

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Conflict of interest

The authors have no conflict of interest to disclose.

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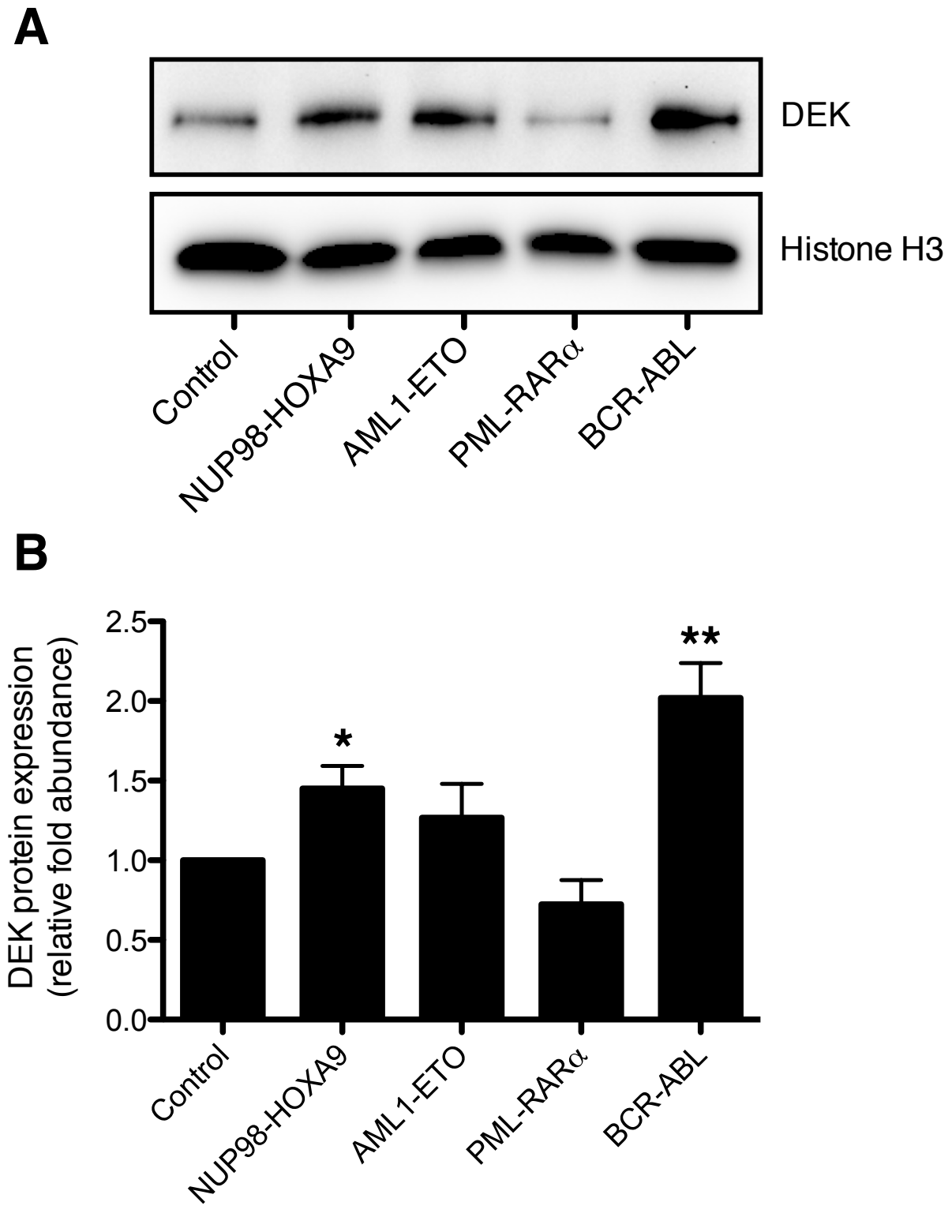
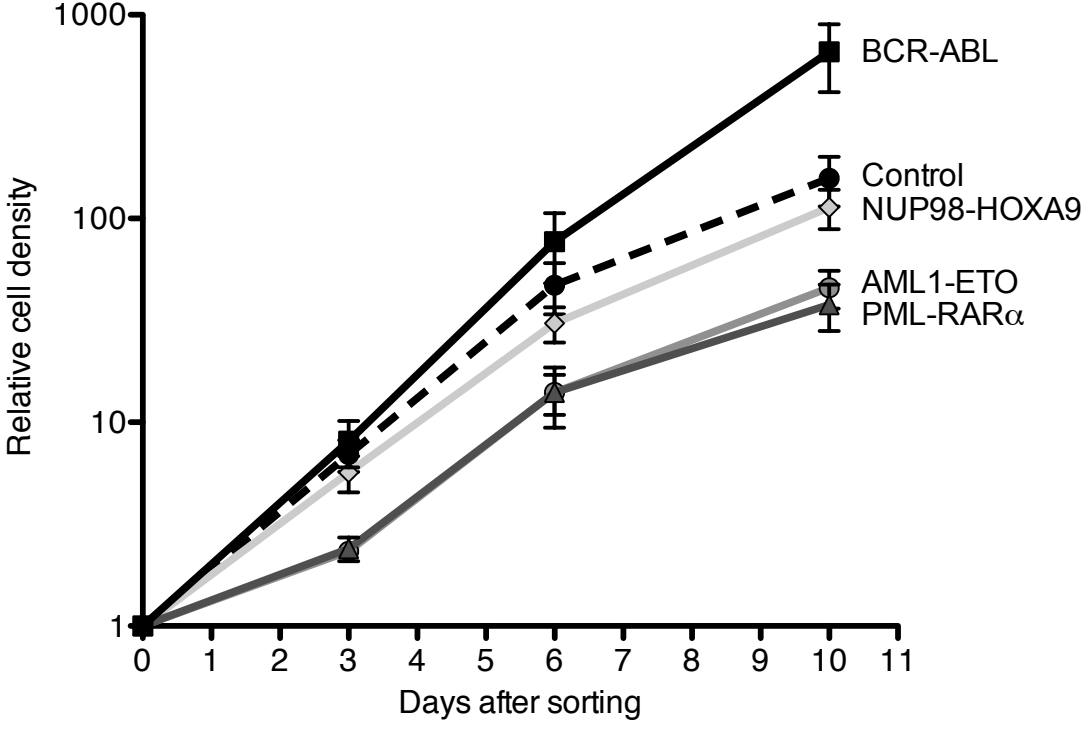
Figure legends

Figure 1. DEK is upregulated by the expression of leukemia-associated fusion genes. (A) Western blot showing the expression of DEK protein 6 days after sorting of primary $CD34^+$ cells transduced with leukemia-associated fusion genes. Expression of histone H3 is used as an equal loading control. One representative experiment out of five. (B) Expression of DEK protein, relative to the expression in cells transduced with empty vector. Mean values were calculated from five independent experiments. Error bars represent S.E.M.

Figure 2. Upregulation of DEK is independent of proliferation. Proliferation of primary human $CD34^+$ cells transduced with the indicated fusion genes, demonstrating that the upregulation of DEK is not a consequence of altered proliferation. Cell viability was consistently above 90% in all cultures. Mean values were calculated from three independent experiments. Error bars represent S.E.M.


A
DEK
Histone H3
Control
NUP98-HOXA9
AML1-ETO
PML-RARα
BCR-ABL
B
DEK protein expression
(relative fold abundance)
2.5
2.0
1.5
1.0
0.5
0.0
*
**
Control
NUP98-HOXA9
AML1-ETO
PML-RARα
BCR-ABL


1000
100
10
1
Relative cell density
0
1
2
3
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Days after sorting
BCR-ABL
Control
NUP98-HOXA9
AML1-ETO
PML-RARα