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Unleashing the Potential of the Human TFome: A Comprehensive Library Revolutionizing Stem Cell Differentiation and Tissue Engineering

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The Human TFome, an extensive library comprising 1,564 transcription factor genes and 1,732 transcription factor splice isoforms. This resource serves as a gateway to systematically explore the realm of transcription factor-mediated programming in human pluripotent stem cells (hPSCs). By screening this comprehensive library across three distinct hPSC lines, the research team unveiled a total of 290 transcription factors, a noteworthy 241 of which had not been previously reported. These transcription factors exhibited the remarkable ability to induce differentiation in just four days, all without the necessity for external cues. Further delving into their capabilities, four of these transcription factors were singled out and subjected to comprehensive study, successfully orchestrating the transformation of hPSCs into neurons, fibroblasts, vascular endothelial-like cells, and oligodendrocytes. Impressively, these cells closely mimicked the properties of their primary counterparts. This study doesn't stop at singular cell type conversions; it also pioneers the concept of parallel programming for hPSCs, enabling the simultaneous generation of multiple cell types within the same culture environment. Moreover, it introduces the notion of orthogonal programming for expediting myelination in cerebral organoids.

This study is, at its core, about the creation of the Human TFome, a library with immense potential in the realm of cell programming. This library is the cornerstone of the research, designed to facilitate a systematic exploration of transcription factor-driven differentiation in human induced pluripotent stem cells (hiPSCs).

During the development of this library, a significant challenge emerged—the constrained expression of transcription factors in the source materials. This roadblock was surmounted by synthesizing the missing open reading frames (ORFs) for 273 transcription factors that were notably absent in existing extensive ORF collections. Merging these synthesized ORFs with the existing transcription factor genes was the key to the successful creation of the Human TFome.

Once the Human TFome library was firmly established, the focus shifted to its practical implementation in hiPSCs. This integration was achieved through the utilization of an all-in-one doxycycline-inducible, puromycin-selectable lentiviral vector. This vector offered precise control over transcription factor expression. Critically, the research team meticulously ensured that each cell received a maximum of just one transcription factor, maintaining a high level of precision in the experimental setup.

With the library in hand, the next step was to introduce it into three different hiPSC lines: PGP1, CRTD5, and ATCC-DYS0100. The selection of these specific hiPSC lines was

guided by their inherent similarities, and the fact that they had been reprogrammed from male fibroblasts using Sendai virus, which helped minimize experimental variability. Importantly, a substantial level of transcription factor coverage was achieved across all three hiPSC lines, providing concrete evidence of the successful integration of the Human TFome library.

This section of the study places significant emphasis on the importance of the Human TFome library in the context of the research. The library serves as the fundamental building block for the subsequent screening and identification of transcription factors capable of instigating hiPSC differentiation. It is this library that enables a comprehensive and systematic exploration of how transcription factors influence cell fate and differentiation in hiPSCs.

Moving on to the exciting aspect of the research, the authors ventured into the realm of transcription factor-driven differentiation in human induced pluripotent stem cells (hiPSCs). Their efforts resulted in the successful induction of differentiation using a remarkable 290 transcription factors, with 65 of these transcription factors inducing differentiation across all three hiPSC lines. To validate their discoveries, the authors conducted in-depth studies on 16 top transcription factors, confirming their potent differentiation-inducing capabilities.

Notable highlights include the identification of ATOH1 as a powerful transcription factor for programming hiPSCs into neurons, effectively achieving rapid neuronal differentiation without the need for specialized culture conditions. These induced neurons displayed functional properties. Additionally, the authors uncovered the efficiency of NKX3-1 in driving hiPSC differentiation into fibroblasts, with these fibroblasts mirroring the characteristics and functionality of primary fibroblasts. Further exploration led to the discovery that ETV2 isoform 2 was highly effective in generating vascular endothelial-like cells, exhibiting remarkable angiogenic potential both in vitro and in vivo. These findings underscore the versatility of transcription factors in steering hiPSC differentiation into specific lineages, offering immense promise in the domains of regenerative medicine and tissue engineering.

This study doesn't limit itself to the transformation of singular cell types; it pioneers the concept of parallel programming. This revolutionary approach enables the simultaneous generation of multiple cell lineages within a single culture environment. To realize this, inducible hiPSCs, capable of differentiating into neurons, fibroblasts, and vascular endothelial cells, were combined in pairs with identical ratios and subsequently induced with the corresponding transcription factors over a span of four days. Co-cultures exhibiting the expression of specific markers for neurons, fibroblasts, and vascular endothelial cells. These findings offer clear evidence of the success of parallel differentiation. Furthermore, when all three cell types were combined in a single culture, they demonstrated the presence of markers for all three lineages. Flow cytometry and single-cell RNA sequencing further validated the existence of distinct populations, each bearing cell-type-specific transcriptomic signatures. Three-dimensional cell culture

experiments added further support to the feasibility of parallel programming, culminating in the generation of spheroids containing all three lineages. These outcomes hint at the exciting potential of differentiating multiple cell lineages in parallel, within the same culture, all without the need for additional soluble factors. This groundbreaking development holds significant promise for the realms of tissue engineering and the construction of complex tissues.

Shifting focus, the study turns its attention to the differentiation of hiPSCs into induced oligodendrocytes. This endeavor was driven by the need to overcome the inherent slow development of certain cell types, particularly mature myelin, in organoid technology, which typically spans over 103-210 days. Armed with the Human TFome library, the research team embarked on this challenge and quickly identified SOX9 as the top performer among 15 transcription factors associated with oligodendrocyte differentiation. Inducing the expression of SOX9 yielded compelling results, as hiPSCs were transformed into oligodendrocyte progenitors within a mere four days. These induced oligodendrocyte progenitors displayed characteristics closely resembling primary oligodendrocytes. What's more, these SOX9-induced oligodendrocytes exhibited promising myelination potential both in in vitro co-culture with neurons and in vivo transplantation into a hypomyelinated mouse model.

Venturing further, the authors introduced the concept of orthogonal cell programming, a groundbreaking approach designed to expedite myelination in cerebral organoids. To put this into practice, inducible SOX9 hiPSCs were combined with unmodified hiPSCs in the formation of cerebral organoids. Following the induction of SOX9 expression, the research team witnessed a rapid surge in myelin formation within the orthogonally programmed organoids. Notably, this included the presence of myelin oligodendrocyte glycoprotein (MOG) and G-ratios akin to those found in healthy human brains. This development opens up a promising avenue for enhancing the development of human brain tissue models.

In conclusion, this study carries significant weight due to its introduction of the Human TFome library and its impactful applications in the world of transcription factor-mediated cell programming. The authors emphasize the potential of this library in conjunction with other methods for cell and tissue engineering. They see opportunities for further refinement through the utilization of computational approaches such as CellNet. Notably, orthogonal programming represents an innovative approach to accelerate myelination in cerebral organoids, slashing the timeline substantially. This novel approach holds the potential to foster the development of more physiologically relevant tissue models, offering exciting prospects for both tissue engineering and genomics. The concept of the Human TFome library 'writing' cellular programs complements the notion of cell atlases that 'read' cell types and states. Together, they enable cross-pollination between the fields of tissue engineering and genomics, facilitating the discovery of cellular recipes for various cell types and states throughout development and aging.

References

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