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TUMORIGENESIS

USP1 keeps ID proteins stable

> USP1 stabilizes ID proteins in osteosarcoma.

Basic helix–loop–helix transcription factors, which are regulators of development and differentiation, can be suppressed by inhibitor of DNA binding (ID) proteins. ID proteins are abundant in embryonic and adult stem cells, and they are overexpressed in some dedifferentiated tumours. Of the four ID proteins, ID1–3 are subject to Lys48-linked ubiquitylation and degradation, a regulatory mechanism that might be aberrant in tumours. Williams *et al.* have now identified USP1 as a deubiquitylating enzyme (DUB) for ID1–3 that stabilizes these proteins and as a result promotes stem cell characteristics in both osteosarcoma cells and mesenchymal stem cells (MSCs).


CORBIS

The authors overexpressed 94 human DUBs in cells and monitored ID2 protein levels. Only USP1 increased the endogenous levels of ID2 and interacted with, and had deubiquitylating activity against, this protein. Furthermore, USP1, but not a catalytically inactive USP1, reduced ID2 ubiquitylation *in vitro* and *in vivo*. Thus, USP1 is a DUB for ID2 and, as indicated by a subset of the above experiments, also for ID1 and ID3.

On carrying out microarray analysis of healthy and diseased tissue, the authors observed that USP1 mRNA and protein levels were increased in a subset of human osteosarcoma biopsy samples, and that this correlated with increased ID2 protein levels. USP1 and ID2 levels were also increased in human osteosarcoma cell lines and primary osteoblasts; short hairpin RNA (shRNA)-mediated knockdown of *USP1* caused a reduction in ID1–3 levels in these cells. So, USP1 stabilizes ID proteins in osteosarcoma.

Osteosarcomas, which might develop from MSCs, consist of all three MSC lineages (that is, osteoblasts, chondrocytes and adipocytes) and express MSC markers rather than osteoblast markers. As ID proteins have been implicated in stem cell maintenance, the authors sought to determine whether their depletion promoted osteoblast differentiation. Indeed, expression of *USP1* or ID shRNAs in osteosarcoma cells decreased the expression of MSC markers and increased the expression of osteoblast markers. Thus, osteogenic differentiation in osteosarcomas is blocked by a USP1-mediated increase in ID proteins. Furthermore, USP1 overexpression in normal human MSCs increased ID protein levels and cell proliferation, and also reduced the expression of osteoblast markers. Therefore, an increase in USP1 levels, and thus ID levels, also prevents the differentiation of normal MSCs, thereby favouring stem cell maintenance.

Finally, the authors examined the effect of altered USP1 expression in animal models. The implantation of NIH3T3 cells overexpressing USP1 or ID2 into mice resulted in the development of tumours; *in vitro* assays confirmed that IDs are essential for the ability of USP1 to transform these cells. So, this study identifies USP1 as a DUB for ID1–3, and shows that it stabilizes these proteins, resulting in the inhibition of differentiation in both MSCs and osteosarcoma cells.

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ORIGINAL RESEARCH PAPER Williams, S. A. *et al.* USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma. *Cell* **146**, 918–930 (2011)