

# Expression of BMP4 mature peptide in eukaryotic cells and its differentiation-inhibiting effect in culturing induced pluripotent stem cells([点击查看pdf全文](#))

《南方医科大学学报》[ISSN:/CN:] 期数: 2012年10期 页码: 1383 栏目: 出版日期: 2012-10-01

Title: 真核表达BMP4成熟肽及其对iPS细胞的分化抑制作用

作者: [丁道芳](#); [王臻](#); [徐浩](#); [徐乐勤](#); [梁倩倩](#); [赵永见](#); [施杞](#); [王拥军](#)

1上海中医药大学龙华医院脊柱病研究所, 上海200032; 2复旦大学肿瘤医院中心实验室, 上海200433

Author(s): -

关键词: [Igk-BMP4](#); [免疫球蛋白K链](#); [过表达](#); [诱导型干细胞](#); [细胞分化](#)

Keywords: [bone morphogenetic protein 4](#); [immunoglobulin kappa chains](#); [over-expression](#); [induced pluripotent stem cells](#); [cell differentiation](#)

分类号: -

DOI: -

文献标识码: -

摘要: 目的研究BMP4因子在诱导型干细胞(iPS细胞)培养过程中的作用和起作用的相关通路。方法通过RT-PCR的方法从小鼠的胎盘组织中扩增BMP4成熟肽, 并且在其N末端连接IgK分泌肽, 此融合片段克隆至pPYCAG载体上。重组质粒pPYCAG-IgK-BMP4转染至293T17细胞中并进行嘌呤霉素的筛选。通过细胞免疫荧光和Western blot方法鉴定出表达BMP4的阳性克隆。为进一步检测上清中BMP4的生物活性, iPS细胞培养于含BMP4因子的细胞培养上清和LIF因子中, 连续培养3代, 并进一步观察细胞表型、三胚层细胞的分化潜能和多潜能相关基因的表达水平。结果Smad1被分泌至上清中的BMP4磷酸化。当培养基中含BMP4同时加入LIF因子后, 可以有效抑制iPS细胞分化。在此种方法培养3代后, 不仅iPS细胞的表型可以有效维持, iPS细胞中多潜能相关的基因表达水平也未受到影响, 同时这些iPS细胞仍具有向三个胚层分化的能力。结论BMP4可高效表达在真核细胞中, 有效地使培养iPS细胞保持其多向分化潜能。

Abstract: Objective To investigate the role of bone morphogenetic protein4(BMP4) in culturing induced pluripotent stem cells (iPSCs) and the related signal pathways.MethodsWe amplified the mature peptide of BMP4from the placenta through RT-PCR, and IgK secretion peptide was ligated to the N-terminal of BMP4 mature peptide. The recombinant plasmid pPYCAG-IgK-BMP4was transfected into293T cells and screened with puromycin, and the positive clones for expressing BMP4were verified by cell immunofluorescence and Western blotting. To test the bioactivity of BMP4, iPSCs were cultured in

[导航/NAVIGATE](#)

[本期目录/Table of Contents](#)

[下一篇/Next Article](#)

[上一篇/Previous Article](#)

[工具/TOOLS](#)

[引用本文的文章/References](#)

[下载 PDF/Download PDF\(2273KB\)](#)

[立即打印本文/Print Now](#)

[推荐给朋友/Recommend](#)

[统计/STATISTICS](#)

[摘要浏览/Viewed](#) 146

[全文下载/Downloads](#) 318

[评论/Comments](#)



the medium supplemented with leukemia inhibitory factor (LIF) plus the supernatant containing BMP4, and the cell phenotype, cell differentiation capacity into lineages of the 3 germ layers and expression levels of pluripotency-associated genes were investigated. Results Smad1 was phosphorylated by BMP4 from the culture medium. iPSCs cultured in the medium supplemented with LIF plus the supernatant containing BMP4 for 3 passages maintained the phenotype of stem cells with the expression levels of pluripotency-associated genes not affected. These iPSCs also maintained the capacity to differentiate into cell lineages of the 3 germ layers. Conclusion BMP4 can be efficiently expressed in mammalian cells to maintain the multipotent differentiation capacity of the iPSCs in vitro culture.

---

## 参考文献/REFERENCES

- [1] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos [J] . *Nature*,1981,292:154-6.
- [2] Watabe T, Miyazono K. Roles of TGF-beta family signaling in stem cell renewal and differentiation [J] . *Cell Res*,2009,19(1):103-15.
- [3] Xu RH, Peck RM, Li DS, et al. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells [J] . *Nat Methods*,2005,2(3):185-90.
- [4] Yu P, Pan G, Yu J, et al. FGF2 sustains NANOG and switches the outcome of BMP4-induced human embryonic stem cell differentiation [J] . *Cell Stem Cell*,2011,8(3):326-334.
- [5] Vallier L, Touboul T, Chng Z, et al. Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways [J] . *PLoS ONE*,2009,4(6): e6082.
- [6] Finley MF, Devata S, Huettner JE. BMP-4 inhibits neural differentiation of murine embryonic stem cells [J] . *J Neurobiol*, 1999, 40(3):271-87.
- [7] Ying QL, Nichols J, Chambers I, et al. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3 [J] . *Cell*, 2003, 115(3): 281-92.
- [8] Qi X, Li TG, Hao J, et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways [J] . *Proc Natl Acad Sci USA*,2004,101(16):6027-32.
- [9] Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors [J] . *Cell*,2006,126(4):663-76.
- [10] Takahashi K, Okita K, Nakagawa M, et al. Induction of pluripotent stem cells from fibroblast cultures [J] . *Nat Protoc*, 2007, 2(12):3081-9.
- [11] Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells [J] . *Nature*,2007,448(7151):313-7.
- [12] Kang L, Wang J, Zhang Y, et al. iPS cells can support full-term development of tetraploid blastocyst complemented embryos [J] . *Cell Stem Cell*,2009,5(2):135-8.
- [13] Zhao XY, Li W, Lv Z, et al. iPS cells produce viable mice through tetraploid complementation [J] . *Nature*,2009,461(7260):86-90.
- [14] Chin MH, Mason MJ, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures [J] . *Cell Stem Cell*,2009,5(1):111-23.
- [15] Deng J, Shoemaker R, Xie B, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming [J] . *Nat Biotechnol*,2009,27(4):353-60.
- [16] Doi A, Park IH, Wen B, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts [J] . *Nat Genet*,2009,41(12):1350-3.
- [17] Samavarchi-Tehrani P, Golipour A, David L, et al. Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming [J] . *Cell Stem Cell*,2010,7(1):64-77.
- [18] Chen J, Liu J, Yang J, et al. BMPs functionally replace Klf4 and support efficient reprogramming of mouse fibroblasts by Oct4 alone [J] . *Cell Res*,2011,21(1):205-12.
- [19] Gassmann M, Donoho G, Berg P. Maintenance of an extrachromosomal plasmid vector in mouse embryonic stem cells [J] . *Proc Natl Acad Sci USA*,1995,92(5):1292-6.
- [20] Camenisch G, Gruber M, Donoho G, et al. A polyoma-based episomal vector efficiently expresses exogenous genes

in mouse embryonic stem cells [J] . Nucleic Acids Res,1996,24(19):3707-13.

[21] Cui Y, Hackenmiller R, Berg L, et al. The activity and signaling range of mature BMP-4 is regulated by sequential cleavage at two sites within the prodomain of the precursor [J] . Genes Dev, 2001, 15(21):2797-802

---

备注/Memo: -

---

更新日期/Last Update: 1900-01-01