Transcriptional Effect of DEFB1 Gene 5′ Untranslated Region Polymorphisms

To the Editor:

Human β-defensin-1 (hBD-1) has been shown to be a candidate tumor suppressor gene by Sun et al. (1) who reported that single nucleotide polymorphisms (SNP) in the *DEFB1* gene are able to modify the transcriptional activity of hBD-1 promoter. In particular, the *−44C/G* SNP was described to enhance transcription up to 2.3 times more than the wild-type sequence in DU145 or TSU-Pr1 cell lines.

Recently, we showed that significant correlation exists between SNPs in the 5′ untranslated region (UTR) of the *DEFB1* gene and the risk of being infected with HIV-1 in Italian and Brazilian children (2, 3).

With the aim of verifying an eventual transcriptional functional effect of the SNPs in the 5′UTR region of *DEFB1* gene, we employed a dual luciferase assay, the same technique used by Sun et al. (1).

Starting from the wild-type *DEFB1* 5′UTR region, we generated three mutated inserts using primers designed to introduce the desired mutation into the wild-type sequence. The four different fragments (wild-type, *−52A*, *−44G*, and *−20A*) have been cloned into pGL3 promoter vector (Promega), and Caco2 cells were transfected for 48 h with 1 μg of the reporter plasmids and 20 ng of control *Renilla* luciferase expression plasmid (phRG-TK, Promega) using 5 μL of GenePORTER transfection reagent; a dual luciferase assay (Promega) was done according to the manufacturer's instruction. To ensure the reproducibility of the results, all tests have been done in quadruplicate and repeated at a later time to confirm the obtained results.

Our results show that the three SNPs do possess a functional activity, leading to an impaired production of the gene downstream to the mutated promoter.[1] Both the *−52A* and the *−20A* alleles cause a 25% mean reduction of expression. Although quantitatively relevant, this reduction was not statistically significant. The *−44G* mutation, contrary to the data presented by Sun et al. (1), drag to a markedly reduced expression of about 53% in our experimental setup.

Based on our results (2, 3) and because all the studied SNPs could hamper an effective production of hBD-1 *in vivo*, we hypothesized that they could be related to reduced response from the innate immune system.

Despite the fact that DU145, TSU-Pr1, and Caco2 are all carcinoma-derived cell lines, their inherent differences could lead to variations in dual luciferase assay results: we believe that the functional effect of the *DEFB1* 5′UTR SNPs is still controversial and deserves a more comprehensive study.

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[1] More detailed information is available at http://www.bbcm.univ.trieste.it/~bbcm/defb1funct/.

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