**Name of journal: World Journal of Stem Cells**

**ESPS Manuscript NO: 7063**

**Columns:** **TOPIC HIGHLIGHTS**

WJSC6th Anniversary Special Issues (2): Mesenchymal stem cells

**Differentiation of mesenchymal stem cells into gonad and adrenal steroidogenic cells**

Yazawa T *et al*. Differentiation of MSCs into steroidogenic cells

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**Author contributions:** Yazawa T, Umezawa A and Miyamoto K designed the research; Yazawa T and Imamichi Y performed the research; Yazawa T and Taniguchi T wrote the paper.

**Supported by** Ministry of Education, Culture, Sports, Science and Technology of Japan No. 23590329, the Terumo Life Science Foundation, and the Smoking Research Foundation

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**Received:** November 2, 2013  **Revised:** December 24, 2013

**Accepted: January 17, 2014**

**Published online:**

**Abstract**

Hormone replacement therapy is necessary for patients with adrenal and gonadal failure. Steroid hormone treatment is also employed in aging people for sex hormone deficiency. These patients undergo such therapies, which have associated risks, for their entire life. Stem cells represent an innovative tool for tissue regeneration and the possibility of solving these problems. Among various stem cell types, mesenchymal stem cells have the potential to differentiate into steroidogenic cells both *in vivo* and *in vitro*. In particular, they can effectively be differentiated into steroidogenic cells by expressing nuclear receptor 5A subfamily proteins (steroidogenic factor-1 and liver receptor homolog-1) with the aid of cAMP. This approach will provide a source of cells for future regenerative medicine for the treatment of diseases caused by steroidogenesis deficiencies. It can also represent a useful tool for studying the molecular mechanisms of steroidogenesis and its related diseases.

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**Key words:** Steroid hormone; Adrenal; Gonad; Steroidogenic factor-1; Liver receptor homolog-1; Mesenchymal stem cells; Differentiation

**Core tip:** Stem cells can be a potential source of cells for regenerative medicine for diseases caused by steroidogenesis deficiency. Among various stem cell types, mesenchymal stem cells have the potential to differentiate into steroidogenic cells both *in vivo* and *in vitro*. This system can also provide a powerful tool for studying the molecular mechanisms of steroidogenesis and its related diseases.

Yazawa T, Imamichi Y, Miyamoto K, Umezawa A, Taniguchi T. Differentiation of mesenchymal stem cells into gonad and adrenal steroidogenic cells.

**Available from:**

**DOI:**

**INTRODUCTION**

In mammals, steroid hormones are produced from cholesterol mainly in adrenal glands and gonads. Steroid hormones are essential for glucose metabolism, the stress response, fluid and electrolyte balance, sex differentiation, and reproduction via binding to cognate receptors in target tissues. Therefore, steroidogenesis abnormality can often be life threatening. Congenital adrenal hyperplasia (CAH) is one of the most common disorders caused by deficiency of any enzyme involved in steroidogenesis in adrenal glands[[1](#_ENREF_1),[2](#_ENREF_2)]. Impaired cortisol and aldosterone production increases adrenocorticotropic hormone (ACTH) secretion from the pituitary gland, leading to adrenal hyperplasia and accumulation of adrenal androgens. Female patients are prenatally virilized because of excess androgen, and neonates of both genders may suffer from life-threatening Addisonian crisis. Steroid hormone deficiency also occurs in aging people by hypogonadism. In males, testosterone concentrations decline with age, causing various clinical symptoms such as obesity and hypertension[[3-6](#_ENREF_3)]. Postmenopausal women often suffer from osteoporosis caused by estrogen deficiency[[7](#_ENREF_7),[8](#_ENREF_8)]. Hormone replacement therapy has been well established for the treatment of such patients, although they require hormone replacement for their entire lifetime. In addition, these patients suffer from various side effects (liver and kidney damage, immune system dysfunction) and risks associated with long-term replacement therapy (cancer). Therefore, another therapy is needed to resolve these problems. Stem cells represent an innovative tool for tissue regeneration and gene therapy, which could possibly solve these problems. In this review, we provide an overview of differentiation and regeneration of steroidogenic cells using mesenchymal stem cells (MSCs), preceded by a description of the development of steroidogenic organs. We also describe molecular events, such as coactivator function and epigenetic modifications, which occur during differentiation.

**DEVELOPMENT OF STERIDOGENIC ORGANS AND NUCLEAR RECEPTOR 5A (NR5A) SUBFAMILY**

Steroidogenesis begins with conversion of cholesterol into pregnenolone in mitochondria by the P450 side chain cleavage enzyme (P450scc/CYP11A1/Cyp11a1), a rate-limiting enzyme in the synthesis of all steroid hormones. Thereafter, various hormones are synthesized by tissue-specific P450 hydroxylases and hydroxysteroid dehydrogenases[[9](#_ENREF_9),[10](#_ENREF_10)]. Although adrenal glands and gonads produce various steroid hormones in adult life, they have a common developmental origin, a so-called adrenogonadal primordium (AGP) that mainly originates from the intermediate mesoderm and is localized on the coelomic epithelia of the developing urogenital ridge[[11-13](#_ENREF_11)]. As development proceeds, AGP separates into two distinct populations, adrenocortical and gonadal primordia, characterized by the existence of chromaffin cell precursors and primordial germ cells, respectively, which originate and migrate from other germ layers. During differentiation, adrenal glands and gonads synthesize tissue-specific steroid hormones by specific expression patterns of steroidogenic enzymes.

Steroidogenic factor-1 (SF-1, also known as Ad4BP) is one of the earliest markers of the appearance of AGP[[11](#_ENREF_11),[14](#_ENREF_14)]. Because SF-1-knockout mice fail to develop go adrenal glands and gonads, SF-1 represents a master regulator of the development of these organs[[15-17](#_ENREF_15)]. SF-1/Ad4BP is also important for steroidogenesis by regulating the transcription of steroidogenic genes. SF-1/Ad4BP was originally discovered by Keith Parker and Ken Morohashi as a transcription factor that binds to the Ad4 sequence in promoter regions of all cytochrome P450 steroid hydroxylase genes for transactivation[[18](#_ENREF_18),[19](#_ENREF_19)]. They concluded from the expression of SF-1 in steroidogenic cells and its regulation of all steroid hydroxylase genes, that SF-1 is a determinant factor in cell-specific expression of steroidogenic enzymes. In addition to steroidogenic enzymes, diverse groups of SF-1 target genes, such as other steroidogenic genes, pituitary hormones and cognate receptors, and sex differentiation-related genes, have been identified thus far[[17](#_ENREF_17),[20](#_ENREF_20),[21](#_ENREF_21)]. SF-1 belongs to the nuclear receptor (NR) superfamily. NRs are lipophilic ligand-dependent and -independent transcription factors and essential for various physiological phenomena[[22](#_ENREF_22), [23](#_ENREF_23)]. A large number of family members have identified from invertebrate to mammals. There are a total of 48 family members on human genome. They share a common structural organization: zinc finger DNA-binding domain and a carboxyl-terminal ligand-binding domain. The NR superfamily can be broadly divided into four classes based on their characteristics (steroid hormone receptors, RXR heterodimers, dimeric orphan receptors and monomeric orphan receptors). SF-1 is categorized into monomeric orphan receptors, although Ingraham and colleague argued the possibility that phosphatidyl inositols are ligands for SF-1[[24](#_ENREF_24)]. SF-1 is very similar to liver receptor homolog-1 (LRH-1). LRH-1 was originally identified in the liver[[25](#_ENREF_25)] and is known to function in metabolism, cholesterol, and bile acid homeostasis by regulating the transcription of a number of genes[[26-29](#_ENREF_26)]. In addition to the liver, LRH-1 is highly expressed in tissues of endodermal origin. It is also expressed in gonads and involved in steroidogenesis; in particular, its ovarian expression levels are the most abundant among tissues[[30](#_ENREF_30)]. These factors constitute one of the NR subfamilies and are designated as NR5A proteins (Table 1, SF-1 is NR5A1 and LRH-1 is NR5A2). SF-1 and LRH-1 have various common characteristics, such as binding sequences, target genes, and cofactors[[24](#_ENREF_24),[31-38](#_ENREF_31)].

Consistent with its role in steroidogenesis, SF-1 expression is detected in adults in three layers of the adrenal cortex (zona reticularis, zona fasciculata, and zona glomerulosa), testicular Leydig and Sertoli cells, ovarian theca, granulosa cells, and to a lesser extent in the corpus lutea[[39](#_ENREF_39),[40](#_ENREF_40)]. In the corpus lutea, LRH-1, rather than SF-1, is highly expressed and is important for progesterone production[[36](#_ENREF_36),[41](#_ENREF_41),[42](#_ENREF_42)]. LRH-1 is also expressed in testicular Leydig cells[[12](#_ENREF_12),[43](#_ENREF_43),[44](#_ENREF_44)].

SF-1-knockout mice die shortly after birth because of adrenal insufficiency, and exhibit male-to-female sex reversal in external genitalia[[15](#_ENREF_15)]. These phenotypes are caused by the complete loss of adrenal glands and gonads. Although the initial stages of adrenal and gonadal development occur in the absence of SF-1, they regress and disappear during the following developmental stage. Because gonads disappear prior to male sexual differentiation, the internal and external urogenital tracts of SF-1-knockout mice are of the female type, irrespective of genetic sex. Heterozygous SF-1-knockout mice show decreased adrenal volume associated with impaired corticosterone production in response to stress[[45-47](#_ENREF_45)], whereas transgenic overexpression of SF-1/Ad4BP increases adrenal size and ectopic adrenal tissue in the thorax[[48](#_ENREF_48),[49](#_ENREF_49)]. Total SF-1 disruption in mice demonstrated that SF-1 is crucial for the determination of steroidogenic cell fate *in vivo*. It has also been shown in Leydig cell- and granulosa cell-specific knockout (LCKO and GCKO, respectively) models that SF-1 plays important roles in steroidogenesis following the development of steroidogenic organs. In LCKO mice, testicular steroidogenic acute regulatory protein (StAR) and Cyp11a1 expression is impaired, indicating a defect in androgen production[[50](#_ENREF_50)]. Consistent with this hypothesis, the testes fail to descend (an androgen-dependent developmental process) and are hypoplastic. In GCKO mice, the ovaries are hypoplastic, adults are sterile, and ovaries show reduced numbers of oocytes and lack corpora lutea[[51](#_ENREF_51)]. Gonadotropin-induced steroid hormone production are also markedly reduced in this model.

LRH-1 knockout mouse embryos die around E6.5–7.5 d[[52](#_ENREF_52),[53](#_ENREF_53)]. Moreover, heterozygous and GCKO models revealed the importance of LRH-1 in steroidogenesis[[41](#_ENREF_41),[54](#_ENREF_54),[55](#_ENREF_55)]. In heterozygous Lrh-1-deficient male mice, testicular testosterone production is decreased along with the expression of steroidogenic enzymes and the development of sexual characteristics[[54](#_ENREF_54)]. In addition, GCKO mice are infertile because of anovulation with impaired progesterone production[[41](#_ENREF_41)]. It has also been demonstrated that LRH-1 has a broader role beyond steroidogenesis in these cells, as they fail to luteinize.

Although SF-1- and LRH-1-deficient models revealed a common function in gonadal steroidogenesis, both factors cannot compensate for the deficiency of the other factor even in cells expressing both factors. These facts indicate that even though SF-1 and LRH-1 control transcription by binding to the same response sequences, each has selective actions on the pattern of gene expression in the development of steroidogenic cells and steroidogenesis.

**DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO STEROIDOGENIC CELLS**

In an early study, forced expression of SF-1 has been shown to direct differentiation of murine embryonic stem cells (ESCs) toward the steroidogenic lineage, and then Cyp11a1 mRNA was expressed after the addition of cAMP and retinoic acid[[56](#_ENREF_56)]. However, the steroidogenic capacity of these cells is very limited and they do not undergo *de novo* synthesis, because progesterone is the only steroid hormone produced in the presence of the exogenous substrate, 20α-hydroxycholesterol. In addition, major differences between these differentiated cells and natural steroidogenic cells have been shown in cholesterol delivery and the steroidogenic pathway, including deficiencies of StAR (cholesterol delivery protein from the outer to inner mitochondrial membrane in steroidogenic cells) and steroidogenic enzymes except for Cyp11a1 and Hsd3b1[[56-58](#_ENREF_56)]. It is also very difficult to isolate clones expressing SF-1 from ESCs and induced pluripotent stem cells[[37](#_ENREF_37),[57](#_ENREF_57),[59](#_ENREF_59)], because SF-1 (and LRH-1) overexpression is cytotoxic to these cells. These studies clearly indicate that SF-1 initiates the fate-determination program of the steroidogenic lineage in stem cells, although it is not completed in pluripotent stem cells.

Based on these results, we focused on MSCs[[57](#_ENREF_57)], multipotent adult stem cells that have been shown to differentiate into mesodermal lineages, such as adipocytes, chondrocytes, osteoblasts, and hematopoietic-supporting stroma, both *in vivo* and *ex vivo*[[60-63](#_ENREF_60)]. Furthermore, MSCs are able to generate cells of all three germ layers, at least *in vitro*. Although MSCs were originally discovered in bone marrow (BM-MSCs)[[60](#_ENREF_60),[64-66](#_ENREF_64)], they have also been isolated from various origins, such as fat, placenta, umbilical cord blood, and other tissues[[62](#_ENREF_62),[63](#_ENREF_63),[67-69](#_ENREF_67)]. In addition to their multipotency, MSCs have attracted considerable interest for use in cell and gene therapies, because they can be obtained from adult tissues and suppress immune responses[[70](#_ENREF_70),[71](#_ENREF_71)]. Indeed, their therapeutic applicability has been assessed in some cases, and particularly in bone tissue engineering[[72](#_ENREF_72),[73](#_ENREF_73)].

***Induction of MSC differentiation into steroidogenic cells in vivo and in vitro***

To investigate the potential of MSCs to differentiate into steroidogenic cells, BM-MSCs from GFP-transgenic rats were transplanted into prepubertal testes (Figure 1A)[[57](#_ENREF_57)]. In testis, there are two different steroidogenic-populations, fetal and adult Leydig cells[[74-76](#_ENREF_74)]. Even though the cells in these two populations share a common characteristic of producing androgen, they are different in their origin, ultrastructure, lifespan, steroidogenic pathway and its regulation. Fetal Leydig cells have multiple origins, and appear in the interstitial space to induce sex differentiation just after the formation of the testis cord. Adult Leydig cells, which originate from mesenchymal precursor cells present in the testicular interstitium, appear to induce puberty. During the postnatal period, fetal Leydig cells are replaced by adult Leydig cells in prepubertal testis. Therefore, it should be possible to use transplanted BM-MSCs in such conditions *in vivo*. Indeed, after 3 weeks, transplanted GFP-positive cells were located in the interstitium and expressed various steroidogenic enzymes for androgen production (P450scc/Cyp11a1, 3β-HSD I and Cyp17). These results indicate that MSCs have the capacity to differentiate into steroidogenic Leydig cells *in vivo*.

Although these data suggest that the injected stem cells differentiated into Leydig cells, the apparent stem cell plasticity may also be explained by possible cell-nuclear fusion between donor and recipient cells. However, purified murine BM-MSC lines spontaneously differentiate into steroidogenic cells *in vitro*[[57](#_ENREF_57)]. A human *CYP11A1* promoter-driven GFP reporter, which consisted of a 2.3-kb fragment that drives reporter gene expression selectively in adrenal and gonadal steroidogenic cells[[77](#_ENREF_77)], has been transfected into BM-MSCs to detect cell populations committed to the steroidogenic lineage. In some transfected cell lines, GFP fluorescence was detected in very small populations that were also positive for Cyp11a1. Further analysis showed that these cells expressed several Leydig cell markers including 3β-HSD type I and VI, and luteinizing hormone (LH) receptor. These observations further support the *in vivo* findings that MSCs have the capacity to differentiate into steroidogenic cells, even under the isolated condition. Therefore, part of population of MSCs can spontaneously differentiate into steroidogenic cells *in vitro*. Interestingly, SF-1 expression was also detected in the GFP-positive cells.

***Differentiation of MSCs into steroidogenic cells induced by SF-1 and LRH-1***

The above mentioned results strongly suggest that SF-1 can effectively direct the differentiation of MSCs into the steroidogenic lineage. Indeed, MSCs completely differentiate into steroidogenic cells and show their phenotype after stable expression of SF-1 (using plasmids or retroviruses) and cAMP treatment (Figure 1B)[[36](#_ENREF_36),[37](#_ENREF_37),[44](#_ENREF_44),[57](#_ENREF_57),[78](#_ENREF_78),[79](#_ENREF_79)]. SF-1 by itself induces morphological changes in BM-MSCs, such as the accumulation of numerous lipid droplets, although these cells hardly express steroidogenic enzyme genes or produce steroid hormones at detectable levels. However, SF-1-expressing cells strongly become positive for CYP11A1/Cyp11a1 after cAMP treatment (Figure 1C). These cells express many other steroidogenesis-related genes (*SR-BI*, *StAR*, *3β-HSD*, and other P450 steroid hydroxylases) and autonomously produce steroid hormones including androgen, estrogen, progestin, glucocorticoid, and aldosterone. Notably, this approach differentiates human BM-MSCs into high cortisol-producing cells in response to ACTH, which are very similar to fasciculata cells in the adrenal cortex (Figure 1B). Adenovirus-mediated transient expression of SF-1 also differentiates BM-MSCs into steroidogenic cells with the capacity of *de novo* synthesis of various steroid hormones[[80-84](#_ENREF_80)]. After transplantation into animal models, these MSC-derived steroidogenic cells can improve symptoms of steroid hormone deficiencies caused by adrenalectomy. However, as mentioned above, these methods are not applicable to ESCs, embryonal carcinoma cells, and terminally differentiated cells such as fibroblasts and adipocytes[[37](#_ENREF_37),[57](#_ENREF_57),[81](#_ENREF_81)]. These results indicate that MSCs are suitable stem cells for differentiation of steroidogenic cells. This hypothesis is supported by the fact that after pre-differentiation into MSCs, ESCs can also be subsequently differentiated into steroidogenic cells using SF-1[[37](#_ENREF_37)].

As in the case of SF-1, introduction of LRH-1 (using retroviruses) into BM-MSCs with the aid of cAMP induced the expression of steroidogenic enzymes and differentiation into steroid hormone-producing cells[[44](#_ENREF_44)]. Expression of SF-1 was never induced in LRH-1-transduced cells, and vice versa. Therefore, LRH-1 could act as another master regulator for determining the MSC fate to the steroidogenic lineage. This phenomenon is likely to represent a situation of active progesterone production in human corpus luteum; LRH-1 is highly expressed, whereas SF-1 is expressed at very low levels[[36](#_ENREF_36),[42](#_ENREF_42)].

**MOLECULAR MECHANISMS OF DIFFERENTIATION**

***Steroidogenic cells derived from various MSCs and their properties***

In addition to BM-MSCs, various MSC types have been differentiated into steroidogenic cells by the above mentioned methods. However, their steroidogenic properties markedly vary and depend on the derivation tissues and species (Table 2)[[36](#_ENREF_36),[42](#_ENREF_42),[57](#_ENREF_57),[83](#_ENREF_83),[84](#_ENREF_84)]. For example, hBM-MSCs differentiated into cortisol-producing adrenocortical-like cells, and umbilical cord blood (UCB)-derived MSCs differentiated into granulosa-luteal-like cells, which produced high levels of progesterone[[36](#_ENREF_36),[57](#_ENREF_57)]. Gondo *et al*[[83](#_ENREF_83)] also reported that steroidogenic profiles of adipose tissue-derived MSCs were markedly different from those of BM-MSCs prepared from the same mouse. However, the cell differentiation fate was consistent in each MSC. These findings suggest that the steroidogenic properties of the differentiated cells depend on the characteristics of the originating MSCs.

To determine the difference between BM-MSCs and UBC-MSCs, the fluctuations in gene expression were investigated by a DNA microarray[[36](#_ENREF_36),[85](#_ENREF_85)]. Among the identified genes, peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) was expressed only in UBC-MSCs at relatively high levels. Consistent with these results, the expression of PGC-1α was observed in ovarian granulosa cells. Overexpression of PGC-1α in granulosa cells induced the genes essential for progesterone synthesis, whereas knockdown of PGC-1α in granulosa cells attenuated the expression of these genes. These results demonstrate that PGC-1α represents one of the important factors for progesterone production in luteinized granulosa cells.

***Epigenetic regulation during differentiation***

Differentiation of stem cells into specialized cells can be viewed as a process in which epigenetic changes result in alterations in genes expressed by the cell as it becomes more specialized[[86](#_ENREF_86),[87](#_ENREF_87)]. Thus, stem cell differentiation is a process that involves a series of epigenetic changes in the genome: histone and DNA modifications cause chromatin structural changes and affect the profiles of gene expression. In fact, such epigenetic modifications contribute to the induction of steroidogenesis-related genes when MSCs differentiate into steroidogenic cells[[44](#_ENREF_44),[88-90](#_ENREF_88)].

The histone code hypothesis predicts that post-translational modifications of histone tails, alone or in combination, function to direct specific and distinct DNA-templated programs[[91](#_ENREF_91)]. Histone acetylation is a positive marker of transcription, while histone methylation correlates with transcriptional activation (H3K4, H3K36) and repression (H3K9, H3K27) that are dependent on their amino acid residues[[92](#_ENREF_92)]. In hMSCs-derived steroidogenic cells, H3K27 acetylation and H3K4 dimethylation (active enhancer markers) increased in the regulatory regions of some steroidogenesis-related genes (glutathione S-transferase A and ferrodoxin reductase) after the introduction of SF-1[[89](#_ENREF_89),[90](#_ENREF_90)]. Conversely, histone eviction, which has been reported in actively transcribed genes[[93](#_ENREF_93)], took place on the promoter and the enhancer regions of the *StAR* gene[[88](#_ENREF_88)]. Because these modifications occurred around the SF-1-binding sites, recruitment of SF-1 to the regulatory regions is likely to induce recruitment of various transcriptional regulators and histone modifiers, which in turn alter chromatin structure and lead to the expression of steroidogenesis-related genes.

In addition to histone modifications, DNA methylation at cytosine residues of the dinucleotide sequence CpG, which induces gene silencing, is essential for differentiation and development[[94](#_ENREF_94),[95](#_ENREF_95)]. In MSC-derived steroidogenic cells, the DNA methylation status changes in the promoter regions of some steroidogenic genes during differentiation[[44](#_ENREF_44)]. In undifferentiated hBM-MSCs, the *CYP11A1* promoter region is hypomethylated, whereas the *CYP17A1* promoter region is highly methylated. In SF-1/LRH-1-introduced MSCs during cAMP treatment, this condition was almost completely unchanged in the *CYP11A1* promoter region, whereas the *CYP17A1* promoter region was progressively demethylated. These methylation patterns of the *CYP11A1* and *CYP17A1* promoters closely paralleled the induction patterns of both genes by cAMP. There is a time lag associated with the induction of steroidogenic enzymes by cAMP treatment in SF-1/LRH-1-introduced MSCs[[44](#_ENREF_44),[57](#_ENREF_57)]. The order of induction of the enzymes is similar to the sequential order of the steroid hormone synthesis pathway; upstream enzymes (CYP11A1 and 3β-HSD) were rapidly induced at earlier time points (6-12 h), whereas downstream enzymes (CYP17A1 and CYP11B1) were induced at later time points (24-48 h). Because this time lag disappeared by treatment with a demethylating agent, the status of DNA methylation in the promoter regions could be important for regulating the expression of steroidogenic enzymes in MSCs.

**CONCLUSION**

It is clear that SF-1 represents a master regulator, not only for the development of steroidogenic organs, but also for steroidogenesis following organogenesis. LRH-1 is also important for steroidogenesis in gonads. In addition, SF-1 and LRH-1 direct differentiation of non-steroidogenic stem cells into steroidogenic cells. Among the various stem cell types, MSCs are suitable stem cells for the differentiation of steroidogenic cells. After pre-differentiation into MSCs, pluripotent stem cells can also be subsequently differentiated into steroidogenic cells using SF-1. These cells may provide a source for regenerative and gene therapies, although various problems should be resolved in future studies. It is essential to delineate the conditions that allow the directed differentiation into specific steroidogenic lineages with the characteristics of testicular Leydig cells, ovarian granulosa and theca cells, as well as various types of adrenocortical cells (reticularis, fasciculata, and glomerulosa). In addition, it is necessary to establish methods for inducing SF-1 and LRH-1 expression in stem cells without gene transfer. Further studies are required for the realization of regenerative of steroidogenic tissues.

MSC-derived steroidogenic cells also provide opportunities for investigating various phenomena involved in differentiation of steroidogenic cells and steroidogenesis. In addition to the molecular mechanisms of differentiation described herein, the conservation and evolution of the androgen metabolic pathway (11-ketotestosterone production) between teleost fish and mammals has been revealed[[78](#_ENREF_78),[96](#_ENREF_96)]. Genome-wide analyses of differentiated cells identified novel target genes regulated by SF-1 and LRH-1[[89](#_ENREF_89),[90](#_ENREF_90),[97](#_ENREF_97),[98](#_ENREF_98)]. In addition, they contributed to the elucidation of one of the causes of steroidogenesis disorders[[99-101](#_ENREF_99)]. Thus, progression of these studies is also important for the understanding of steroidogenesis and its related disorders.

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**P-Reviewers:** Holan V, Lalli E, PixleyJS **S-Editor:** Qi Y

**L-Editor: E-Editor:**

**Figure 1** **Differentiation of mesenchymal stem cells into steroidogenic cells.** A: Transplantation of GFP-positive MSCs into prepubertal testis. Double staining of frozen sections from the testis 5 wk after MSC transplantation with anti-GFP and anti-P450scc antibodies; B: Protocol for generating steroidogenic cells from MSCs, and gene expression pattern of steroidogenic cells derived from hBM-MSCs; C: Fluorescence images of DAPI staining and P450scc immunostaining of SF-1-introduced BM-MSCs cultured with or without cAMP. ST: Seminiferous tubule.