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Cell lines
Valuable tools or useless artifacts

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Cell lines are often used in place of primary cells to study biological processes. However, care must be taken when interpreting the results as cell lines do not always accurately replicate the primary cells. In this article, we will briefly talk about advantages and disadvantages of cell lines and then discuss results using the mouse Sertoli cell line, MSC-1, compared with primary mouse Sertoli cells. MSC-1 cells resemble Sertoli cells morphologically and possess several biochemical markers associated with Sertoli cells. Studies have demonstrated that the function and regulation of retinoic acid receptor α (RARα) is similar between MSC-1 and rat Sertoli cells. However, MSC-1 cells lack some of the immune privilege properties associated with primary Sertoli cells, including survival in animals with a fully functional immune system. Therefore, it has to be kept in mind that cell lines do not behave identically with primary cells and should not be used to replace primary cells. In order to strengthen the findings, key control experiments using primary cells should always be performed.

Immortal cell lines are often used in research in place of primary cells. They offer several advantages, such as they are cost effective, easy to use, provide an unlimited supply of material and bypass ethical concerns associated with the use of animal and human tissue. Cell lines also provide a pure population of cells, which is valuable since it provides a consistent sample and reproducible results. Cell lines have revolutionized scientific research and are being used in vaccine production, testing drug metabolism and cytotoxicity, antibody production, study of gene function, generation of artificial tissues (e.g., artificial skin) and synthesis of biological compounds e.g., therapeutic proteins.[1-3] Cell line popularity can be estimated by the numerous publications using cell lines and American Type Culture Collection (ATCC) Cell Biology Collection which consists of over 3,600 cell lines from over 150 different species. However, despite being a powerful tool, one must be careful when using cell lines in place of primary cells. Cell lines should display and maintain functional features as close to primary cells as possible. This may particularly be difficult to determine as often the functions of the primary cells are not entirely understood. Since cell lines are genetically manipulated this may alter their phenotype, native functions and their responsiveness to stimuli. Serial passage of cell lines can further cause genotypic and phenotypic variation over an extended period of time and genetic drift can also cause heterogeneity in cultures at a single point in time. Therefore, cell lines may not adequately represent primary cells and may provide different results. The other major problems associated with cell lines are contamination with other cell lines and mycoplasma. The bitter truth of cross-contamination of cell lines either inter or intraspecies was exposed by Walter Nelson-Rees in the early 1970s. He showed that at that time point the majority of cell lines being used worldwide and distributed by cell banks were contaminated with HeLa cells.[4] This still remains a problem even after 40 y.[5,6] When contamination of a cell line occurs whereby a very rapidly proliferating cell line is introduced, it only takes a few passages until the culture is entirely taken over by the contaminating cell line. HeLa cell contamination is well known to cause such problems. Additionally, mycoplasma contamination can persist undetected in cell cultures for a long period of time and cause extensive alterations in gene expression and cell behavior. Based on submissions to cell banks, 15–35% of cell lines were estimated to be contaminated with mycoplasma.[7,8] Therefore, great care should be taken when using cell lines and experiments where key findings are confirmed in primary cultures should always be included.

Herein we share our experience using an immortalized mouse Sertoli cell line (MSC-1), that was developed in 1992 by Peschon et al.[9] This cell line was isolated from transgenic mice containing Sertoli cells transformed by the small and large T-antigens of the SV40 virus, which were targeted to Sertoli cells using the promoter for Mullerian inhibiting substance. MSC-1 cells were similar to primary Sertoli cells morphologically and expressed many of the same genes as primary Sertoli cells.[9,10] Although, follicle-stimulating hormone receptor (FSHr) and Mullerian inhibiting substance were not detected in MSC-1 cells.[9,10]

Previously, MSC-1 cells were used to study the function and regulation of retinoic acid receptor α (RARα). In these studies, retinoic acid, activation of protein

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kinase C (PKC) and mitogen activated protein kinase (MAPK) were shown to increase the nuclear localization and transcriptional activity of RARα.[11] Additionally, peroxisome proliferators inhibited the retinoic acid-induced nuclear localization and transcriptional activity of RARα, while increasing the nuclear localization and transcriptional activity of peroxisome proliferator-activated receptor α (PPARα) in MSC-1 cells.[12] Importantly, the results were confirmed in primary Sertoli cells isolated from 20-d old rats,[11,12] which verified that RARα nuclear localization and transcription were regulated by retinoic acid, PKC, MAPK and peroxisome proliferators. This demonstrates that RARα regulation and function is similar in MSC-1 and primary Sertoli cells and that MSC-1 cells can be used as a model to study RARα regulation in primary Sertoli cells.

However, not all results using the MSC-1 cell line are consistent with results from primary Sertoli cells as illustrated by studies on immune privilege.[13,14] Immune-privileged sites are anatomical sites where foreign tissues survive for extended periods of time because immune surveillance is reduced, and thus foreign antigens can be tolerated without evoking a detrimental immune response. The testis is an immune-privileged site that results in protection of the auto-immunogenic germ cells (when germ cells are removed from the testis and injected at a different site in the same animal, the cells are rejected).[15] Sertoli cells play an important role in creating this immune-privileged environment by expressing several immunoregulatory factors.[16-19] Moreover, isolated Sertoli cells survive and prolong the survival of co-transplanted cells when transplanted as allografts[20,21] or xenografts.[22] Similarly, Sertoli cells grafted alone across species survive longer than other cell types.[23,24]

To compare the immunoprotective properties of MSC-1 cells with primary Sertoli cells, MSC-1 cells were co-transplanted with BALB/c pancreatic islets as allografts into diabetic C3H mice. The islets were rejected in 32.8 ± 8.4 d, which was not significantly different from control mice that received allogeneic islets alone (26.9 ± 2.1 d). In contrast, co-transplantation of BALB/c primary Sertoli cells with BALB/c islets as allografts into diabetic C3H mice significantly prolonged islet graft survival (> 61.1 ± 6.9 d), with 59% of the Sertoli cell/islet co-grafts surviving throughout the study period.[14] In addition, 100% graft survival was observed when primary Sertoli cells were transplanted alone as allografts into naïve BALB/c mice for 20 d.[13] In contrast, MSC-1 cells were unable to protect co-grafted cells in diabetic animals and were themselves rejected when transplanted into naïve mice with a fully functional immune system.[13,14] This emphasizes the importance of being cautious before assuming results obtained from cell lines are the same as those obtained using primary cells.

Interestingly, MSC-1 cells did survive in 66% of the recipient diabetic mice even though the islet grafts were rejected.[14] This is most likely due to the suppressed immune system associated with diabetes and suggests MSC-1 cells express some immunoprotective factors but lack or have lower expression of the key factors needed for immune protection of co-grafted cells and for fully functional immune privilege. Thus, MSC-1 cells may not mimic the survival and immune privilege properties of primary Sertoli cells but are useful as a control cell line to identify the key mechanisms or factors important for primary Sertoli cell immune privilege. To identify genes and immune-related functional pathways that are differentially regulated in these cells gene expression profiles of primary mouse Sertoli cells and MSC-1 cells were compared by microarray and ontological analyses.[13] We found that 2,369 genes were expressed with a ± 4-fold or higher level in primary Sertoli cells than in MSC-1 cells. Genes involved in immune functions were identified and differentially expressed.[13] While the Sertoli cells and MSC-1 cells express many of the same genes, they were expressed at different levels which appear to result in different immune regulatory functions. This confirms that the MSC-1 cell line is substantially different from primary mouse Sertoli cells and reiterates the importance of being cautious when making conclusions based on the results from cell lines.

As mentioned earlier, FSHr was not detected in MSC-1 cells.[10] FSHr is activated by follicle-stimulating hormone (FSH) and is important for Sertoli cell proliferation, macromolecular synthesis, morphological structure, and ultimately the spermatogenic capability.[25] However, the role of FSH in creation of an immune privileged environment is not clear. In one study there was an increase in testicular graft size/survival after transplanting to oophorectomized rodents that correlated with FSH and luteinizing hormone (LH) levels.[26] Additionally, Selawry et al., demonstrated that media collected from rat Sertoli cells cultured at 37°C for 24 h supplemented with FSH significantly inhibited the ConA stimulated proliferation of spleen lymphocytes, suggesting FSH may be important for Sertoli cells immune protection.[27] In contrast, the same group also demonstrated that protection of cellular grafts within the testis was not dependent on FSH or LH as treatment of rats with a gonadotropin-releasing hormone (GnRH) analog or hypophysectomy had no effect on the survival of transplanted intratesticular islet allografts.[28]

Since FSHr is known to be important for the function of primary Sertoli cells and MSC-1 cells lack FSHr, the survival of MSC-1 cells stably transfected with FSHr (MSC-1FSHr) was examined after allotransplantation. MSC-1FSHr cells were shown previously to express functional FSHr as demonstrated by northern blot analysis and increased *c-fos* mRNA after FSH treatment.[29] Prior to transplantation, the expression of FSHr was confirmed by RT-PCR and as expected, FSHr mRNA was not detected in MSC-1 cells (**Fig. 1A**, lane 3) while MSC-1FSHr cells expressed FSHr mRNA (**Fig. 1A**, Lane 2). Four million MSC-1 or MSC-1FSHr cells were cultured as aggregates (**Fig. 1C and D**) and transplanted into naïve BALB/c mice as allografts. Graft-bearing kidneys were removed 20 d post-transplantation and examined for cell survival by immunohistochemistry for SV-40 large T antigen and RT-PCR for FSHr. Consistent with the previous survival data in naïve mice, MSC-1 cell grafts were rejected (0/6) by 20 d post-transplantation (**Fig. 1F**). Similarly,

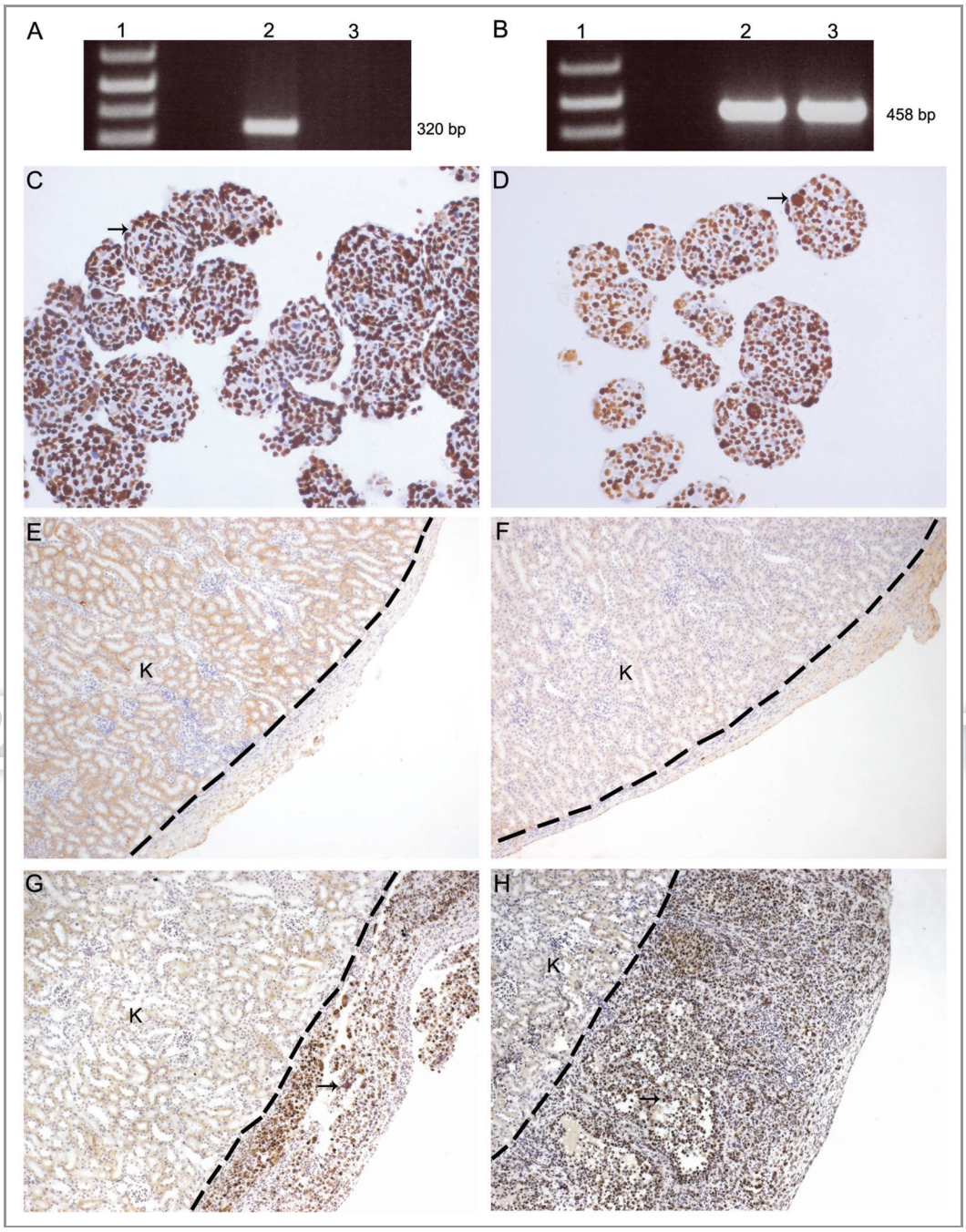

Figure 1. FSHr mRNA expression and survival of MSC-1 and MSC-1FSHr cells as allografts. MSC-1 cells stably transfected with rat FSHr cDNA were obtained from Dr. Griswold (Washington State University, Pullman, WA).[29] MSC-1FSHr cells were maintained and cultured essentially the same as MSC-1 cells with the exception of the addition of 250 mg/ml G418 (Invitrogen, Carlsbad, CA). A and B) RT-PCR was performed for FSHr (A), Lanes 2 and 3; Primers-For 5′CCA TTG TGT CCT CAT CAA GC, Rev 5′CAT GGA AGT TGT GGG TAG CG) or cyclophilin (B), Lanes 2 and 3; Primers-For 5′CCC ACC GTG TTC TTC GAC, Rev 5′ATC TTC TTG CTG GTC TTG CC) with RNA isolated from MSC-1FSHr (A and B, Lane 2) or MSC-1 cells (A and B, Lane 3). Lane 1 (A and B) is 1 kb Plus DNA Ladder (Invitrogen). (C and D) MSC-1 (D) or MSC-1FSHr (C) cells were cultured as aggregated for 48 h. Aggregates were fixed, dispersed in agar, embedded in paraffin, sectioned and immunostained for large T antigen (brown color) and hematoxylin (blue color). (E-H) Four million of these aggregated cells were transplanted under the kidney capsule of naïve (E and F) and diabetic (G and H) BALB/c mice. The grafts were collected at day 20 post-transplantation, and tissue sections were immunostained for MSC-1 cell marker, large T antigen (brown color, E-H). All sections were counterstained with hematoxylin (blue color). A dotted line separates the kidney from the graft. K, kidney; Arrow, large T antigen positive cells. Care and maintenance of animals described in (E-H) was performed in accordance with the Institute for Laboratory Animal Research Care and Use of Laboratory Animals, and Texas Tech University Institutional Animal Care and Use Committee-approved protocols.

MSC-1FSHr grafts were also rejected in naïve BALB/c animals and no large T antigen positive MSC-1FSHr cells or FSHr mRNA were detected at 20 d post-transplantation (0/7) (**Fig. 1E**, data not shown). In contrast, both MSC-1 (2/2) and MSC-1FSHr (4/4) cells survived in diabetic mice at 20 d post-transplantation as shown by large T antigen staining (**Fig. 1G–H**) and RT-PCR for FSHr mRNA (MSC-1FSHr only; data not shown). However, the MSC-1FSHr grafts were slightly smaller than the MSC-1 cell grafts (**Fig. 1**, compare G and H). This indicates that the addition of functional FSHr to MSC-1 cells does not compensate for the loss of immune privilege.

Immune privilege involves a complex interplay between immunoregulatory factors, the transplant environment and the host's immune system. Thus, addition of just one factor e.g., FSHr to a cell line does not make it immune-privileged. Other studies have identified several potential pathways or factors that may contribute to Sertoli cell immune privilege.[13] For example, Sertoli cells express or secrete complement inhibitors, apoptosis inhibitors and factors that modulate the immune response. Thus, it seems likely that a combination of several factors is required to make Sertoli cells immune-privileged. Overall, the MSC-1 cell line may serve as a good comparison cell line to study key factors/mechanisms required for primary Sertoli cell immune privilege but they should not be used in place of primary Sertoli cells to study survival mechanisms.

A different MSC-1FSHr cell line was created by Eskola et al.[30] In this cell line, intact FSHR signaling and function, similar to Sertoli cells was verified by cAMP response to FSH and PKC. Antiproliferative effects of FSH on MSC-1FSHr further demonstrated that these cells resemble adult Sertoli cells and thus can be used a model to study post-transcriptional regulation of FSHR and its signal transduction.[30] However, regulation of inhibin-α expression in response to FSH was different from primary Sertoli cells. In a separate study, the basal and cAMP regulated expression of PKA subunits was compared in MSC-1 cells to rat Sertoli cells.[31] This study demonstrates that the RIIβ mRNA basal levels, magnitude of induction of RIIβ mRNA by cAMP, half-life after cAMP removal and mRNA induction independent of protein synthesis is different from primary rat Sertoli cells.[31] These results further demonstrate that even though a Sertoli cell line retains major characteristics of primary Sertoli cells; they do not completely replicate primary Sertoli cells.

In conclusion, cell lines are a powerful tool and offer several advantages over primary cells. However, it must be understood that cell lines do not completely mimic primary cells. Therefore, great caution should be taken when designing experiments to assure that the conclusions drawn from cell line are sound. Key experiments should also be replicated in primary cells.

Finally, it should be recognized that a weakness of in vitro cell cultures, both primary cells and cell lines, is that they are being studied in the absence of their local environment that often includes interactions with other cell types that may be critical to the hypothesis being tested. Sertoli cells are well known to interact with other cell types in the local environment and therefore these cells are particularly vulnerable to deficiencies of the isolated or enriched culture environment.

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