Journal of Reproductive Health and Medicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Reproductive Health and Medicine

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

Review article

Gene profiling the window of implantation: Microarray analyses from human and rodent models

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ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form 8 November 2016
Accepted 13 November 2016
Available online xxx

Keywords:
Endometrial receptivity
Implantation site
Gene expression
Unexplained infertility
Recurrent implantation failure

ABSTRACT

Poor uterine receptivity leads to implantation defects or failure. Identification of uterine molecules crucial to uterine receptivity and/or embryo implantation provides the opportunity to design a diagnostic screening toolkit for uterine receptivity or targeted drug discovery for treating implantation-based infertility. In this regard, gene-profiling studies performed in humans and rodents have identified numerous genes involved in the transcriptional regulation of uterine receptivity and embryo implantation. In this article, we compared available uterine microarray datasets collected during the time of uterine receptivity and implantation in humans, mice and hamsters to uncover conserved gene sets. We also compared the transcriptome signature of women with unexplained infertility (UIF) and recurrent implantation failure (RIF) to gain insight into genes potentially dysregulated during endometrial receptivity or embryo implantation. Among numerous differentially expressed genes, few were revealed that might have molecular diagnostic screening potential for identifying the uterine receptive state during the time of implantation. Finally, functional annotation of gene sets uncovered altered uterine apoptosis or cell adhesion pathways in women with UIF and RIF, respectively. These conserved or divergent gene sets provide insights into the uterine receptive state for supporting blastocyst implantation.

1. Introduction

Blastocyst implantation failure in naturally-occurring and assisted human reproduction occurs in up to 2/3 of all cases, and has been attributed to delayed or failed receptivity. Defective endometrial receptivity is also considered a major cause of unexplained infertility (~10% of reproductive women) and abnormal pregnancies (~25 to 40%).[1–6] Past studies have demonstrated that certain morphological parameters and regulation of several uterine genes are associated with successful uterine receptivity and implantation. Predictors of the uterine receptive state are needed to better understand the causes of uterine-based infertility and help women in whom a poor uterine receptive state is considered a limiting factor for blastocyst implantation and pregnancy success. Identification of endometrial molecular signatures will provide the opportunity to design diagnostic screening tests for detecting uterine receptivity status, as well as therapeutic drug discovery for treating implantation-based infertility/pregnancy defects.

Embryo implantation is a multifaceted process beginning with attachment of the blastocyst to the uterine wall. Importantly, embryo implantation occurs during a specific "window" of time when the hormonally prepared uterus becomes receptive and the embryo has reached its proper developmental state.[7,8] In women, the uterus becomes receptive ~7 to 10 days after ovulation or the LH surge.[9] In mice, the uterus achieves receptivity after a transient pre-implantation estrogen (E_2) rise occurring around noon of day 4 of pregnancy.[10] Following the short window of implantation, the uterus becomes non-receptive to the implantation-competent blastocyst.[7,11] A challenging question has always been how to distinguish the normal or defective uterine receptive state from the non-receptive state.

Since embryo implantation is the initial event defining mammalian pregnancy, it is possible that this event is regulated by conserved gene functions across species. Shared features of embryo implantation in humans and mice include stromal

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http://dx.doi.org/10.1016/j.jrhm.2016.11.006
2214-420X/

decidualization and hemochorial mode of placentation. Differences in implantation include: (1) hormonal preparation of the receptive uterus, (2) mode of embryo implantation, (3) trophoblast attachment side (polarity) to the luminal epithelium (LE) and (4) timing of decidualization.[12] The uterus of humans, rhesus monkeys, pigs, rabbits, guinea pigs and hamsters require only ovarian progesterone (P_4) to prepare the uterus for blastocyst implantation, suggesting luteal estrogen may play a permissive role in these species.[13–21] However, the uterus of gerbils, rats and mice requires an active role of both P_4 and estrogen (E_2) to achieve its receptive state.[7,10,22] Blastocyst implantation in humans is interstitial (invasive), where the blastocyst completely embeds within the uterine stroma by displacing the underlying epithelium; while mice and hamsters exhibit an eccentric (displacement) type of implantation, where the blastocyst lies within a uterine crypt and causes loss of the underlying epithelial cells.[12] Despite these differences, the implantation process in most species involves an initial interaction between the trophectoderm of the blastocyst and the apical surface of the uterine LE.[23] Normally, the apical surface of the pre-receptive LE does not allow blastocyst attachment. However, the uterine transition from pre-receptive to receptive state permits fundamental structural and functional changes in epithelial cell organization,[24] allowing for successful blastocyst attachment.

Early gene expression analyses and gene targeting technology in mice yielded a substantial amount of information on the importance of individual genes required for uterine receptivity and blastocyst implantation. Such genes included a number of growth factors, cytokines, transcription factors, as well as others.[1,25–27] Given the complexity of blastocyst implantation in a receptive uterus, this most likely involves the actions of multiple gene families and gene-environment interactions. Identification of this genetic environment and genetic signaling networks remained a particular challenge for quite sometime, but the development of microarray and RNA-sequencing technologies has helped make such identifications possible.

To gain insight into the necessary genes for embryo implantation in humans, studies have compared the pre-receptive and receptive endometrium of non-conception cycles in order to avoid ethical constraints of collecting endometrial samples from conception cycles in which the embryo is present in the uterus. However, there is a single study to date that inadvertently collected endometrium from a conception cycle to study endometrial gene expressions in humans.[28] Studies using mouse and hamster models have added insight into the molecular basis of human implantation because of the existence of some important shared features. Over the past two decades, a variety of platforms have been implemented for measuring gene expression: oligonucleotide chips, cDNA microarrays, serial analysis of gene expression (SAGE) and, more recently, RNAseq. Each technology, utilized by multiple studies, has revealed many differentially expressed genes (DEGs) between: pre-receptive and receptive endometrium of humans,[29–34] rhesus monkeys,[35–37] rabbits[38] and mice[39]; post-implantation sites and non-implantation sites in hamsters[40] and mice.[41,42] However, the number of common DEGs is relatively small, as a result of either: (1) conservation of DEGs; (2) differences in experimental design and forms of technology used as older technology examine considerably smaller subsets of genes compared to current technology; and (3) variation in data analysis tools, such as use of various normalization or expression methods may yield different results.

The goal of this review is to provide a comprehensive analysis of available gene expression profiling data sets comparing pre-receptivity, receptivity and post-implantation endometria to elucidate genes needed for receptivity as well as embryo implantation. Data sets were restricted to microarray studies for reasons of: simplicity of comparison and lack of complete RNA-sequencing studies performed on mouse and hamster embryo implantation sites.

2. Endometrial receptivity transcriptomic profiling

2.1. Healthy, natural-cycling women

Many underlying causes of human infertility have been circumvented by in vitro fertilization (IVF) and embryo-transfer techniques. Despite this, implantation rates remain low, likely the result of transferring embryos into non-receptive endometrium. Therefore, several studies have compared the transcriptomics of the human endometrium in different phases of the menstrual cycle, including within the receptivity phase.[29–34] These studies demonstrated the existence of differential gene expression patterns in different phases, allowing classification of the endometrium based on its molecular signature. Exploitation of the endometrial signature at the receptivity phase has led to an endometrial receptivity array (ERA) as a clinically-utilized diagnostic tool to differentiate phases of the menstrual cycle, including the window of implantation.[43] The ERA has been used to demonstrate a shift in the window of implantation of patients with repeated implantation failure (RIF) and to guide their personalized embryo transfer as a novel therapeutic strategy.[44,45] Although improved by this approach, implantation rates of patients with RIF remain suboptimal.

The genes included in the ERA were selected from one study comparing DEG between the pre-receptive and receptive endometrium.[46] It is possible that the strength of the ERA could be improved by inclusion of DEGs obtained from other studies comparing the pre-receptive and receptive endometrium. To this end, we have combined the DEGs obtained from five studies (Table 1, green box) with the ERA, resulting in a 1541 non-overlapping 'Human Endometrial Receptivity' gene signature of healthy, naturally-cycling women. Of these 1541 DEGs, a total of 241 gene transcripts were shared by two or more microarray studies. We then compared the similarity of the 'Human Endometrial Receptivity' transcriptome signature to the endometrial transcriptome of patients with Unexplained Infertility or Recurrent Implantation failure, which is discussed below.

2.2. Patients with recurrent implantation failure (RIF)

Recurrent implantation failure (RIF) is diagnosed when high-quality embryos fail to implant following several IVF treatment cycles. In the absence of recognizable genital tract, embryonic and endocrine factors, studies have sought to identify genes whose aberrant expression is consistently associated with implantation failure.[47] These studies hypothesize that the pattern of endometrial gene expression during the receptive period may differ between women who have had successful versus failed embryo implantation following repeated embryo transfers.[47,48]

To date, two studies performed transcriptome analysis to identify DEGs in endometrial samples from women with RIF compared to spontaneously fertile women and patients with successful IVF treatment.[47,48] Endometrial samples were collected during a receptive period of induced endometrial cycle (used for IVF/embryo transfer) using exogenous E_2 and P_4. The authors state that the stimulation protocol performed before the endometrial sample collection was the same for all participating women in their studies. Thus, the differential transcript profile in patients with RIF suggests a long-term dysregulation of endometrial gene expression rendering it not suitable for embryo implantation.[47] These studies had a total of 6 overlapping DEGs [complement component 4 binding protein, alpha (C4BPA), Clusterin (CLU), Immunoglobulin

Please cite this article in press as: Herington JL, et al. Gene profiling the window of implantation: Microarray analyses from human and rodent models, *J Reprod Health Med.* (2016), http://dx.doi.org/10.1016/j.jrhm.2016.11.006