Stem Cell Reports
Article

ISSCR

OPEN ACCESS

Retinoic Acid Is Sufficient for the In Vitro Induction of Mouse Spermatocytes

Si Wang,[1,2] Xiuxia Wang,[1] Longfei Ma,[1,3] Xiwen Lin,[1] Daoqin Zhang,[1,3] Zhen Li,[1,3] Yujian Wu,[1] Chunwei Zheng,[1,3] Xue Feng,[1,4] Shangying Liao,[1] Yanmin Feng,[1,3] Jian Chen,[1,3] Xiangjing Hu,[1,3] Min Wang,[1,5] and Chunsheng Han[1,*]

[1]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[2]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[3]University of Chinese Academy of Sciences, Beijing 100049, China
[4]Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing 100191, China
[5]Center for Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
*Correspondence: hancs@ioz.ac.cn
http://dx.doi.org/10.1016/j.stemcr.2016.05.013

SUMMARY

Meiosis is the key step in gametogenesis. However, the mechanism of mammalian meiosis remains poorly understood due to the lack of an in vitro model. Here, we report that retinoic acid (RA) is sufficient for inducing leptotene/zygotene spermatocytes from cultured mouse spermatogonial stem cells. Multiple genes regulated by RA were identified by RNA sequencing. RA in combination with pup Sertoli cell co-culture resulted in a higher induction efficiency of 28%. Comparisons in the transcriptomic profiles of the induced spermatogenic cells and the isolated ones revealed the progressive induction of the germ cells. Using this model, we showed that *Stra8*, *Agpat3*, *Fam57a*, *Wdr91*, and *Sox30* contributed to the proliferation and meiosis initiation differentially. In conclusion, we have efficiently generated spermatocytes using an RA/pup Sertoli cell-based in vitro model and provided proof-of-concept evidence for its application in identifying genes involved in mammalian meiosis.

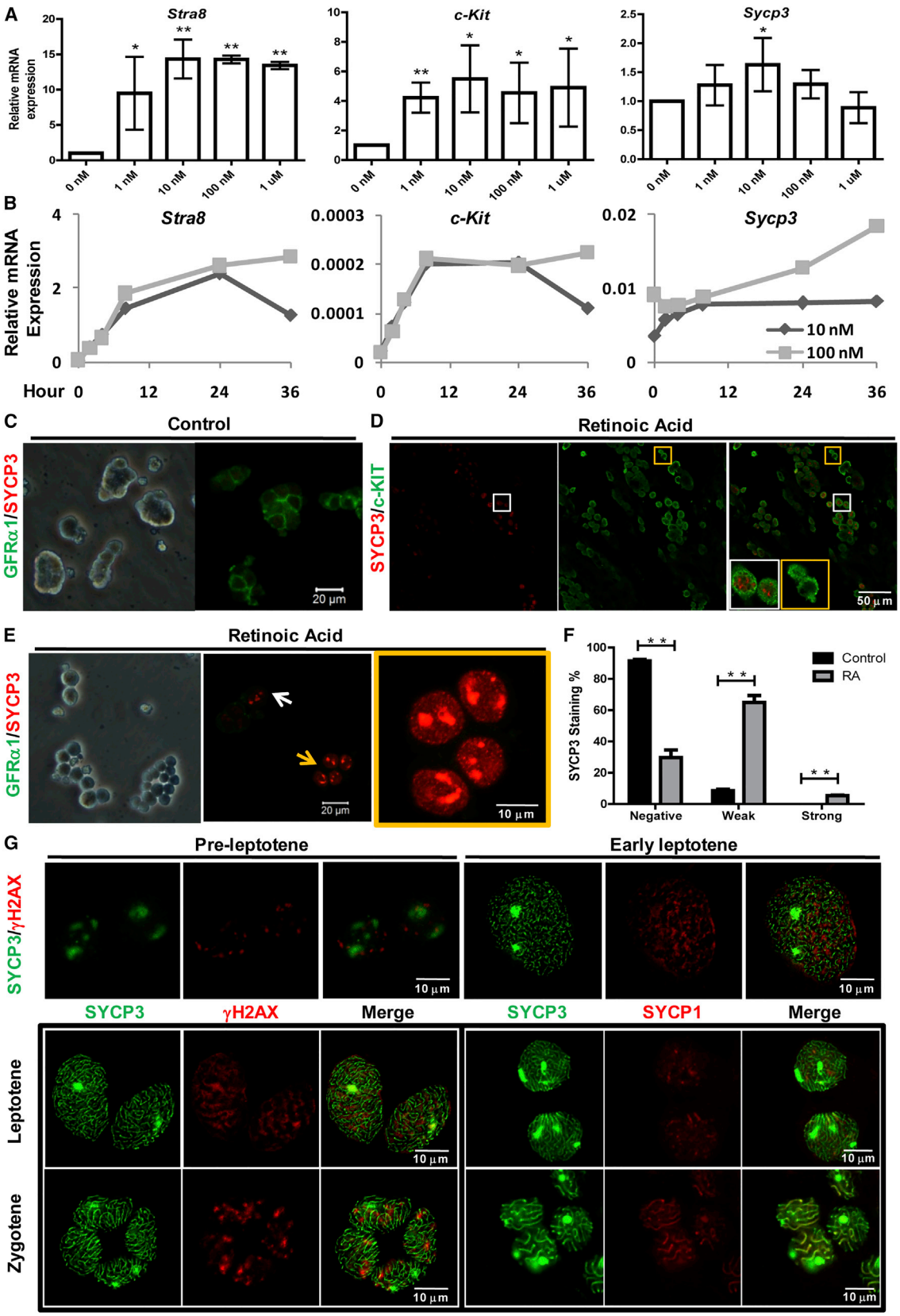
INTRODUCTION

Meiosis is the key step of gametogenesis, which ensures the production of haploid gametes from their diploid precursors and the recombination of genetic materials from the parents. For lower eukaryotes such as yeasts, meiosis is initiated under unfavorable environmental conditions whereby the extracellular signals are integrated at the transcriptional level of master regulatory genes, which in turn activate the expression of downstream targets (van Werven and Amon, 2011). In mammals, meiosis initiation is believed to be mainly regulated by the production, storage, and metabolism of retinol and its metabolite retinoic acid (RA) (Griswold et al., 2012). RA signaling is mediated by its target genes such as *Stra8*, which is essential for the initiation and progression of meiosis (Anderson et al., 2008; Baltus et al., 2006; Bowles et al., 2006; Koubova et al., 2006; Mark et al., 2008). Intrinsic factors such as DAZL (deleted in azoospermia-like) prepare diploid germ cells ready for meiosis initiation when extracellular signals are received (Lin et al., 2008).

Somatic cells play important roles in mammalian meiosis initiation, partially because these cells govern the production and degradation of RA. Female germ cells initiate meiosis shortly after sex determination, while their male counterparts enter a quiescent state because their surrounding somatic cells, the Sertoli cells, express RA-metabolizing enzymes, such as CYP26b1, which effectively degrades RA before it can act upon the germ cells (Bowles et al., 2006; Koubova et al., 2006; MacLean et al., 2007). Paracrine factors such as FGF9 and intrinsic ones such as NANOS2 are also essential for meiosis inhibition and sex-specific gene expression in males (Bowles et al., 2010; Saba et al., 2013). After birth, a subpopulation of male germ cells turn into spermatogonial stem cells (SSCs), which undergo life-long self-renewal and differentiation to produce sperm.

The attempts to generate gametes in vitro can be dated back to almost a century ago. During the early phase of this long journey, the strategy of organ/tissue culture of the gonads was used and several studies reported the derivation of spermatocytes using rodent testes or human testicular biopsy (Song and Wilkinson, 2012). This strategy reached maturity when the Ogawa group reported that pup testis explants supported the derivation of functional spermatids and sperm from either autologous spermatogonia or transplanted mouse SSCs (mSSCs) (Sato et al., 2011a, 2011b). However, this ex vivo model is not ideal for elucidating the mechanisms of meiosis due to the complex constituents of the culture system. Another direction of the effort was to use dissociated testicular cells to derive gametes. Although several studies reported that pachytene spermatocytes or even haploid spermatogenic cells can be derived in the germ cell/Sertoli cell co-cultures, the low efficiency also limits its value for research and practical applications (Sa et al., 2008; Tres and Kierszenbaum, 1983). Moreover, none of these studies used long-term SSC cultures, which make large-scale amplification and gene modification of germ cells possible.

The third strategy for gamete generation is to induce gametes directly from pluripotent stem cells. About a



(legend on next page)