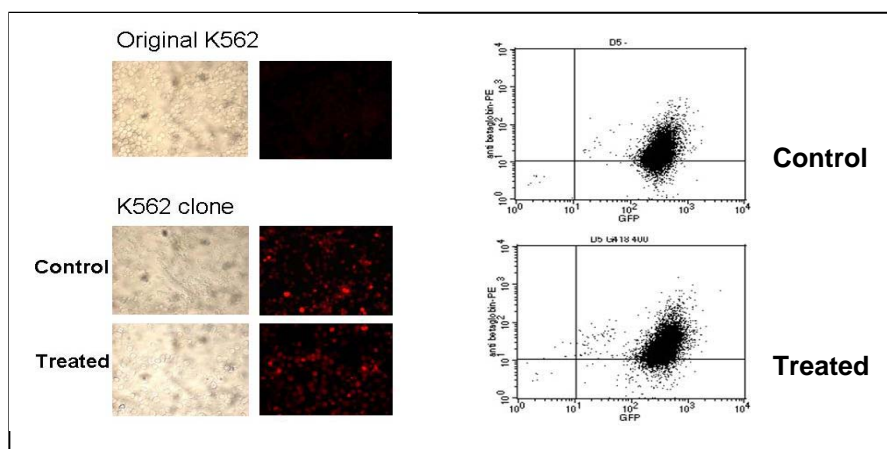


Cellular model to study β thalassemia

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The University of Ferrara developed a cellular model expressing normal β -globin for the study of new potential therapeutic approaches for the treatment of the β thalassemia. These approaches include the use of aminoglycosides for the translational correction of nonsense mutations, gene therapy, gene correction and induction of fetal and adult haemoglobin.

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 wt.PGW) carrying a GFP gene under the control of the PGK promoter and the β wt-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 wt clones the β -globin mRNA accumulated is produced by the transcription of the introduced β -globin genes. The accumulation of the β -globin mRNA is detectable by qRT-PCR while the accumulation of the protein is detectable by labeling the cells with the PE-labeled β globin MoAb and analysing the labeled cells by FACS.

2. Innovative aspects of the product

Nowadays, a corrective therapy for the β thalassemia is not available, but several therapeutic approaches are being developed. Accordingly, the development of experimental model systems useful for the screening of such approaches is of great interest for the possible identification of drugs or techniques to be employed for the experimental therapy of inherited diseases. At the same time, the production of cell lines expressing normal β -globin at different levels, being used as positive controls, is very important.

The cellular model here proposed allows a simple and quite rapid assay to determine the effect on the β -globin gene expression of lots of compounds, with a hypothetical HbA

induction activity, resulting in an increase of fluorescence released by the PE-labeled β globin MoAb, used for the labelling of the treated cells. 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 and positive control models is necessary to permit an easy and fast screening of lots of compounds and techniques.

The cellular model here proposed 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 as a positive control for therapeutic strategies for the β thalassemia but also for the characterization of potential HbA inducers. 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, wild type β -globin, HbA inducers, lentiviral vector.

5. Current stage of development

The cellular model is being tested as positive control and with lots of potential HbA inducers.

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, Feriotta G, Destro F, Breda L, Rivella S, Gambari R. Biotechnology and applied biochemistry (2008). Submitted.

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