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Editorial

New and old techniques in cell and developmental biology

During last year's editorial board meeting, the staff of *Differentiation* agreed that it was timely to organize a Special Issue on Techniques in Cell and Developmental Biology that would encompass both historic and well as contemporary methods, spelling out in some detail the "tricks" required for success. Accordingly, we have compiled a broad spectrum of papers that cover a disparate collection of techniques from classic mesenchymal–epithelial interactions (Cunha and Baskin) to generation of genome-edited epiblast stem cells via a detour through embryonic stem cell-chimaeras (Osteil et al.) and to generation and purification of stem cell-derived cardiomyocytes (Schwach and Passier).

Three related papers deal with an exceptionally useful method for studying *in vivo* development, cancer modeling and translational cancer research, namely transplantation of organ rudiments (animal or human) or human primary cancers under the renal capsule of immune-deficient mice. The basic methodology of renal capsule grafting (Cunha and Baskin) is described in detail. Through use of immune-compromised mouse hosts it is possible to grow human fetal organs, adult human tissue, primary human tumors and heterospecific tissues recombinants (*e.g.*, mutant mouse mesenchyme combined with human fetal epithelium) to assess human organogenesis under experimental conditions, to assess direct versus indirect effects of mesenchymal gene knockout on human epithelial development and to establish new tumor transplant lines that are particularly informative of tumor cell biology.

The widespread use of the mouse as an animal model of human development is predicated on the generally unproven assumption that the molecular mechanisms of human and mouse develop are similar/identical. This is certainly not true in some instances. For example, up-regulation of uterine epithelial progesterone receptor is induced in humans by estrogens (Kurita et al., 2005). In contrast, mouse uterine epithelial progesterone receptor is induced as a result of ovariectomy (the absence of estrogen) (Kurita et al., 2000). While mouse development has progressed to the molecular mechanistic level, our understanding of human development has not reached this level of sophistication, which emphasizes the need for modern detailed analysis of human development. Cunha et al. describe how developing human fetal organs can be isolated from abortus specimens and grown under the renal capsule of host mice where normal development proceeds. Such human fetal organ grafts can then be subjected to a wide array of analytic techniques, and the mouse hosts bearing grafts of human fetal organs can be treated with environmentally relevant agents known or suspected of eliciting adverse developmental effects based upon purely animal studies.

Wang et al. describe how the sub-renal capsule graft site can be used to generate patient-derived tumor transplant models that continue to maintain a high degree of biologic fidelity, which includes histopathological and molecular characteristics, tumor heterogeneity, metastatic ability, and response to treatment. In addition, the authors describe the isolation of new transplant lines from heterogeneous primary tumors whose metastatic activity is either minimal or prominently expressed. In so doing the Wang lab has developed new human tumor transplant models for studies of (a) cancer progression, metastasis and drug resistance, (b) evidence-based precision cancer therapy, (c) preclinical drug efficacy testing and (d) discovery of new anti-cancer drug candidates.

All organs composed of an epithelial parenchyma develop as a result of interactions between epithelium and mesenchyme in which the mesenchyme induces and specifies epithelial development. The first paper on mesenchymal–epithelial interactions in mammals was published by Clifford Grobstein in the 1950s (Grobstein, 1953). The basic concepts of mesenchymal–epithelial interactions in development have emerged as a result of hundreds of papers, but new opportunities for using this classic technique have emerged with advent of gene knockout mice. Germline knockout mice with phenotype in epithelial development/differentiation beg the question as to whether the epithelial phenotype is due to absence of the gene in the epithelium, mesenchyme or both. Tissue recombinants composed of mutant mesenchyme plus wild-type epithelium or wild-type mesenchyme plus mutant epithelium provide a quick answer to this question without the need of generating conditional gene knockout mice. Cunha and Baskin describe the basic methodology of mesenchymal–epithelial interactions.

Another paper related to mesenchymal–epithelial interactions and *in vivo* development under the renal capsule deals with the isolation and analysis of discreet adult human prostatic epithelial and stromal populations. The paper by Strand et al. provides an excellent conceptual background of prostatic epithelial cells (secretory luminal cells, basal cells and neuroendrine cells) and prostatic stromal cells (fibroblasts, smooth muscle cells, vasculature cells and myofibroblasts), their origin and biological properties. Methods for isolating and characterizing these cells are described, which can be applied to human prostate as well as other human organs. Isolated human epithelial cells can be used to prepare heterospecific mouse/human tissue recombinants (Hayward et al., 1998) that are useful in analyzing the molecular mechanisms of human epithelial differentiation, particularly when such heterospecific mouse/human tissue recombinants are grafted under the renal capsule of immune-deficient mice.

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The paper by Rosario et al. deals with gene expression in the murine uterine compartments (luminal epithelium, glandular epithelium and stroma) whose coordinated function is essential for embryo attachment, subsequent invasion into the endometrial stroma and embryo/fetal development. Unraveling this incredibly complex process requires analysis of the specific signaling mechanisms in the individual cellular compartments of the uterus. The authors describe methods for the isolation of murine luminal uterine epithelium and subsequent analysis of gene expression/signaling pathways. Methods are described for the analysis of RNA and proteins by qPCR, immunostaining and Western blotting. These methods can be applied to the cellular compartments of the uterus during embryo invasion and endometrial development and to other organs.

Organ culture has been an established technique for growing embryonic organs. The advantage of this technique is that it preserves tissue architecture and maintains cell–cell contacts and signaling relationships. In addition, it provides the opportunity of assessing the effects of exogenous factors such as inhibitors, growth factors and morpholinos to probe the mechanisms of organogenesis. McClelland and Bowles review the three established techniques for *ex vivo* culture of embryonic organs: (a) filter culture, (b) agar block culture and (c) hanging drop culture. Advantages and disadvantages of each of these techniques are reviewed. While all 3 techniques have merit, the key disadvantage of agar-block and filter based culture is the large amount of media required which can make these approaches expensive. Hanging drop culture, most commonly used to enable the aggregation of embryonic stem cells into embryoid bodies, can be employed for *ex vivo* organ culture. This method requires only 40 μL of media per drop and isolates every explant to a trackable unit. Methods for each of these techniques are described along with media formulations to provide the user with clues to optimal culture methods for their needs.

The in utero development of mammals drastically reduces the accessibility of the mammalian embryo and limits the range of experimental manipulations possible in the study of gene function and signaling pathways during embryonic development. Gonçalves et al. describe simple culture techniques for studying and manipulating mid-gestation mouse embryos explanted on top of a floating membrane filter in defined culture media. Viability of short-term cultured embryos as assessed by apoptosis and proliferation demonstrated only a slight increase in apoptosis after 12 h of culture compared to embryos developing in utero. Significantly, differentiation and morphogenesis proceed normally as assessed by 3D imaging of the transformation of the myotome into deep back muscles. Not only does muscle cell differentiation occur as expected, but also extracellular matrix organization is normal, and the myotome splits normally into its normal 3 epaxial muscle groups. The method allows for the culture and manipulation of mammalian embryo explants and permits the manipulation of developmental events in a controlled serum-free environment. Explants grown under these ex utero conditions simulate real developmental events that occur in utero.

One of the persistent problems in working with small embryonic rudiments is obtaining paraffin sections for histology, immunohistochemistry and in situ hybridization with the appropriate “section orientation” to facilitate interpretation. A second paper by McClellan et al. describes agarose/gelatin immobilization of tissues or embryo segments for orientated paraffin embedding and sectioning. This simple method solves a persistent problem that many embryologists have endured.

Tissue ablation experiments have been used for many years as a method for studying early embryonic patterning and regenerative properties of embryonic tissues during organogenesis. Surgical ablation procedures based on removal of tissues during organ formation depend on the individual skills of the researcher, are difficult to reproduce, and often result in extensive tissue disruption and embryonic death. Palmquist-Gomes et al. describe a method to generate precise, discrete, locally restricted and highly reproducible wounds in the developing chick embryo using a liquid nitrogen-cooled metallic probe. This *in ovo* procedure allows for study of organ-specific tissue responses to damage, such as compensatory cell growth, cell differentiation, and reparative/regenerative mechanisms throughout the embryonic lifespan. This method could also be used on organ culture explants.

Kelder et al. present a paper of general interest to those interested in studying in ovo development in chick embryos, but with a specific focus on the developing cardiac conduction system. The authors provide a concise review of heart morphology and cardiac development in the chick. The avian embryo is noted for its ease of accessibility making it particularly suitable for *in ovo* microsurgery and manipulation. The authors summarize a variety of techniques used to study development of the cardiac conduction system in avian embryos. Based on the large amount of relevant data arising from experiments in avian embryos, the avian embryo will continue to be a powerful model system to study development in general and the developing cardiac conduction system in particular.

The paper by Ipulan et al. focuses on establishment of sexual dimorphism within developing male and female reproductive tracts. The authors provide an excellent conceptual background for development of sexual dimorphism that emerges during pre- and early postnatal periods in the mouse with particular emphasis on external genitalia. Experimental techniques involving hormonal modulation of sexual dimorphism as well as the critical time windows for hormonal modulation are described. The authors provide detailed information on the various transgenic mice useful for investigating sexual dimorphism of reproductive structures. In addition, the authors emphasize those mutants and hormonal treatments that generate phenotype relevant to hypospadias.

Use of immunohistochemistry or in situ hybridization on tissue sections or wholemounts of embryonic organs is useful, but has its limitations. High-resolution three-dimensional (3D) analysis can provide a clear global view of gene expression that for some purposes is superior to section- or wholemount-based procedures. Wright and Horn describe a method of 3D analysis of the mouse cochlea that permits imaging of the sensory cells of the cochlea. The method utilizes confocal imaging of immunostained wholemount preparations followed by 3D analysis using the Imaris software. The 3D analysis of confocal stacks can be successfully used for investigating a number of mouse tissues and developmental processes.

The Hippo pathway is a key regulator of tissue and organ development and tissue regeneration, and when disrupted, promotes tumorigenic processes. For instance, in the early embryo, regulation of the Hippo pathway is important for specifying trophectoderm and the pluripotent embryonic cells of the inner blastula, while in the late gastrula, Hippo pathway inactivation supports notochord maintenance. The Hippo pathway also interacts with and controls the activity of other signaling pathways such as the TGFβ/Smad pathway, in which Hippo pathway activity influences the subcellular localization of Smad transcription factors. Narimatsu et al. describe techniques for examining crosstalk between Hippo and TGFβ signaling in polarizing mammary epithelial cells. In addition, the authors describe methods for analyzing the subcellular localization of the Hippo pathway effectors, Taz and Yap, using both *in vitro* cultured epithelial cells and in pre-gastrulation mouse embryos.

Mouse epiblast stem cells are typically derived directly from the epiblast or the ectoderm germ layer of post-implantation embryos. Osteil et al. describe a method for generating self-

renewing multipotent epiblast-like stem cells by conversion of embryonic stem cells through culture conditions. This method obviates the need for post-implantation embryos, which are difficult to process and involves deriving epiblast stem cells using genome-edited embryonic stem cells. This strategy enables the production of epiblast stem cells where (a) no genetically modified animals or embryonic stem cells are available, (b) the impact of the genetic modification on post-implantation development is requisite knowledge for using the epiblast stem cells for a specific investigation, and (c) multiple editing of the genome is desirable to modify the biological attributes of the epiblast stem cells for studying, for example, the gene network activity on the trajectory of lineage differentiation and tissue morphogenesis.

Efficient and reproducible generation and purification of human stem cell-derived cardiomyocytes is crucial for regenerative medicine, disease modeling, drug screening and study of developmental events during cardiac specification. Established methods to generate cardiomyocytes from human pluripotent stem cells typically result in heterogeneous populations comprised of both cardiomyocytes and uncharacterized non-cardiac cell-types. Therefore, generation of pure populations of stem cell-derived cardiomyocytes is of fundamental importance for basic cardiac research and pre-clinical and possible clinical applications. In the past purification of cardiomyocytes from heterogeneous populations employed fluorescent activated cell sorting (FACS). However, FACS-based isolation of cardiomyocytes has several disadvantages, such as undesired contamination and low viability of target cells. Schwach and Passier describe a convenient and rapid procedure for the purification of human pluripotent stem cell-derived cardiomyocytes under sterile culture conditions, resulting in high purity and viability of sorted cardiomyocytes. Purification with VCAMI-coupled magnetic Dynabeads results in robust enrichment of cardiomyocytes. In addition, this method will also be beneficial for the standardization and reproducibility of human stem cell-derived assays in the fields of cardiac disease modeling, drug discovery and therapy.

Adult mammalian stem cells support tissue renewal and are endowed with the ability to self-renew and to generate a spectrum of differentiated cell types appropriate to their tissue of origin. Despite their remarkable properties, adult stem cells are generally present in small numbers and are relatively quiescent. Therefore, their identification and analysis rely heavily upon methods for their isolation in high purity. The paper by Belenguer et al. deals with isolation of neural stem cells from sub-ependymal tissue of the adult mouse brain. While the procedures are complicated, the purified cultures of adult neural stem cells provide a powerful tool for understanding the fundamental properties and behavior of different types of neural stem cells that may be of potential in regenerative medicine.

Finally, the paper by Tanigawa and Perantoni deals with methodologies for the differentiation of pluripotent stem cells into renal progenitors. Significant advances have been made in defining the niche conditions for sustaining renal progenitors from embryonic and neonatal metanephric kidneys. Such renal stem cells have enhanced our understanding of kidney development and provide the prerequisites for establishing viable approaches to kidney regeneration. In their article, the authors provide an excellent review of metanephric development, review the culture techniques and models historically used for the study of metanephric development, describe the signaling mechanisms driving renal cell progenitor self-renewal, and discuss current efforts to generate de novo functional tissues.

The wide range of topics (techniques) reviewed in this special issue covers a spectrum of methods, both old and new, that should be of interested to investigators. We hope that the readership will find this issue a valuable resource that will facilitate future research.

Acknowledgments

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