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Regular paper

Overexpression of *ID1* reverses the repression of human dental pulp stem cells differentiation induced by *TWIST1* silencing

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Multiple studies showed that the cessation of *TWIST1* expression is the prerequisite for osteoblasts' maturation. However, recent reports revealed that the function of TWIST1 is different in the dental pulp stem cells (DPSCs), where a high level of *TWIST1* expression promoted DPSCs' differentiation. The aim of the study was to investigate the impact of TWIST1 and ID1 on the differentiation process in the human DPSCs. Methods: *TWIST1* and *ID1* expression in the DSPCs was modulated by lentivirus transduction. Genes expression was assessed with qRT-PCR. The proteins level was evaluated by Western blot. The DPSCs differentiation was assessed with the proliferation, alkaline phosphatase (ALP) activity, and calcium concentration assays. Results: *TWIST1* silencing suppressed the expression of *ID1* and both the early and late markers of odontoblasts' differentiation detected at the transcript and protein level. The forced overexpression of *ID1* increased the expression of the late markers of odontoblasts differentiation but diminished the expression of the early markers. DPCSs with the silenced *TWIST1* and subsequent *ID1* overexpression displayed an increase in the expression of the late markers of odontoblasts differentiation. Cells with silenced *TWIST1* and overexpressing *ID1* had increased activity of ALP, higher calcium concentration and decreased proliferation rate. The high level of *ID1* expression might be a critical factor stimulating DPSCs differentiation and it might compensate the repressed differentiation of DPSCs caused by *TWIST1* silencing. Conclusion: The mutual correlation between the expression level of *TWIST1* and *ID1* might be a critical factor driving the process of the human odontoblasts' differentiation.

Key words: dental pulp stem cells, TWIST1, ID1, odontoblast differentiation, DSPP, DMP1

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Abbreviations: DPSC, dental pulp stem cell; TWST1, Twist Family BHLH Transcription Factor 1; ID1, Inhibitor of DNA binding 1

Table 1S Nucleotide sequences of human primers used for qRT-PCR.

Gene	Primer	Product size (bp)	Annealing temperature (^{0}C)	Accession number
ID1	CTGGACGAGCAGCAGGTAA CTCCAACTGAAGGTCCCTGA	156	60	NM_002165
TWIST1	AGTCCGCAGTCTTACGAGGA CCAGCTTGAGGGTCTGAATC	160	60	NM_000474
DSPP	GACCTCAAGTAGCTGGAAGCA TTGGGATCATCTCCTTGCAT	164	60	NM_014208
DMP1	CCTGAGGAGCGTATAGAAGGACCCA CCCTCCCTCGTTCTCCAACGC	166	60	NM_001079911
OCN	ATGAGAGCCCTCACACTCCT CCCAGCCATCATTGATACAGGTAG	230	60	NM_199173
OSF2	ATGGGAGGAGCAGTCTTTGA AGATCCGTGAAGGTGGTTTG	215	60	NM_00113594
GAPDH	GAGTCAACGGATTTGGTCGT GACAAGCTTCCCGTTCTCAG	185	60	NM_001256799
BSP	CTGAGCAAAATTAAAGCAGTCTTCA TGCCTTGAGCCTGCTTCCT	79	60	NM_004967
ON	TCTTCCCTGTACACTGGCAGTTC AGCTCGGTGTGGGAGAGGTA	73	60	NM_003118
ALP	ACTGGCGAGACCAAGCGCAA AGGCCTCAGGGGGCATCTCG	506	60	NM_004784
TSP1	GACCAAAGCCTGCAAGAAG TTGGACAGTCCTGCTTGTTG	85	60	NM_003246

Table 2S Antibodies used in Western blot.

Antibody	Vendor	Concentration used in Western blot
Goat polyclonal anti-ID1	R&D Systems, Abingdon, UK	1.0 μg/ml
Rabbit polyclonal anti-TWIST1	Abcam, Cambridge, UK	2.5 μg/ml
Rabbit polyclonal anti-DSP #LF-154	Dr. Larry Fisher (National Institutes of Health/National Institute of Dental and Craniofacial Research)	1:500
Mouse polyclonal anti-DMP1, #LF-148	Dr. Larry Fisher (National Institutes of Health/National Institute of Dental and Craniofacial Research)	1:500
Mouse monoclonal anti-OSF2	Sigma-Aldrich, Poznan, Poland	2.4 μg/m
Mouse monoclonal anti-OCN/BGLAP	Sigma-Aldrich, Poznan, Poland	1 μg/ml
Rabbit polyclonal anti-BSP	Abcam, Cambridge, UK	2.0μg/m
Mouse monoclonal anti-ON/SPARC	Abcam, Cambridge, UK	0.36 μg/m
Rabbit polyclonal anti-TSP1	Abcam, Cambridge, UK	1.0 μg/m
Mouse monoclonal anti-β-actin	Sigma-Aldrich, Poznan, Poland	0.8μg/m

TWIST1 (Twist basic helix-loop-helix transcription factor 1),ID1 (inhibitor of DNA binding 1), DSP (dentin sialoprotein), DMP1 (dentin matrix acidic phosphoprotein protein 1), BSP (bone sialoprotein), and OSF2 (periostin), ON/SPARC (osteonectin/secreted protein acidic and cysteine rich), TSP1 (thrombospondin 1), OCN/BGLAP (osteocalcin/bone gamma-carboxyglutamate protein).