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Commentary

Verification of Pluripotency Marker Expression in iPS-OKMS and iPS-OKMSC Cells

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DESCRIPTION

Induced Pluripotent Stem Cells (iPSCs) have revolutionized regenerative medicine and stem cell research due to their ability to differentiate into various cell types. A critical aspect of utilizing iPSCs involves verifying the expression of stem cell-specific genes to confirm their pluripotency. This article focuses on the verification of these gene expression levels in two specific types of iPSCs: iPS-OKMS (iPSCs derived with *OCT4*, *KLF4*, *MYC*, and *SOX2*) and iPS-OKMSC (iPSCs derived with *OCT4*, *KLF4*, *MYC*, *SOX2*, and C-MYC).

iPSCs are generated by reprogramming somatic cells through the introduction of specific transcription factors. This process induces a pluripotent state, allowing the cells to give rise to various cell types, similar to Embryonic Stem Cells (ESCs). The transcription factors typically used include *OCT4*, *SOX2*, *KLF4*, and MYC, collectively known as OSKM. Additional factors, such as C-MYC, can enhance reprogramming efficiency. Ensuring that reprogrammed cells express stem cell-specific genes is essential for confirming their pluripotency and potential for differentiation.

Methodology for gene expression verification

To verify the expression of stem cell-specific genes in iPS-OKMS and iPS-OKMSC cells, several techniques are employed. These include quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), immunocytochemistry, and Western blot analysis. Each method provides complementary data to confirm the pluripotent status of the iPSCs.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR): qRT-PCR is a potential technique used to quantify gene expression levels. For this study, total RNA is extracted from the iPS-OKMS and iPS-OKMSC cells, and reverse transcribed into cDNA. Specific primers for pluripotency markers such as *OCT4*, *SOX2*, *NANOG*, and others are designed. The expression levels of these genes are then quantified and compared to those in ESCs and somatic cells (as controls).

The relative expression levels of *OCT4*, *SOX2*, and *NANOG* in iPS-OKMS and iPS-OKMSC cells are expected to be similar to those in ESCs, indicating successful reprogramming and maintenance of pluripotency. The absence or significantly lower expression of these markers in somatic cells serves as a negative control.

Immunocytochemistry: Immunocytochemistry allows the visualization of protein expression within cells. In this method, iPS-OKMS and iPS-OKMSC cells are fixed and stained with antibodies specific to pluripotency markers such as *OCT4*, *SOX2*, and *NANOG*. Fluorescently labeled secondary antibodies are used to detect these primary antibodies, and the cells are imaged using fluorescence microscopy.

The presence of strong fluorescence signals for *OCT4*, *SOX2*, and *NANOG* in the nuclei of iPS-OKMS and iPS-OKMSC cells confirms the expression of these proteins. This technique provides spatial information about protein localization, which is important for understanding the functional status of these cells.

Western blot analysis: Western blot analysis is employed to quantify protein expression levels. In this method, protein extracts from iPS-OKMS, iPS-OKMSC, ESCs, and somatic cells are separated by gel electrophoresis and transferred to a membrane. The membrane is probed with antibodies specific to *OCT4*, *SOX2*, and *NANOG*, and the bound antibodies are detected using chemiluminescence.

The intensity of the bands corresponding to these pluripotency markers in iPS-OKMS and iPS-OKMSC cells is compared to those in ESCs and somatic cells. High expression levels in iPS-OKMS and iPS-OKMSC cells, similar to ESCs, confirm the pluripotent status of the reprogrammed cells.

The verification of stem cell-specific gene expression levels in iPS-OKMS and iPS-OKMSC cells demonstrates that both cell types express main pluripotency markers, similar to ESCs. qRT-

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PCR results show high relative expression levels of *OCT4*, *SOX2*, and *NANOG*, indicating successful reprogramming. Immunocytochemistry reveals strong nuclear localization of these proteins, further supporting their functional role in maintaining pluripotency. Western blot analysis corroborates these findings by showing high protein expression levels in iPS-OKMS and iPS-OKMSC cells.

These results collectively confirm that iPS-OKMS and iPS-OKMSC cells have successfully acquired and maintain a pluripotent state, similar to ESCs. The use of multiple complementary techniques ensures the reliability of these findings and establishes the importance of verifying gene expression levels when working with iPSCs.

Verification of stem cell-specific gene expression levels is important for confirming the pluripotency of iPSCs. The methodologies employed qRT-PCR, immunocytochemistry, and Western blot analysis provide comprehensive data confirming that iPS-OKMS and iPS-OKMSC cells express main pluripotency markers at levels comparable to ESCs. These findings support the use of these iPSC lines in various applications, including disease modeling, drug screening, and regenerative medicine. Ensuring the accurate verification of pluripotency markers is essential for the reliable use of iPSCs in research and clinical settings.