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Review article

Mesenchymal-epithelial interaction techniques

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ABSTRACT

This paper reviews the importance of mesenchymal-epithelial interactions in development and gives detailed technical protocols for investigating these interactions. Successful analysis of mesenchymal-epithelial interactions requires knowing the ages in which embryonic, neonatal and adult organs can be separated into mesenchymal and epithelial tissues. Methods for separation of mesenchymal and epithelial tissues and preparation of tissue recombinants are described.

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1. Introduction

Interactions between epithelium and connective tissue play a central role in development of all organs composed of an epithelial parenchyma whether the epithelium is derived from ectoderm, endoderm or mesoderm. Such interactions are involved in development of the integument (ectoderm) and all of its derivatives such as the mammary gland, salivary glands, pituitary gland, preputial glands, hair, teeth, nails, feathers, claws and other derivatives. Likewise, the development of the endodermal gastrointestinal system and its derivatives (lungs, liver, pancreas, urinary bladder, prostate, gall bladder and the urethra) are dependent upon mesenchymal-epithelial interactions. Organs composed of mesoderm-derived epithelium (kidney, epididymis, vas deferens, seminal vesicle, uterus, cervix and vagina) also owe their development to mesenchymal-epithelial interactions. Thus, interactions between mesenchyme and epithelium are one of the fundamental developmental mechanisms responsible for the development of the majority of organs in the body.

In the embryo these interactions are called mesenchymal-epithelial interactions, signifying interactions between epithelium and undifferentiated embryonic connective tissue (mesenchyme). Such interactions between epithelium and connective tissue

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

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continue throughout life, but postnatally are called stromal-epithelial interactions (Cunha et al., 1985). The significance of embryonic mesenchymal-epithelial interactions is that during development the mesenchyme induces and specifies epithelial identify, and development (Cunha et al., 1983a; Haffen et al., 1987; Saxen, 1987; Birchmeier and Birchmeier, 1993; Baskin et al., 1996; Cunha and Hom, 1996), a process that includes specifying the types of secretory products produced by the epithelium (Cunha et al., 1995; Aboseif et al., 1999).

There are two types of tissue recombinants: homotypic and heterotypic. Homotypic tissue recombinants are composed of epithelium and mesenchyme derived from the same organ. For homotypic tissue recombinants the expectation is that the epithelium and mesenchyme will continue their normal development provided that both tissues are derived from wild-type mice. Of course, for homotypic tissue recombinants one tissue may be derived from a mutant mouse to assess the role of gene knockout on development. Heterotypic tissue recombinants, composed of epithelium and mesenchyme derived from different organ rudiments, are constructed with the expectation that the mesenchyme may reprogram epithelial development (Table 1). Reprogramming of epithelial development by heterotypic mesenchyme has been shown to involve: (a) Epithelial morphogenesis, (b) epithelial cytodifferentiation, (c) specification of keratin expression, (d) regulation of epithelial proliferation and apoptosis, (e) specification of secretory proteins, (f) induction of epithelial androgen receptor, (g) epithelial expression of heparin sulfate proteoglycans, (h) epithelial expression of p63, (i) epithelial expression of Hox genes, and (j) regulation of epithelial progesterone receptor (Cunha et al., 1997; Kurita et al., 2001a; Kurita and Cunha, 2001; Kurita et al., 2001b). Surprisingly, there appears to be germ layer restrictions on the ability of an epithelium to respond to a heterotypic mesenchyme. For example, urinary bladder epithelium is derived from endoderm and when combined with mesenchyme from either the urogenital sinus (a prostate inductor) or seminal vesicle, the result is prostatic differentiation (Table 1). Seminal vesicle and urogenital sinus mesenchyme are closely related anatomically and both are glandular inductors. While seminal vesicle mesenchyme can induce glandular differentiation in endodermal bladder epithelium, the endpoint is prostate and not seminal vesicle, perhaps because prostatic differentiation is within the development repertoire of endoderm, while seminal vesicle is not. Likewise, urogenital sinus mesenchyme induces seminal vesicle differentiation when combined with mesoderm-derived epithelia of the epididymis, ductus deferens and ureter (Table 1), again perhaps because seminal vesicle differentiation is within the development repertoire of mesoderm, while prostate is not. Thus, while the result in such tissue recombinants in a dramatic reprogramming of epithelial differentiation, the result is specified by the germ layer origin of the epithelium. An apparent exception to this rule is the induction of preputial gland differentiation by preputial gland mesenchyme in bladder epithelium (Taylor et al., 2009).

Based upon decades of research on mesenchymal-epithelial interactions, the following concepts have emerged. (a) Embryonic epithelial development and differentiation is impaired or completely abrogated in the absence of mesenchyme even though an appropriate extracellular matrix can substitute for living mesenchyme for certain aspects of epithelial development. (b) Mesenchyme induces and specifies epithelial development. (c) Mesenchyme itself undergoes a differentiation process, which is dependent upon an interaction with epithelium (Cunha et al., 1989; DiSandro et al., 1998). Thus, mesenchymal-epithelial interactions involve reciprocal signaling of mesenchyme to epithelium and epithelium to mesenchyme. (d) Temporal factors play an important role in morphogenetic mesenchymal-epithelial interactions, in so far as initially the epithelium is undifferentiated and not committed to a particular differentiation endpoint. Subsequently, as a result of mesenchymal induction, epithelial identity is covertly specified, that is, determined but not yet expressed. Continued mesenchymal-epithelial interactions promote specific morphogenetic programs such as ductal branching morphogenesis followed by specific forms of cytodifferentiation and ultimately the production of epithelial specific proteins, such as tissue-specific secretory proteins (Higgins et al., 1989; Cunha et al., 1995; Aboseif et al., 1999).

Mesenchymal specification of epithelial identity requires that the epithelium is uncommitted in regard to a particular differentiation endpoint, that is, capable to responding to inductive cues from heterotypic mesenchyme. The typical scenario is that early in development, the epithelium is developmentally plastic, uncommitted and thus capable of being reprogrammed by heterotypic mesenchyme. For example, Fig. 1 illustrates a series of tissue recombinants between neonatal epithelium and mesenchyme from the mouse uterus and vagina, each of which exhibits a unique histodifferentiation and a unique molecular profile. In this experiment uterine mesenchyme induced vaginal epithelium to undergo uterine differentiation, and vaginal mesenchyme induced uterine epithelium to undergo vaginal differentiation (Cunha, 1976). Subsequent studies have demonstrated that the newly induced uterine and vaginal cytodifferentiation is associated with expression of vaginal- and uterine-specific molecular markers (Kurita, 2011). The initial mesenchyme-induced covert commitment to a new phenotype may be an irreversible event in so far as the covertly determined epithelium may continue to express its

Table 1
Heterotypic inductions in which the mesenchyme elicits a new developmental fate in the epithelium.

Mesenchyme	Epithelium	Epithelial germ layer	Induced epithelial differentiation	Reference
Urogenital sinus	Adult bladder	Endoderm	Prostate	(Cunha et al., 1983b)
Urogenital sinus	Urethra	Endoderm	Prostate	(Boutin et al., 1991)
Seminal vesicle	Bladder	Endoderm	Prostate	(Donjacour and Cunha, 1988)
Seminal vesicle	Adult ureter	Mesoderm	Seminal vesicle	(Cunha et al., 1991; Lipschutz et al., 1996)
Seminal vesicle	Adult ductus deferens	Mesoderm	Seminal vesicle	(Cunha et al., 1991)
Seminal vesicle	Wolffian duct	Mesoderm	Seminal vesicle	(Higgins et al., 1989)
Seminal vesicle	Adult epididymis	Mesoderm	Seminal vesicle	(Turner et al., 1989; Cunha et al., 1992)
Uterus	Vagina	Mesoderm	Uterine	(Cunha, 1976)
Vagina	Uterus	Mesoderm	Vagina	(Cunha, 1976)
Mammary gland	Epidermis	Ectoderm	Mammary gland	(Cunha et al., 1995)
Rectum	Bladder	Endoderm	Rectum	(Li et al., 2000)
Back skin	Plantar foot skin	Ectoderm	Hair follicles	(Kollar, 1970)
Tooth	Plantar foot skin	Ectoderm	Tooth	(Kollar and Baird, 1970)
Duodenum	Stomach	Endoderm	Duodenum	(Ishizuya-Oka and Mizuno, 1984; Yasugi and Mizuno, 2008)
Preputial gland	Bladder	Endoderm	Preputial gland	(Taylor et al., 2009)