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Selectable Marker Gene Bsrm Induces Death of Keratinocytes

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Gene transfer into cultured mammalian cells and the identification of transformed cells with a selectable marker are common steps in gene transfer and gene therapy protocols. The blasticidin S resistance gene (bsr), isolated from Bacillus cereus K55-S1 strain, is used as a dominant selectable marker in gene transfer to mammalian cells. Recently, we reported a construction of improved form of bsr (bsrm), which provided higher protection against BS in the cells transduced with that gene by retroviral vector. Keratinocytes are powerful target cells to ex vivo gene therapy protocols, although the selection process still needs to be improved due to the extreme sensitivity of these cells to drugs. Therefore, we decided to use the bsrm gene as a selectable marker to transfer therapeutic genes into the mouse keratinocytes BALB/MK cells. Surprisingly, the transduced cells died within 6 days of culture. Cell cycle analysis by flow cytometry on the 6th day demonstrated that the majority of cells were in the sub-G0 phase. A ring-shaped condensed chromatin was also observed upon staining of bsrm-transduced BALB/MK cells with HOECHST 33258 dye after 6 days of culture. Additionally, the caspases-3 was also activated on day 6. These findings indicate that BALB/MK transduced with bsrm gene died by apoptosis. Most importantly, whereas primary human keratinocytes also died in response to bsrm, rat vascular smooth muscle cells and NIH3T3 mouse fibroblasts were resistant to the same manipulation. Taken together, our results suggest that the toxic effect of bsrm is somehow specific for keratinocytes. Therefore, we proposed that even if the bsrm/BS selection system appears to be powerful to eliminate non-transduced cells efficiently, care should be taken to employ it in gene transfer experiments, at least the ones involving keratinocytes. Supported by FAPESP.

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