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Research Article

Kruppel Like Factor 4(Klf-4), A Key Regulator of Induced Pluripotent Stem Cells (iPS) and its Function at Developmental Role

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Abstract

Pluripotency is a very important concept in stem cell therapy to regenerate damaged or required tissues and organs. Specific differentiation cells do not have differentiation potential. But in 2006, Yamanaka created the concept of inducible pluripotent cells (iPS). It is a fully differentiated cell that can be reprogrammed into its original pluripotent cell, which contains differentiation into different types of cells. This phenomenon can be triggered by four factors: Oct-4, c-Myc, Sox-2 and Klf-4. However, the structure of a factor such as Kruppel (Klf-4) is very similar to that of Kruppel. This interacts with the GC rich sequence of the major orchards of cells into the zinc finger binding domain. Unlike other E-KLF proteins, this KLF inhibits the differentiation process. Some studies show that naive splenic B cells express high levels of the transcription factors KLF4 and KLF9. Interestingly, molecular associations between Wnt/beta-catenin signaling and the expression of the telomerase subunit Tert have recently emerged. Beta-catenin also regulates Tert expression through interaction with Klf4, a key component of a versatile transcriptional network. These studies show that Klf-4 can participate in the developmental stage in the actual role of multipotentialities. This suggests that inhibition of Klf-4 may be important in attenuating telomerase mutant cancer in metabolites.

Keywords: Kruppel; Klf-4; Stem Cells

Introduction

Induced pluripotent stem cells are derived from mouse fibroblasts by the introduction of four transcription factors, Octa/4, Sox2, c-Myc and Klf4, and subsequent selection for Fbx15 expression [1]. Can be. These iPS cells (hereinafter referred to as Fbx15 iPS cells) are similar to ES cells in their morphology, proliferation and teratoma formation. However, they differ in terms of gene expression and DNA methylation patterns, and do not produce adult chimeras. Okita., *et al.* [2] showed that selection for Nanog expression induced germ cell competent iPS cells with increased ES cell-like gene expression and DNA methylation pattern compared to Fbx15 iPS cells. Four transgene genes were strongly silenced in Nanog iPS cells. Okita., *et al.* [2] obtained adult chimeras from seven Nanog iPS cell clones, and one clone was transmitted to the next generation through the reproductive system. Approximately 20% of offspring developed tumors due to reactivation of the c-Myc transgene. Okita., *et al.* [2] concluded that iPS cells suitable for gonadal chimeras could be obtained from fibroblasts but concluded that retroviral introduction of c-Myc should be avoided clinically.

Wernig., *et al.* [3] independently demonstrated that transcription factors Oct4, Sox2, c-Myc, and Klf4 could reprogram somatic genomes into embryonic pluripotency states. Using microarray analysis, Good and Tangye [4] showed that naive splenic B cells express more of the transcription factors KLF4, KLF9 and PZLF compared to memory B cells. The age-old condition of these cells is related to the pluripotency associated with the state of embryonic stem cells. Activation of purified B cells through CD40 and B cell receptors decreased expression of these cytostatic-related transcription factors. Overexpression of KLF4, KLF9 and PZLF in memory B cells delayed entry into cell division and proliferation. Good and Tangye [4] showed that memory B cells undergo regrowth and significantly decrease the activation threshold compared to pure B cells, resulting in faster onset of division and differentiation into Ig-secreting plasma cells and more rapid cleavage I conclude that I can produce antibodies.

All KLF family members feature three Cys2 His2 zinc fingers located at the C-terminus separated by a highly conserved H/C link. DNA binding studies have proven that KLF has a similar affinity for sites with different GC-rich or CACCC homologies to

each other and can compete against each other for occupations in those areas (figure 2). KLFs also share high homology between the specific protein families of zinc-finger transcription factors and bind similarly, although not in similar regions in many genes.

In this study, we will characterize factors such as Kruppel in terms of universality. Factors such as Kruppel have regions similar to the Drosophila melanogaster Kruppel gene. The Kruppel gene is well known as an important factor in fruitfly development.

Results

Identification of Klf4 structure as a transcription factor

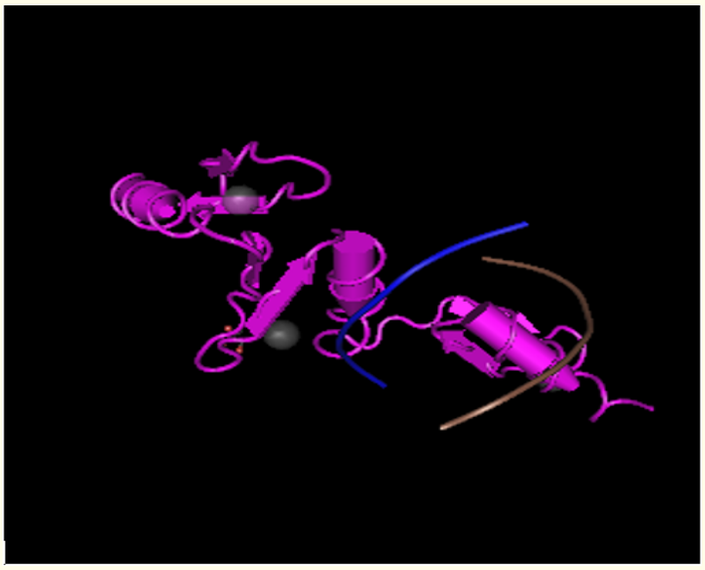

Figure 1: Klf-4 bound to DNA. In contrast to other members of the EKLF family, which are transcriptional activators, KLF4 functioned as a transcriptional repressor in transient transfection studies. The authors identified both the repression domain and the activation domain within KLF4. **

****Source from**

Madej T, Addess KJ, Fong JH, Geer LY, Geer RC, Lanczycki CJ, Liu C, Lu S, Marchler-Bauer A, Panchenko AR, Chen J, Thiessen PA, Wang Y, Zhang D, Bryant SH. "MMDB: 3D structures and macromolecular interactions." Nucleic Acids Res. 2012 Jan; 40(Database issue): D461-4.

Protein Nucleotide Chemical

Molecules and interactions

Label	Count	Molecule	Interactions
Protein and interactions (1 molecule)			
A	1	Krueppel-like Factor 4 zf-H2C2_2 superf COG5048 Show annotation ▼	5'-d(*gp*ap*gp*gp*cp*gp*cp)-3' 5'-d(*gp*cp*gp*cp*cp*tp*cp)-3' Glycerol Zinc Ion
Nucleotides and interactions (2 molecules)			
F	1	5'-d(*gp*ap*gp*gp*cp*gp*cp)-3'	5'-d(*gp*cp*gp*cp*cp*tp*cp)-3' Krueppel-like Factor 4
G	1	5'-d(*gp*cp*gp*cp*cp*tp*cp)-3'	5'-d(*gp*ap*gp*gp*cp*gp*cp)-3' Krueppel-like Factor 4
Chemicals and interactions (4 molecules)			
1	3	Zn^{++} (Zinc Ion)	Krueppel-like Factor 4
	1	Glycerol	Krueppel-like Factor 4

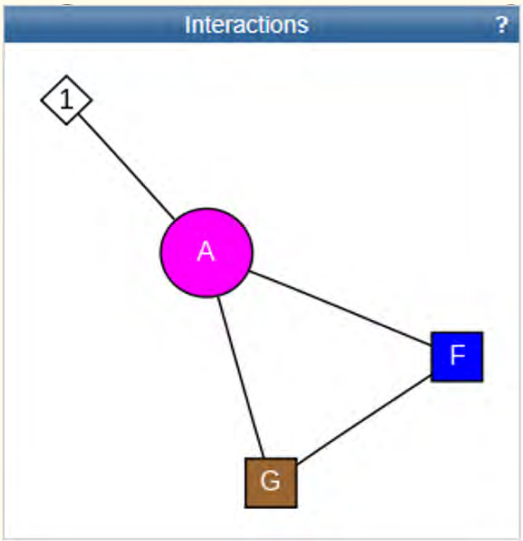

Figure 2: Klf Binding domains and interaction. Klf4 has a zinc finger binding motif-like activity as a transcription factor. It has a lot of similar homological properties with Kruppel, a gene that is used in embryoninc development.

Source from Pubmed

**The major difference is the following: In contrast to other members of the E-KLF family, which are transcriptional activators, KLF4 functioned as a transcriptional 'repressor' in transient transfection studies.

Discussion

Stem cells can be reprogrammed and induced by a variety of factors. Using Oct4, Sox2, Klf4, and Myc (Park., *et al*. [5]), iPS cells are derived from fetal, neonatal and adult human primary cells, including skin fibroblasts isolated from skin biopsies of healthy subjects. Human iPS cells are similar to embryonic stem cells in their ability to form teratomas in morphology and gene expression and immunodeficient mice. Defined factors can multiply reprogram human cells and establish how patient specific cells can be established in culture.

Kim., *et al.* [6] reported that adult mouse neural stem cells exhibit higher endogenous levels of Sox2 and c-Myc than embryonic stem cells and that exogenous Oct4 together with Klf4 or c-Myc produces pluripotent stem (iPS) cells derived from neural stem. It was shown to be sufficient for the cell. These two-factor iPS cells are similar to embryonic stem cells at the molecular level, contribute to the development of the germline, and form chimeras. Kim., *et al.* [6] suggested that the number of reprogramming factors can be reduced when using somatic cells that endogenously express an adequate level of supplementation in induction of pluripotency. Generation of mouse iPS cells is reported independently without viral vectors. Repeated transfection of mouse embryo fibroblasts with two expression plasmids containing the Oct3/4, Sox2 and Klf4 cDNAs and containing c-Myc cDNA resulted in iPS cells without evidence of plasmid integration, [2]. Production of virus-free iPS cells begins with embryonic fibroblasts but addresses important safety concerns for the potential use of iPS cells in regenerative medicine.

Some studies have shown that the two LIF signaling pathways are connected to the core circuitry necessary to maintain pluripotency through different transcription factors. In mouse embryonic stem cells, Klf4 is mainly activated by the Jak-Stat3 pathway and preferentially activates Sox2 while Tbx3 is first regulated by the phosphatidylinositol-3-OH kinase-Akt and mitogen-activated protein kinase pathways, Artificial expression of Klf4 or Tbx3 was sufficient to maintain pluripotency while maintaining the expression of Oct 3/4. In particular, the overexpression of Nanog supported the regeneration of the fiber-free factor of mouse embryonic stem cells in the absence of Klf4 and Tbx3 activity. Thus, we conclude that KLF4 and TBX3 are involved in delivering the LIF signal to the central nervous system but conclude that embryonic stem cells are not directly involved in maintenance of pluripotency because they remain pluripotent without expression in certain situations [7].

In addition to the pluripotency reducing nature of Klf-4, it is associated with development. The genes were regulated in retinal ganglion cells (RGCs), and KLF4 was identified as a transcriptional repressor of axon progenitors in RGCs and other central nervous system (CNS) neurons. KLF4 deficient RGCs showed increased axonal growth after *in vitro* and *in vivo* optic nerve damage. Related KLF family members have suppressed or improved axon growth to some extent and some growth inhibitory KLFs have increased postnatally while growth promoting KLFs have decreased. Simultaneous expression of KLF4 or KLF9 blocked the growth promoting effect of KLF6 or KLF7 *in vitro*. Thus, coordinated activities of different KLFs regulate the regenerative capacity of CNS neurons [8].

Using bone marrow chimeric Klf -/- mice and flow cytometry analysis, it was found that Klf4 was used for Th17 cells and IL17 production [9]. This implies that iPS and development can be connected at specific mechanisms. Th 17 cells are completely

differentiated, but the development of these cells may require a common process based on embryonic bases. They performed a chromatographic immunoprecipitation analysis and showed that Klf4 binds to the Il17 promoter. Wildtype and Klf4 -/- T cells similarly elevated Rorgt expression during Th17 polarization, suggesting that the two transcription factors are independently regulated. However, transfer of T cells from Klf4 -/- mice did not result in the development of experimental autoimmune encephalomyelitis. Lebson., *et al.* [9] concluded that KLF4 plays a role in Th17 cell differentiation.

Hoffmeyer., *et al*. (2012) showed that beta-catenin regulates Tert expression through interaction with Klf4, a key component of a versatile transcriptional network. This is quite surprising in the fact that development and iPS have a special linkage to control the overall pluripotentcy in a particular organ. Beta-catenin binds to the Tert promoter in murine intestinal tumor models and human carcinoma cells. Hoffmeyer., *et al.* (2012) identified an unknown association between stem cell and tumorigenicity by controlling beta-catenin expression in Tert, thereby identifying telomere lengths that may be important in human regenerative therapy and cancer.

Finally, there was a report discussing the direct role of Klf-4 in the development mechanism. Hoffmeyer., *et al.* (2012) reported a molecular association between the expression of Wnt / beta-catenin signaling and the telomerase subunit Tert. Beta-catenin deficient mouse embryonic stem (ES) cells have short telomere. Conversely, ES cells expressing an activated form of beta-catenin (beta-catenin (delta Ex3/+)) have long telomeres.

This indicates that organ designation of apoptosis can be achieved through the regulation of beta-catenin through the regulation of telomere (Hanna., *et al.* (2009)). Reprogramming with Oct4, Sox2, Klf4 and Myc transcription factors demonstrates that almost all mouse donor cells continue to be a continuous stochastic process that causes iPS cells to express transcription factors. Further inhibition of the p53 p21 pathway or overexpression of Lin28 increased the rate of cell division and accelerated iPS cell formation. This is directly proportional to the increase in cell proliferation. Quantitative analysis defined clear cell fraction-dependent and non-independent types to accelerate confirmatory reprogramming processes and suggested that the number of cell divisions is a key parameter to induce progeny genetic reprogramming into pluripotency.

Materials and Methods

Generation of Induced Pluripotent Stem Cells

Induced pluripotent stem cells were first produced by the Shinya Yamanaka team at Kyoto University in Japan in 2006. Yamanaka used genes that were found to be particularly important in embryonic stem cells (ESCs) and used retroviruses to deliver the selection of these genes to mouse fibroblasts. Eventually, four key multipotent genes essential for the production of pluripotent stem cells were isolated. Oct-3/4, SOX2, c-Myc and Klf4. Cells were isolated by antibiotic selection of Fbx15 + cells. However, this iPS cell line showed DNA methylation errors compared to the original pattern of the ESC line, and injection into a developing embryo did not produce a viable chimera.

Identification and usage of Klf4

Klf4 of the Klf gene family is described by Yamanaka., *et al.* Jaenisch., *et al*. As a production factor of mouse iPS cells, Yamanaka., *et al.* As a factor of production of human iPS cells, however, Thomson., *et al*. Reported that Klf4 is unnecessary for the production of human iPS cells and does not actually produce iPS cells. Klf2 and Klf4 were found to be the factors capable of producing iPS cells and the related genes Klf1 and Klf5 were similarly low in efficiency.

Conclusion

In conclusion additional research, including the structure and its binding mechanisms, should be ensured to conclude the exact link between reverse programming and forward development because of its relevance to developmental studies and the multifunctional activities of Klf-4. IPS combined with normal phase cell lines will be a great tool for revealing this mechanism.

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