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LETTER TO THE EDITOR

Overexpression of SNORD114-3 marks acute promyelocytic leukemia

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A global downregulation of small nucleolar RNAs (snoRNA) was reported in acute lymphoblastic (ALL) and acute myeloid leukemias (AML) by Valleron *et al.*[1] The authors also identified a specific snoRNA signature in acute promyelocytic leukemia (APL) with overexpression of snoRNA clusters *SNORD112-114*. Recently, Cohen *et al.*[2] reported a marked overexpression of *SNORD113-3*, *SNORD113-4*, *SNORD114-2* and *SNORD114-3* in three additional cases of APL.

We have compiled a hematological gene expression data set of neoplastic and non-neoplastic samples hybridized to Affymetrix HG-U133 Plus 2.0 GeneChips, all downloaded from the GEO microarray repository. The data were collected as described earlier by Heinäniemi *et al.*[3] Of all the genes on the microarray, 15 were identified as snoRNAs (Table 1). They represent various types of snoRNAs, including H/ACA and CD box types, small Cajal body-specific RNAs (scaRNA), intergenic and intronic snoRNAs, and even one snoRNA without any known target RNA (so-called 'orphan' snoRNA).

To study the expression of the 15 snoRNAs, we focused on 883 cases of pediatric leukemias, which were subdivided into three classes: early B-ALL, T-ALL and AML. In addition, we considered 35 non-neoplastic samples consisting of hematopoietic stem cells (HSCs) along with naive B- and naive T-lymphocytes. In most cases, the snoRNAs were expressed in a relatively uniform manner across all leukemia subtypes and normal blood cells (Figure 1a). In our limited set of 15 snoRNAs, we did not observe a lower expression in acute leukemias as compared with HSCs and naive lymphocytes. Two snoRNAs, *SNORA70* and *SNORD104*, were expressed consistently higher than other snoRNAs in leukemic as well as healthy samples. They were expressed at a level approximately fourfold higher relative to other snoRNAs (Figure 1a). Interestingly, *SNORA25* and *SNORA61* were expressed strongly in naive, or unstimulated, B cells compared to HSCs, naive T cells and leukemias, suggesting B-cell differentiation-dependent regulation of expression (Figure 1a).

At single patient level, the expression of individual snoRNAs remained again rather constant except for *SNORD114-3* (Figure 1a and Supplementary Figure S1). Among the 237 pediatric AML patients, 15 were found to have increased expression of *SNORD114-3* when the cut-off level in $\log_2$-expression was set at 7 (Figure 1b). Interestingly, 13 out of these 15 patients harbored t(15;17) translocation, the hallmark of APL. In the two remaining t(15;17)-negative patients, the expression was only slightly above the threshold level of 7. These two samples did not fall into any major cytogenetic subtype of AML, but one of them had an internal tandem duplication of FLT3 and the other carried a mutation in either *NRAS* or *KRAS*. Among the cases with normal *SNORD114-3* expression, only six patients out of 222, or 2.7%, were identified as t(15;17)-positive patients. Four of these six APL cases were carrying FLT3-ITD. A similar pattern emerged also in the cohort of 1117 adult AML patients in our database, 69 of which were of promyelocytic subtype (Figure 1c). One-hundred and nine adult AML samples were found with an elevated *SNORD114*-3 expression (keeping the threshold at 7). Out of them, 50 were from APL patients. From the adult samples with normal *SNORD114-3* expression, only 19, or 1.9%, were classified as APL. The expression level of *SNORD114-3* differentiated the APL-positive and -negative cases among both pediatric ($P < 10^{-8}$) and adult AML ($P < 10^{-28}$). Mann–Whitney *U*-test was used to assess statistical significance.

Table 1. Characteristics of 15 selected snoRNAs

snoRNA	*Type*	*Host gene*	*Chromosome*	*Target*	*Probes*
SCARNA2	Small cajal body	Independent transcriptional unit	1q13.1	U2 snRNA C61 and U2 snRNA G11	11
SCARNA17	Small cajal body	Independent transcriptional unit	18q21.1	U4 snRNA C8	11
SNORA25	Box H/ACA	*Homo sapiens* cDNA AK128061	11q21	18S rRNA U801 and 18S rRNA U814	6
SNORA61	Box H/ACA	SNHG12 small nucleolar RNA host gene 12 (non-protein coding)	1p35.3	28S rRNA U2495	7
SNORA64	Box H/ACA	RPS2 (ribosomal protein S2)	16p13.3	28S rRNA U4975	10
SNORA65	Box H/ACA	RPL12 (ribosomal protein L12)	9q34	28S rRNA U4373 and 28S rRNA U4427	4
SNORA66	Box H/ACA	RPL5 (ribosomal protein L5)	1p22.1	18S rRNA U119	5
SNORA68	Box H/ACA	RPL18A (ribosomal protein L18a)	19p13	28S rRNA U4393	22
SNORA70	Box H/ACA	RPL10 (ribosomal protein L10)	Xq28	18S rRNA U1692	4
SNORA71A	Box H/ACA	SNHG17 small nucleolar RNA host gene 17 (non-protein coding)	20q11.23	18S rRNA U406	4
SNORA71B	Box H/ACA	SNHG17 small nucleolar RNA host gene 17 (non-protein coding)	20q11.23	18S rRNA U406	22
SNORA74A	Box H/ACA	SNHG4 small nucleolar RNA host gene 4; MATR3 alt Matrin3 mRNA	5q31.2	28S rRNA U3741, 28S rRNA U3743 and U3 snRNA U8	33
SNORD8	Box C/D	CHD8 chromodomain helicase DNA binding protein 8	14q11.2	U6 snRNA A53	5
SNORD104	Box C/D	Independent transcriptional unit	17q23.3	28S rRNA C1327	5
SNORD114-3	Box C/D	LOC100507242, snoRNA gene cluster	14q32	Unknown	4

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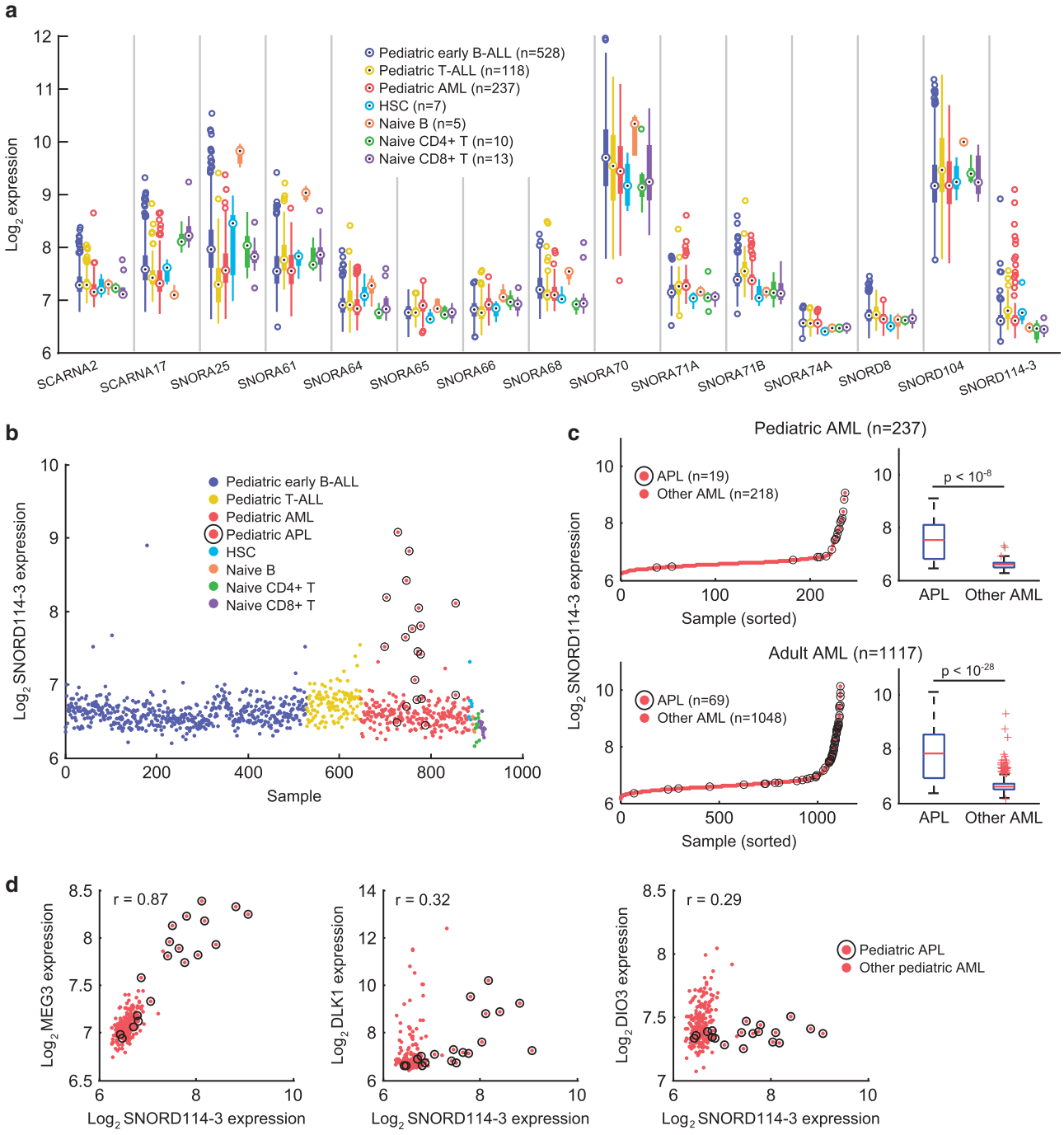

Figure 1. Overexpression of *SNORD114-3* in APL. (**a**) Expression of the 15 studied snoRNAs in three types of pediatric leukemias and four healthy cell types. (**b**) The expression of *SNORD114-3* in pediatric leukemias. (**c**) When pediatric and adult AML patients are sorted according to *SNORD114-3* expression, APL cases are clearly enriched among the patients with high expression. By using Mann–Whitney *U*-test, differential *SNORD114-3* expression between APL and other AML was deemed statistically significant. (**d**) *SNORD114-3* expression is strongly correlated with *MEG3* expression, but weakly with *DLK1* and *DIO3*. Pearson's correlation coefficients were calculated using $\log_2$ expressions.

The snoRNA cluster *SNORD114* lies in the genomically imprinted domain DLK1-DIO3 in the 14q32 region. Genomic imprinting gives rise to mono-allelic, parent-of-origin-specific gene expression. Imprinted genes are susceptible to errors, as a single genomic or epigenetic change may alter or even ablate their function. Imprinted small RNAs are involved in the development and metabolism, and are also frequently perturbed in malignancies.[4] The paternal allele of DLK1-DIO3 domain expresses three protein-coding genes (*DLK1*, *RTL1* and *DIO3*), whereas the maternal counterpart expresses two non-coding transcripts (*MEG3* and *RTL1AS*) accompanied by 53 microRNAs and two snoRNA clusters (*SNORD113* and *-114*) constituting 9 and 31 snoRNAs, respectively.[4] To gain some insight on how deregulation of DLK1-DIO3 affects other genes it houses, we looked at the expressions of *DLK1*, *DIO3* and *MEG3*. Besides *SNORD114-3*, these were the only genes in this domain to be represented on the microarray. The expression of *MEG3* (*Maternally Expressed Gene 3*) was strongly correlated with that of *SNORD114-3*, whereas such correlation was

not observed between *SNORD114-3* and *DLK1* or *DIO3* (Figure 1d). This reflects the fact that *MEG3* is expressed from the same (maternal) allele as *SNORD114*, whereas *DLK1* and *DIO3* are expressed from the other (paternal) one. Furthermore, it suggests that the deregulation may affect the maternal DLK1-DIO3 locus as a whole.

As it happens, *SNORD114-3*, like all the copies in *SNORD114*, is orphan. The function of such snoRNAs lacking RNA targets is unknown but they might have gene regulatory roles as microRNA precursors or by being involved in gene splicing events.[5] Valleron *et al.*[1] showed that *SNORD114-1* is capable of modulating cell growth and proliferation in APL cells, thus suggesting leukemogenicity of *SNORD114*. Recently, Chu *et al.*[6] found that *ACA11*, an orphan snoRNA encoded in an intron of the *WHSC1* gene, is overexpressed in multiple myeloma patients with the translocation t(4;14). In these cells, overexpression of *ACA11* increased proliferation, suppressed oxidative stress and conferred chemoresistance.

Our findings in regard to APL support and increase the robustness of those reported by Valleron *et al.*[1] and Cohen *et al.*,[2] as we used data obtained with a different platform and from a substantially larger cohort of patients. Collectively, they reveal an intricate regulation of snoRNA expression[7] and add to the mounting evidence implicating DLK1-DIO3 locus in tumorigenesis. Seven microRNAs of this locus were found to be upregulated in APL by Dixon-McIver *et al.*,[8] and other transcripts of DLK1-DIO3 domain have been associated with lung cancer,[9] hepatocellular carcinoma[10] and the pluripotency levels of stem cells.[11]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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