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Review article

Use of sub-renal capsule transplantation in developmental biology

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ABSTRACT

The sub-renal capsule graft site for in vivo growth and development of developing organs can be used to great advantage in the "rescue" of organ rudiments from "embryonic" or "birth" lethal mutant mice, which permits examination of the full impact of gene knockout in all phases of development from morphogenesis to adult functional differentiation. Another use of the sub-renal capsule graft site is the examination of normal and "chemically perturbed" development of human fetal organs. Tissue recombinants composed of various types of epithelium and mesenchyme, when grafted under the renal capsule undergo normal development and in 3–4 weeks achieve full adult functional cytodifferentiation. The investigator can control many of the developmental parameters of the graft such as endocrine status of the host and treatment of the host with a variety of biologically active agents to assess their effects on development and differentiation.

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1. Introduction

Transplantation of tissues and organ rudiments under the renal capsule is an extremely useful technique for study of development and differentiation as it allows the investigator to examine organogenesis from its inception through all stages to final functional (secretory) cytodifferentiation under experimental conditions in which the investigator has control many of the developmental parameters. For example, embryonic urogenital sinuses of both male and female mouse embryos when transplanted under the renal capsule of adult male hosts undergo prostatic development and achieve secretory function following 1 month of growth under the renal capsule (Cunha, 1975). During this process solid prostatic buds emerge from grafts of the ambisexual urogenital sinus a few days post-transplantation. Within a week post-transplantation, solid prostatic buds canalize to form patent ducts. One month after transplantation the epithelium matures into an adult-like secretory epithelium producing prostate-specific secretory proteins (Donjacour and Cunha, 1993). Of course, in the case of androgen-dependent prostatic development, manipulation of the hormonal status of the male host is an important variable to be controlled by the investigator (intact versus castrated host, treatment with testosterone versus anti-androgens [flutamide], or treatment with environmentally relevant estrogenic chemicals such as bis-phenol A). The ideal conditions of the renal capsule transplantation site are due to the extremely high vascular density of the graft site, which gives the kidney its bright red color. Accordingly, the take rate of grafts under the renal capsule approaches 100%. The method can be learnt in an afternoon and with experience high take rates can be achieved.

Abbreviations: DES, diethylstilbestrol; BPA, bis-phenol A

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2. Rescue of organ development

An important use of renal capsule transplantation is the rescue to organ development in germ-line knockout mice, which may be either "embryonic lethal" or "birth lethal". In such mutant mice it may be impossible to follow development of organs sufficiently to reveal the full impact of gene knockout due to their early demise. There are two solutions to this problem. A conditional gene knockout mouse line can be constructed and maintained at great expense. Alternatively, the germline mutant organ rudiment can be isolated from "embryonic lethal" or "birth lethal" mice and transplanted under the renal capsule of the appropriate host mouse. This approach was used to study the role of p63 and basal epithelial cells in prostatic biology. Prostatic basal epithelial cells normally express p63, and thus it was suspected that prostates of p63 knockout mice might lack basal cells. This possibility could not be tested directly in p63 knockout mice due to lethality at birth, well before prostatic differentiation. Accordingly, late gestation or newborn p63 knockout prostatic rudiments were grown under the renal capsule of male hosts and formed mature prostatic tissue lacking basal epithelial cells. Such p63 knockout prostates exhibited a variety of abnormalities (Kurita et al., 2004). This important observation was achieved rapidly at expense.

Well before conditional Rb knockout mice were available, we created Rb-KO prostate, also at minimal expense, through a sub-renal capsule transplantation strategy. Germ-line Rb-KO mice die around day 12 of gestation, well before the prostatic rudiment exists. The prostate develops from the endoderm of the urogenital sinus, which is derived from the cloaca. Accordingly, we transplanted cloacas from 11- to 12-day embryonic Rb-KO mice under the renal capsule. After 1 month a considerable amount of Rb-KO prostate was present in the grafts from which Rb-KO prostatic epithelium was isolated and studied (Day et al., 2002).

For several of our embryonic organ rescue experiments, the mutant tissues were obtained from collaborators who had constructed the mutant mice. Mutant mouse lines are frequently used for very limited purposes, and consequently most of the mutant mouse is discarded at the end of the day. Organs of developing mutant mice are a valuable resource, and typically the investigator with the mutant mouse colony is willing to make available unused mouse parts to others. We have found that embryonic, neonatal or even adult mouse organs/organ rudiments remain viable for up to 3 days (probably longer) if stored at 0–4 °C in medium containing 10% fetal bovine serum. This means that discarded mutant mouse parts from investigators can be shipped overnight for transplantation the next day. For this purpose, it is best to place mouse parts in 50 ml tubes filled to near the top with medium. This averts the possibility that the tube of medium will freeze solid thus destroying the mouse tissue because ice taken directly from an ice machine is typically maintained well below 0 °C.

A preferred solution to embryonic or birth lethality in germline KO mice is to construct a conditional knockout mouse using, for example, an "epithelial tissue specific" promoter. The problem is that there are very few "epithelial tissue specific" promoters that can be used to delete a gene from all of the cell types within an epithelium. More disturbing is the fact that many investigators fail to distinguish "epithelial tissue-specific" versus "epithelial cell-specific" promoters. This problem is seen frequently in the mammary and prostatic fields, but is not unique to either of these fields. The problem lies in the fact that both mammary and prostatic epithelia contain more than one cell type. For example, mammary and prostatic epithelium contained columnar secretory cells as well as myoepithelial or basal cells. Conditional knockout of a gene in the prostate using the probasin promoter deletes the gene of interest from the secretory luminal cells, but not the basal cells. Likewise, conditional knockout of a gene in the mammary gland using the whey acidic protein promoter or the beta-lactoglobulin milk protein promoter deletes the gene of interest from the secretory luminal cells, but not the myoepithelial cells (Chapman et al., 2000; Walton et al., 2001). Thus, in such mice the epithelium is mosaic with respect to the gene of interest. Accordingly, the strategy of conditional gene knockout has to take into consideration whether the promoter is a tissue-specific or a cell-specific promoter. Of course, the use of germline gene knockout mice and renal capsule transplantation eliminates this problem.

3. Growth of human fetal organs under the renal capsule

With the advent of the athymic nude mouse and other immune-compromised mutant mice, the transplantation of human fetal organs has become possible. In the early 1980s Dr. Stan Robboy and I embarked on a study on the effect of diethylstilbestrol (DES) on development of human female fetal reproductive tract organs as an experimental approach to the human DES episode. Over 1 million young women were exposed in utero to DES from the 1940s to ~1970, and later developed upper and lower genital tract anomalies and in rare cases vaginal or cervical clear cell adenocarcinomas (Herbst et al., 1971, 1975; Kaufman et al., 1977, 1980; Jefferies et al., 1984). To directly explore the effects of DES on human reproductive tract development in vivo, human fetal reproductive tracts consisting of Fallopian tubes, uterine corpus, cervix and vagina were transplanted under the renal capsule of female athymic mice. Human fetal female reproductive tracts grown as grafts in intact (untreated) female athymic mice grew in size and developed normally. In this xenograft model the following results were obtained: (a) the paired Mullerian ducts fused to form a single uterovaginal canal, (b) endometrial and tubal mesenchyme differentiated normally into stromal and smooth muscle layers, (c) tubal and endometrial mucosa underwent plication, (d) endometrial glands formed, and (e) the simple columnar Mullerian vaginal epithelium underwent normal squamous differentiation (Robboy et al., 1982; Taguchi et al., 1983; Cunha et al., 1987a, 1987b). In contrast, human fetal female reproductive tract organs grafted into DES-treated female athymic mice developed abnormally exhibiting: (a) partial obliteration of the upper genital tract, (b) inhibition of differentiation of utero-tubal mesenchyme into stromal and smooth muscle layers, (c) inhibition of plical development in the Fallopian (uterine) tube, (d) inhibition of normal transformation of caudal Mullerian epithelium into a stratified squamous vaginal epithelium and (e) induction of vaginal adenosis (Robboy et al., 1982; Taguchi et al., 1983). Subsequent studies showed that certain "fertility drugs" (Clomid) had adverse developmental effects similar to that of DES on developing human female reproductive tracts (Cunha et al., 1987a). Today certain consumer products contain chemicals such as bis-phenol A (BPA, a weak estrogen) alleged to be harmful to human development based upon animal studies and correlational epidemiologic studies (Rochester, 2013; Vandenberg, 2014). Whether BPA actually can elicit an adverse effect on developing human organs has never been directly assessed. Such experiments are possible using transplants of human fetal reproductive tracts into mice treated with BPA. Finally, over many years the Cunha and Baskin labs have used the renal capsule site for the transplantation of tissue recombinants as described in this volume.

4. Materials and methods

The method of sub-renal capsule transplantation is illustrated in the NIH website on mammary gland biology (http://mammary.nih.gov/tools/mousework/Cunha001/index.html) and will be