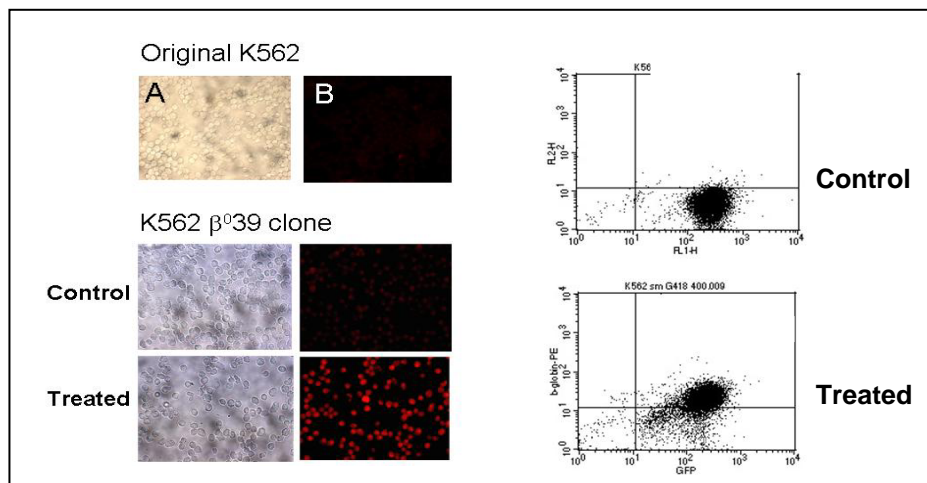


Cellular model to study β^{039} thalassemia

Prof. Roberto Gambari - University of Ferrara

The University of Ferrara developed a β^{039} thalassemia cellular model, using K562 cells, for the study of new potential therapeutic approaches for the treatment of the β^{039} thalassemia. These approaches include the use of aminoglycosides for the translational correction of nonsense mutations.

Immunohistochemistry and FACS analysis after treating the cell clone with aminoglycoside and labelling with antibody anti- β -globin



1. Description of the product

The cellular model was obtained by the transduction of K562 cells with a lentiviral vector (pCCL. β^{039} .PGW) carrying a GFP gene under the control of the PGK promoter and the β^{039} -globin gene under the control of the β -globin promoter.

The K562 cells do not express the β -globin gene both in their uninduced state as well as after erythroid differentiation stimulated by a variety of chemical inducers, so in the β^{039} clones the β -globin mRNA accumulated is almost only β^{039} , that cannot be translated to β -globin. After a potential correction of the nonsense mutation, the accumulation of the β -globin is detectable by labelling the cells with the PE-labeled β globin MoAb and analysing the labelled cells by FACS.

2. Innovative aspects of the product

Nonsense mutations promote premature translational termination and are the leading cause of up to 30% of inherited diseases, including β^{039} -thalassemia.

The development of experimental model systems useful for the screening of molecules facilitating translation is of great interest for the possible identification of drugs to be employed for the experimental therapy of diseases caused by stop codons. In the last years, in order to characterize potential translational correctors, many experimental systems were

produced, which are very useful to identify new compounds and to analyze their effects on the translation but are time-consuming, laborious and expensive.

The cellular model here proposed allows a simple and quite rapid assay to determine the read-through effect of lots of compounds on the β^{039} nonsense mutation.

The most innovative aspect of the model developed is the use of a lentiviral vector for the transduction of the K562 cells. This vector permits to obtain very stable clones, because of its ability to integrate into the cellular genome. Moreover, this vector contains two silencing sequences that reduce the effects both of the carried promoters on the surrounding chromatin and of the chromatin on the vector.

3. Main advantages of the offer

Nowadays, several therapeutic approaches for the β thalassemia are being developed. For this reason, the obtainment of proper cellular models is necessary to permit an easy and fast screening of lots of compounds and techniques.

Our cellular model is easy to use, because the culture of the K562 cells do not need particular attentions, and the effects obtained by the treatments are rapidly detected by FACS analysis.

Besides, this model is very versatile. First, it can be used not only for the screening of different translational correctors but also for the development of a gene correction strategy for the β thalassemia. Second, it can be useful for the study of a corrective therapy for other genetic diseases, subsequently a change in the gene carried by the vector and in the antibody used for the FACS analysis.

4. Technology keywords

Cellular model, β thalassemia, aminoglycosides, read-through, lentiviral vector.

5. Current stage of development

The cellular model is being tested with lots of compound for the read-through of nonsense mutations..

Technical and scientific publications

Development of K562 cell clones expressing β -globin mRNA carrying the β^{039} thalassemia mutation for the screening of correctors of stop codon mutations. Salvatori F, Cantale V, Breveglieri G, Zuccato C, Finotti A, Bianchi N, Canella A, Pinotti M, Borgatti M, Feriotto G, Destro F, Breda L, Rivella S, Gambari R. Biotechnology and applied biochemistry (2008). Submitted.

CONTACT

info@biopharmanet.eu

Tel.: +39 0521 905073 - Fax: +39 0521 905006