МОЛЕКУЛЯРНАЯ БИОЛОГИЯ, 2023, том 57, № 4, с. 668–670

МОЛЕКУЛЯРНАЯ БИОЛОГИЯ КЛЕТКИ

УДК 57.085.23:57.032

КОМБИНИРОВАННАЯ СВЕРХЭКСПРЕССИЯ *Foxa3* и *Hnf4a* УСИЛИВАЕТ ПРОЛИФЕРАЦИЮ И ПРОДЛЕВАЕТ ФУНКЦИОНАЛЬНУЮ СОХРАННОСТЬ ПЕРВИЧНЫХ ГЕПАТОЦИТОВ[1]

© 2023 г. J. Y. Fan[a, b, c, 2], G. Dama[a, d, 2], Y. L. Liu[a, b], W. Y. Guo[b], J. T. Lin[a, b, e, *]

[a]*Stem Cell and Biotherapy Engineering Research Center of Henan, Henan Joint International Research Laboratory of Stem Cell Medicine, Xinxiang Medical University, Xinxiang, 453003 China*

[b]*College of Life Science and Technology, Xinxiang Medical University, Henan, Xinxiang, 453003 China*

[c]*Shandong Tianchuan Precision Medical Technology Co. Ltd., Dezhou, 253084 China*

[d]*Department of Community Health, Advanced Medical and Dental Institute, University Sains Malaysia, Kepala Batas, 13200 Malaysia*

[e]*College of Biomedical Engineering, Xinxiang Medical University, Henan, Xinxiang, 453003 China*

*****e-mail: linjtlin@126.com*

Поступила в редакцию 05.09.2022 г.
После доработки 07.11.2022 г.
Принята к публикации 06.12.2022 г.

В системе культивирования *in vitro* пролиферативная активность первичных гепатоцитов обычно низкая и сопровождается снижением жизнеспособности и потерей специфических для гепатоцитов функций. Ранее показано, что комбинированное введение определенных гепатоцитспецифических факторов транскрипции приводит к конвертированию фибробластов в функциональные гепатоцитоподобные клетки. Однако комбинированное использование факторов транскрипции в первичной культуре гепатоцитов еще недостаточно изучено. Белки FoxA3 (forkhead box protein A3) и Hnf4α (forkhead box protein A3 hepatocyte nuclear factor 4α) представляют собой факторы транскрипции, которыми обогащена печень, и играют жизненно важную роль в дифференцировке и поддержании гепатоцитов. В представленном исследовании в гепатоцитах крысы получили одновременную избыточную экспрессию двух генов: *Foxa3* и *Hnf4a*. Показано, что комбинированное усиление экспрессии двух транскрипционных факторов: FoxA3 и Hnf4α – приводит к повышенной скорости пролиферации и стабилизации специфических функций первичных гепатоцитов в течение длительного периода культивирования.

Ключевые слова: гепатоциты, пролиферация, печень, FoxA3, Hnf4α

DOI: 10.31857/S0026898423040031, **EDN:** QKQQUW

СПИСОК ЛИТЕРАТУРЫ

1. Taub R. (2004) Liver regeneration: from myth to mechanism. *Nat. Rev. Mol. Cell. Biol.* **5**, 836–847.
2. Li A.P. (2007) Human hepatocytes: isolation, cryopreservation and applications in drug development. *Chem. Biol. Interact.* **168**, 16–29.
3. Farinati F., Cardin R., D'Errico A., De Maria N., Naccarato R., Cecchetto A., Grigioni W. (1996) Hepatocyte proliferative activity in chronic liver damage as assessed by the monoclonal antibody MIB1 Ki67 in archival material: the role of etiology, disease activity, iron, and lipid peroxidation. *Hepatology.* **23**, 1468–1475.
4. Michalopoulos G.K., De Frances M.C. (1997) Liver regeneration. *Science.* **276**, 60–66.
5. Block G.D., Locker J., Bowen W.C., Petersen B.E., Katyal S., Strom S.C., Riley T., Howard T.A., Michalopoulos G.K. (1996) Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGF alpha in a chemically defined (HGM) medium. *J. Cell. Biol.* **132**, 1133–1149.
6. Mitaka T. (1998) The current status of primary hepatocyte culture. *Int. J. Exp. Pathol.* **79**, 393–409.
7. Wang J., Xu L., Chen Q., Zhang Y., Hu Y., Yan L. (2015) Bone mesenchymal stem cells overexpressing FGF4 contribute to liver regeneration in an animal

[1] Статья представлена авторами на английском языке.
[2] Эти авторы внесли равный вклад в выполнение работы.

model of liver cirrhosis. *Int. J. Clin. Exp. Med*. **8**, 12774–12782.
8. Berthiaume F., Moghe P.V., Toner M., Yarmush M.L. (1996) Effect of extracellular matrix topology on cell structure, function, and physiological responsiveness: hepatocytes cultured in a sandwich configuration. *FASEB J.* **10**, 1471–1484.
9. Takashi H., Katsumi M., Toshihiro A. (2007) Hepatocytes maintain their function on basement membrane formed by epithelial cells. *Biochem. Biophys. Res. Commun*. **359**(1), 151–156.
10. Cho C.H., Berthiaume F., Tilles A.W., Yarmush M.L. (2008) A new technique for primary hepatocyte expansion *in vitro*. *Biotechnol. Bioeng.* **101**, 345–356.
11. Tan G.D., Toh G.W., Birgersson E., Robens J., van Noort D., Leo H.L. (2013) A thin-walled polydimethylsiloxane bioreactor for high-density hepatocyte sandwich culture. *Biotechnol. Bioeng.* **110**, 1663–1673.
12. Paul D., Hohne M., Pinkert C., Piasecki A., Ummelmann E., Brinster R.L. (1988) Immortalized differentiated hepatocyte lines derived from transgenic mice harboring SV40 T-antigen genes. *Exp. Cell. Res.* **175**, 354–362.
13. Wege H., Le H.T., Chui M.S., Liu L., Wu J., Giri R., Malhi H., Sappal B.S., Kumaran V., Gupta S., Zern M.A. (2003) Telomerase reconstitution immortalizes human fetal hepatocytes without disrupting their differentiation potential. *Gastroenterology.* **124**, 432–444.
14. Tsuruga Y., Kiyono T., Matsushita M., Takahashi T., Kasai H., Matsumoto S., Todo S. (2008) Establishment of immortalized human hepatocytes by introduction of HPV16 E6/E7 and hTERT as cell sources for liver cell-based therapy. *Cell. Transplant.* **17**, 1083–1094.
15. Sekiya S., Suzuki A. (2011) Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. *Nature.* **475**, 390–393.
16. Naldini L., Blomer U., Gallay P., Ory D., Mulligan R., Gage F.H., Verma I.M., Trono D. (1996) *In vivo* gene delivery and stable transduction of non dividing cells by a lentiviral vector. *Science.* **272**, 263–267.
17. Kingston R.E., Chen C.A., Okayama H. (2003) Calcium phosphate transfection. *Curr. Protoc. Cell. Biol.* **20**, 20–23.
18. Bowles N.E., Eisensmith R.C., Mohuiddin R., Pyron M., Woo S.L. (1996) A simple and efficient method for the concentration and purification of recombinant retrovirus for increased hepatocyte transduction *in vivo*. *Hum. Gene. Ther.* **7**, 1735–1742.
19. Lecluyse E.L., Alexandre E. (2010) Isolation and culture of primary hepatocytes from resected human liver tissue. *Methods Mol. Biol.* **640**, 57–82.
20. Schmittgen T.D., Livak K.J. (2008) Analyzing real-time PCR data by the comparative C (T) method. *Nat. Protoc.* **3**, 1101–1108.
21. Yamada T., Yoshikawa M., Kanda S., Kato Y., Nakajima Y., Ishizaka S., Tsunoda Y. (2002) *In vitro* differentiation of embryonic stem cells into hepatocyte-like cells identified by cellular uptake of indocyanine green. *Stem Cells*. **20**, 146–154.
22. Shulman M., Nahmias Y. (2013) Long-term culture and coculture of primary rat and human hepatocytes. In: *Epithelial Cell Culture Protocols*: Second Edition. Eds Randell H.S., Fulcher L.M. Totowa, NJ: Humana Press, pp. 287–302.
23. Schrem H., Klempnauer J., Borlak J. (2002) Liver-enriched transcription factors in liver function and development. Part I: The hepatocyte nuclear factor network and liver-specific gene expression. *Pharmacol. Rev.* **54**, 129–158.
24. Huang P., He Z., Ji S., Sun H., Xiang D., Liu C., Hu Y., Wang X., Hui L. (2011) Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature.* **475**, 386–389.
25. Du Y., Wang J., Jia J., Song N., Xiang C., Xu J., Hou Z., Su X., Liu B., Jiang T., Zhao D., Sun Y., Shu J., Guo Q., Yin M., Sun D., Lu S., Shi Y., Deng H. (2014) Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. *Cell Stem Cell.* **14**, 394–403.
26. Huang P., Zhang L., Gao Y., He Z., Yao D., Xu J., Hou Z., Su X., Liu B., Jiang T., Zhao D., Sun Y., Shu J., Guo Q., Yin M., Sun D., Lu S., Shi Y., Deng H. (2014) Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. *Cell Stem Cell.* **14**, 370–384.
27. Kim J., Kim K.P., Lim K.T., Lee S.C., Yoon J., Song G., Hwang S.I., Schöler H.R., Cantz T., Han D.W. (2015) Generation of integration-free induced hepatocyte-like cells from mouse fibroblasts. *Sci. Rep.* **5**, 15706.
28. Tomizawa M., Shinozaki F., Motoyoshi Y., Sugiyama T., Yamamoto S., Ishige N. (2016) Transcription factors and medium suitable for initiating the differentiation of human induced pluripotent stem cells to the hepatocyte lineage. *J. Cell. Biochem.* **117**, 2001—2009.
29. Naiki T., Nagaki M., Asano T., Kimata T., Moriwaki H. (2005) Adenovirus-mediated hepatocyte nuclear factor-4α overexpression maintains liver phenotype in cultured rat hepatocytes. *Biochem. Biophys. Res. Commun.* **335**, 496–500.
30. Cirillo L.A., Lin F.R., Cuesta I., Friedman D., Jarnik M., Zaret K.S. (2002) Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol. Cell.* **9**, 279–289.
31. Wangensteen K.J., Zhang S., Greenbaum L.E., Kaestner K.H. (2015) A genetic screen reveals Foxa3 and TNFR1 as key regulators of liver repopulation. *Genes Dev.* **29**, 904–909.
32. Li J., Ning G., Duncan S.A. (2000) Mammalian hepatocyte differentiation requires the transcription factor HNF-4α. *Genes Dev.* **14**, 464–474.
33. Shen W., Scearce L.M., Brestelli J.E., Sund N.J., Kaestner K.H. (2001) *Foxa3* (hepatocyte nuclear factor 3γ) is required for the regulation of hepatic GLUT2 expression and the maintenance of glucose homeostasis during a prolonged fast. *J. Biol. Chem.* **276**, 42812–42817.
34. Parviz F., Matullo C., Garrison W.D., Savatski L., Adamson J.W., Ning G., Kaestner K.H., Rossi J.M., Zaret K.S., Duncan S.A. (2003) Hepatocyte nuclear factor 4α controls the development of a hepatic epithelium and liver morphogenesis. *Nat. Genet.* **34**, 292–296.
35. Liu K., Guo M.G., Lou X.L., Li X.Y., Xu Y., Ji W.D., Huang X.D., Yang J.H., Duan J.C. (2015) Hepatocyte nuclear factor 4α induces a tendency of differentiation and activation of rat hepatic stellate cells. *World J. Gastroenterol.* **21**, 5856–5866.
36. Klocke R., Gomez-Lechon M.J., Ehrhardt A., Mendoza-Figueroa T., Donato M.T., López-Revilla R., Castell J.V., Paul D. (2002) Establishment and characterization of immortal hepatocytes derived from various transgenic mouse lines. *Biochem. Biophys. Res. Commun*. **294**, 864–871.

Combinational Overexpression of *Foxa3* and *Hnf4a* Enhance the Proliferation and Prolong the Functional Maintenance of Primary Hepatocytes

J. Y. Fan[1, 2, 3], G. Dama[1, 4], Y. L. Liu[1, 2], W. Y. Guo[2], and J. T. Lin[1, 2, 5, *]

[1]Stem Cell and Biotherapy Engineering Research Center of Henan, Henan Joint International Research Laboratory of Stem Cell Medicine, Xinxiang Medical University, East of JinSui Road 601, Xinxiang, 453003 China

[2]College of Life Science and Technology, Xinxiang Medical University, Henan, Xinxiang, 453003 China

[3]Shandong Tianchuan Precision Medical Technology Co. Ltd., Dezhou, 253084 China

[4]Department of Community Health, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas, 13200 Malaysia

[5]College of Biomedical Engineering, Xinxiang Medical University, Henan, Xinxiang, 453003 China

**e-mail: linjtlin@126.com*

In an *in vitro* culture system, primary hepatocytes usually display a low proliferation capacity, accompanied with a decrease of viability and a loss of hepatocyte-specific functions. Previous studies have demonstrated that the combination introductions of certain hepatocyte-specific transcription factors are able to convert fibroblasts into functional hepatocyte-like cells. However, such combinational usage of transcription factors in primary hepatocytes culture has not yet sufficiently studied. The forkhead box protein A3 (FoxA3) and hepatocyte nuclear factor 4α (Hnf4α) are liver-enriched transcription factors that play vital roles in the differentiation, and maintenance of hepatocytes. Thus, we simultaneously overexpressed the two genes, *Foxa3* and *Hnf4a*, in rat hepatocytes and observed that the combinational augmentation of these two transcription factors have enhanced the proliferation and stabilized the hepatocyte-specific functions of primary hepatocytes over a long-term culture period.

Keywords: hepatocytes, proliferation, liver, FoxA3, Hnf4α