In Vitro Expression of the −87 β-Globin Thalassemic Mutations

To the Editor:

Naturally occurring β-thalassemia mutations can be divided into two main categories, β-β and β-+β-thal, depending on the complete or partial loss of β-globin chain production from the affected gene. The β-+β-thal mutations result from point mutations affecting the splicing signal consensus sequence, the polyadenylation addition signal, the cap site or the promoter region within the consensus sequences for the TATA or CACCC boxes. The CACCC box of the β-globin gene is duplicated in man. A number of β-+β-thalassemia mutations have been defined within the proximal element, (positions −92, −88, −87, and −86) as well as in the distal one (−101).

The CACCC box is a crucial element in determining the developmentally regulated high-level expression of the adult β-globin gene. Two transcription factors bind to the CACCC box, i.e., the ubiquitous Spl protein and the erythroid and specific factor EKLF (erythroid Kruppel-like factor). Inactivation of the EKLF gene in mice by gene targeting has shown that the EKLF transacting factor is of crucial importance for the expression of the β-globin gene in the adult stage of development. The binding of EKLF is highly specific and the point mutations above mentioned negatively affect the binding of this transacting factor to the CACCC box. All possible nucleotide substitutions (C-G, C-T, and C-T) have been described at position −87. The C-G mutation, which accounts for 0.2% of the β-thal alleles in the Mediterranean population, when in combination with a β- or severe β-+β-thal allele results in a mild, usually nontransfusion-dependent, clinical phenotype. By contrast, expression studies in HeLa cells have showed an opposite pattern. In this assay, the C-G mutation has indeed a residual expression of 20% to 30% compared with the wild-type β-globin gene, whereas the C-T has a 45% to 51% residual expression.

Herein, we report the results of transient expression studies, which have been performed for elucidating the reasons for the apparent
discrepancy between the observed phenotype and the data on the residual output of expression of the affected gene in vitro. Systemic expression studies were performed both in Cos7, a nonerythroid cell line, and K562, an erythroid cell line. By site-specific mutagenesis, performed using the megaprimer method, we created the C-G, C-T, C-A point mutations at position -87 in a 450-bp β-globin promoter fragment. The mutated β-globin promoter fragment was then cloned into two different reporter expression vectors. The first, an ESL vector, which was used to transfect K562 cells, contains the firefly cDNA sequence and a Simian virus 40 (SV40) promoterless derivative of the pSV2ALΔ5 containing the cDNA of the firefly luciferase gene as well. Transfection has been performed by lipofection. The plasmid pCH110, which carries a β-gal gene, has been cotransfected in each experiment as an internal control for transfection efficiency.

The data presented here show that, although there is a good agreement between the clinical severity observed in vivo and the relative transcription level in vitro using the erythroid expression system HSI/ESL/K562, no correlation has been found using the SV40/Cos7 none erythroid expression system. This result suggests that the previously reported expression data on HeLa cells (a none erythroid cell line) did not faithfully measure the residual transcription activity of the β-thal gene. Even though in the K562 cell line the expression of the EKLF transcription factor is low, the presence of the HSII core of the LCR most likely creates an expression system that more closely reproduces the in vivo situation.

Table 1. Transcriptional Activity of β-thal Proximal CACCC Box Promoter Mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>RTL in Cos7* (%)</th>
<th>RTL in K562* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C-G</td>
<td>40</td>
<td>14.6</td>
</tr>
<tr>
<td>C-T</td>
<td>35</td>
<td>8.0</td>
</tr>
<tr>
<td>C-A</td>
<td>243</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Abbreviation: RTL, relative transcription factor.

* Mean of the different -87 β-thal mutants RTL in Cos7 and K562 cells. The RTLs are given as a percentage of the wild-type β-globin gene expression level.

REFERENCES

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