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Epigenetic control of gene expression in human mesenchymal stem cells during osteogenic differentiation

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Adult stem cells are widely used in cellular therapy not only because of their intrinsic potential but also because their use does not raise ethical issues. Dental pulp is an interesting source of postnatal progenitor cells/stem cells [1].

Stem cell lineage commitment and differentiation are regulated by epigenetic mechanisms. Epigenetic modification of DNA and DNA-associated histone proteins has been demonstrated to control and regulate the renewal and pluripotency of stem cell populations. The activities of the nuclear enzymes, histone deacetylases, are increasingly being recognized as potential targets for pharmacologically inducing stem cell differentiation[2; 3].

The aim of this study was to evaluate the osteogenic differentiation of dental pulp stem cells (DPSCs) treated with different histone deacetylase inhibitors (HDACi): Valproic acid (VPA), Entinostat (MS275), Trichostatin A (TSA) and Vorinostat (SAHA).

The effects of these inhibitors on cell proliferation, viability, bone-associated gene expression and matrix mineralization were determined.

VPA was found to be the drug that most induced osteogenic differentiation: at low concentration it was sufficient to significantly enhance matrix mineralization by increasing osteopontin and bone sialoprotein expression. In contrast, osteocalcin levels were decreased, an effect induced at the transcriptional level, and were strongly correlated with inhibition of HDAC2. In fact, HDAC2 silencing with shRNA produced a similar effect to that of VPA treatment on the expression of osteoblast-related markers.

Our in vitro data were confirmed by in vivo studies. H&E and Alizarin red stainings have highlighted a strong trend of VPA-treated cells to form a dense connective tissue. Within this tissue is visible a rather large portion that is even more dense and organized, highly colorable (very eosinophilic), similar to a bone ossification center. Immunofluorescence analysis to evaluate the expression of OPN and OC confirmed the results obtained in vitro and in vivo.

By means of RT-PCR, immunofluorescence and Western blotting, a series of biomarkers involved in the osteogenic pathway have been screened, identifying the glucocorticoid receptor (GCr), which is upregulated during the treatment with VPA and shHDAC2 cells, as the main responsible for the downregulation of osteocalcin.

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References

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Key words

Dental pulp stem cells, epigenetic, bone differentiation.