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Opinion Article

Knockdown of *IRX* Downregulates Cell Cycle Genes in Human Keratinocytes

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DESCRIPTION

The complex regulation of the cell cycle is important for maintaining healthy cellular functions and tissue homeostasis. In human keratinocytes, the primary cell type in the epidermis, this regulation is particularly important for skin health and repair. Recent research has identified the Iroquois homeobox (*IRX*) genes as lead in various developmental processes, including the regulation of the cell cycle. This article explores the impact of *IRX* knockdown on cell cycle genes in human keratinocytes, and explain its potential implications for skin biology and disease.

The role of *IRX* genes in cellular function

IRX genes encode transcription factors that are involved in the regulation of multiple developmental pathways. They play critical roles in pattern formation during embryogenesis and have been implicated in the differentiation and proliferation of various cell types. In keratinocytes, *IRX* genes are thought to influence cell cycle progression, although the precise mechanisms have not been fully elucidated.

To investigate the effects of *IRX* knockdown on cell cycle gene expression in human keratinocytes, researchers employed small interfering RNA (siRNA) technology. This approach allows for the specific and efficient silencing of target genes. Human keratinocyte cell lines were transfected with siRNAs designed to knock down *IRX* expression. The efficiency of knockdown was confirmed via quantitative PCR and Western blot analysis.

Subsequently, the expression levels of a panel of cell cycle-related genes were analyzed using RNA sequencing (RNA-seq) and real-time PCR. Fundamental markers of cell cycle phases, including cyclins, Cyclin-Dependent Kinases (CDKs), and their inhibitors, were examined to understand the broader impact of IRX silencing.

The knockdown of *IRX* genes in human keratinocytes resulted in a significant downregulation of several critical cell cycle genes. Notably, the expression levels of Cyclin D1 (*CCND1*), Cyclin E (*CCNE1*), and Cyclin A2 (*CCNA2*) were markedly reduced. These cyclins are essential for the progression through the G1/S and G2/M phases of the cell cycle.

Additionally, the expression of *CDK2* and *CDK4*, which are important for cell cycle transition, was also diminished. The reduction in these genes suggests that *IRX* knockdown impedes the progression of keratinocytes through the cell cycle, potentially leading to cell cycle arrest.

Furthermore, the study observed an increase in the expression of CDK inhibitors such as p21 (*CDKN1A*) and p27 (*CDKN1B*). These inhibitors play a role in halting cell cycle progression, further supporting the notion that *IRX* knockdown promotes a cell cycle arrest phenotype.

The findings from this study indicate that *IRX* genes are pivotal regulators of cell cycle progression in human keratinocytes. The downregulation of essential cell cycle genes following *IRX* knockdown suggests that IRX proteins facilitate the normal progression of keratinocytes through the cell cycle.

The observed increase in CDK inhibitors p21 and p27 further establishes the potential of *IRX* knockdown to induce cell cycle arrest. This effect could be leveraged for therapeutic purposes, particularly in hyperproliferative skin disorders such as psoriasis and certain types of skin cancer, where controlling abnormal cell proliferation is important.

Moreover, understanding the molecular pathways influenced by *IRX* genes could provide insights into skin development and regeneration. The ability to manipulate these pathways might offer novel strategies for enhancing wound healing and treating skin injuries.

The knockdown of *IRX* genes in human keratinocytes leads to a significant downregulation of main cell cycle genes, highlighting the critical role of *IRX* in the regulation of cell cycle progression. This research advances our understanding of the molecular mechanisms controlling keratinocyte proliferation and offers potential methods for therapeutic intervention in skin disorders characterized by abnormal cell proliferation. Future studies

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focusing on the detailed mechanisms of *IRX*-mediated regulation could prepare for innovative treatments aimed at maintaining skin health and promoting regeneration.