Differentiation 91 (2016) 42–49

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

Review article

Gene expression analysis in the compartments of the murine uterus ☆

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ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form
16 October 2015
Accepted 16 October 2015
Available online 2 December 2015

Keywords:
Luminal epithelium
Stroma
Immunofluorescence
Microarray
Uterus
LIF

ABSTRACT

Embryo implantation, a key critical feature of mammalian pregnancy, involves co-ordinate interplay between an incoming blastocyst and a receptive uterus. Aberrations in signaling cascades during this process result in pregnancy loss in mammals, including women. Analysis of the complete uterus at any given point either during preparation for implantation or during and after embryo attachment and invasion makes it difficult to assign specific signaling mechanism to the individual cellular compartments of the uterus. Here, we describe methods for the specific isolation of the luminal epithelium (LE) and subsequent analysis of gene expression/signaling pathways during embryo attachment. We further describe the analysis of RNA and proteins by specific techniques of quantitative PCR (qPCR), immunostaining and Western blotting of uterine tissues. These methods can be applied to the other cellular compartments of the uterus and embryo invasion and endometrial development. These techniques will be beneficial to investigators for delineating the mechanisms involved during embryo attachment and female reproduction as well as providing a means to studying highly dynamic changes in gene expression in tissues.

Contents

☆Funding source: Funded by the Singapore Biomedical Research Council and the Singapore Agency for Science, Technology and Research (A*STAR) to CLS.
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http://dx.doi.org/10.1016/j.diff.2015.10.009
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0301-4681/

1. Introduction

The mammalian uterus is essential for female reproduction in supporting the development of the embryo to term. A critical stage during pregnancy is embryo implantation. Embryo implantation is an intricate process involving interplay between a competent blastocyst and a receptive uterus. Failure at this critical stage mostly leads to embryo loss in mammals, and in human pregnancy it has been estimated that some 30% of conceptions are lost around the time of implantation. The uterus is composed of different cellular compartments: the myometrium; smooth muscle layer and the endometrium. The endometrium is comprised of the luminal epithelium (LE), the glandular epithelium (GE) and the stroma. The LE, GE and stroma undergo a cyclical series of changes, involving cell proliferation and differentiation, in preparation for pregnancy, that are called estrous cycles in mice and rats and the menstrual cycle in women.

In the murine reproductive cycle, cells in the LE, GE and stroma undergo sequential rounds of proliferation that are initiated by the ovarian steroid hormone, estrogen (E_2). Subsequently epithelial cell proliferation is inhibited and cell differentiation initiated by the other ovarian steroid hormone progesterone (P4) (Finn and Martin, 1974; Groothuis et al., 2007; Pan et al., 2006; Psychoyos, 1973; Tong and Pollard, 2002). Such sequential transformation of the endometrium is a prerequisite to prepare the uterus for embryo implantation. These same events initiated by the ovarian hormones or during embryo implantation can be reproduced in a highly defined way by first ovariectomizing adult mice and then priming them with a timed regimen of exogenous hormones that culminate in creating a uterine environment, primed for implantation that is similar to that occurring naturally (Chen et al., 2005; Rosario et al., 2014; Tong and Pollard, 1999, 2002). Such protocols have been essential for determining dynamic changes in the molecular pathways the two ovarian hormones either alone or in combination have in regulating the preparation of the uterus for implantation.

The LE, GE and stroma all respond to ovarian hormones during uterine preparation for implantation; however the functions of these different tissues differ. The LE is the first tissue of contact for the incoming embryos. In mice, despite adequate endometrial development in response to ovarian hormones, it requires a small surge of ovarian E_2 (named nidatory E_2) on morning of day 4 of gestation to establish receptivity to the blastocysts (Finn and Martin, 1974; McCormack and Greenwald, 1974; Psychoyos, 1973). The key function of nidatory E_2 is to induce the synthesis of Leukemia Inhibitory Factor (LIF) by the GE. LIF is a pro-inflammatory cytokine belonging to interleukin-6 (IL-6) family (Chen et al., 2000; Stewart et al., 1992). LIF binds to LIFR and gp130 heterodimeric complex that is expressed in the LE and activates the JAK–STAT3 pathway (Cheng et al., 2001; Rosario et al., 2014). Activation of JAK–STAT3 pathway induces a slew of downstream events that convert the LE from being non-receptive to being receptive for blastocyst implantation (Rosario et al., 2014). Using ovariectomized, hormonally primed females it is therefore possible to accurately define the temporal and spatial changes in gene and protein expression at the apposition and attachment stages of the implantation window in the response in the LE, GE and stroma to LIF or nidatory E_2.

The adherence of the blastocyst to the LE triggers the underlying stroma to undergo sequential proliferation and differentiation and convert from a stroma to a decidua – a process called decidualization. The primary functions of the decidual cells is to provide both nutrition to the developing embryo until a functional placenta is formed, as well as to restrain proliferation and invasion of the embryonic trophoblast (Robb et al., 1998).

There are at least 2 important issues regarding the molecular and physiological analysis of how the uterus regulates embryo implantation. The first is that it is extremely difficult, if not impossible, to recreate an effective and informative in vitro model system to study implantation. Secondly, almost all studies on the changes in uterine gene expression in preparation for or at embryo implantation, with few exceptions, have utilized the entire uterus for analysis (Afshar et al., 2012; Franco et al., 2010; Pan et al., 2006; Vitiello et al., 2008). Use of the entire uterus complicates any analysis by making it difficult to assign any change in gene expression to a specific tissue or cell type without detailed subsequent analysis. In fact, the LE comprises of only about 5% of the total number of cells within the uterus. Purified LE or stromal cells are therefore more suited to study alterations in gene expression specific to that particular uterine compartment. Laser capture microdissection (LCM) and enzyme digestion methods are two ways to obtain purified populations of LE or stroma (Bigsby et al., 1986; Campbell et al., 2006; Chen et al., 2006; Niklaus and Pollard, 2006; Sherwin et al., 2004; Yoon et al., 2004). In addition, LE can also be mechanically isolated (Pan et al., 2006).

Here, we provide a detailed description of the methods we have used to study the molecular changes in the uterus, following hormonal priming, in presence and absence of embryo, isolation of the LE, as well as techniques, particularly immunostaining, for analyzing the dynamic changes in RNA and protein expression in tissues and at the subcellular level. These techniques will help in global analysis of the events that take place in the LE during uterine development or embryo attachment. The techniques are also applicable to studying changes in gene expression in other tissues.

2. Methods

2.1. Mice

All animal experimentation was approved by the Institutional Animal Care and Use Committee (IACUC). The mice were maintained at the A*STAR Biological Resource Centre (BRC) facility.

2.2. Hormonal priming of mice

2.2.1. Non-pregnant mice

(1) 8–10 week old B6C3HF1 mice are ovariectomized (ovxd) under tri-bromo-ethanol anesthesia and rested for 12 days to eliminate endogenous ovarian hormones from the circulation (Tong and Pollard, 1999).
(2) On days 1–3, the mice are primed daily with 100 ng of E_2