

Understanding the mechanisms of human tubal ectopic pregnancies: new evidence from knockout mouse models

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Ectopic pregnancy, a worldwide health problem, is potentially life-threatening and occurs in approximately 1.5–2% of all pregnancies in the western world; however, the precise mechanisms underlying the initiation and development of tubal ectopic pregnancy are unknown. Tubal abnormalities and dysfunction, such as altered contractility or abnormal ciliary activity, have been speculated to lead to tubal ectopic pregnancy. To elucidate the cellular and molecular mechanisms of the tubal transport process, several knockout (KO) mouse models have been developed. This review summarizes what has been learned from studies of the Fallopian tube in caspase-1, cannabinoid receptor and Dicer1 KO mice. Our understanding of the mechanisms which contribute to tubal ectopic pregnancy in humans may be enhanced through further study of these KO mouse models.

Key words: tubal ectopic pregnancy / animal models / knockout mice / Fallopian tube / endocannabinoids

Introduction

The aetiologies of many female reproductive tract diseases are unknown. Approximately 21 of 1000 reported pregnancies and 1 of 1000 women between 15 and 44 years old are diagnosed with ectopic pregnancy annually in the USA (Van Den Eeden *et al.*, 2005). However, throughout the western world ectopic pregnancy is estimated to occur in approximately 1.5–2% of all pregnancies (Barnhart, 2009). More than 95% of all ectopic pregnancies are located in the Fallopian tube (Corpa, 2006). Women with tubal ectopic pregnancy (tEP) not only have an increased rate of infertility, but also have an increased risk for tEP in future pregnancies (Farquhar, 2005). Fallopian tube transport of the gamete and early embryo into the uterus is crucial for the establishment of pregnancy in mammals (Jansen, 1984). Abnormalities in the structure and function of the Fallopian tube can interfere with the gamete/early embryo transport process and lead to tEP (Farquhar, 2005); however, the molecular mechanisms underlying this interference are not well characterized. Although several related risk factors have been proposed, such as pelvic infections, past or ever smoker and endometriosis (Farquhar, 2005; Corpa, 2006), the lack of appropriate animal models to study

tubal transport limits our understanding of the development of human tEP at the cellular and molecular levels. This paper provides an overview of current progress in our understanding of the molecular mechanisms responsible for tubal dysfunction gained from studies of genetically modified mice along side clinical studies, and a perspective on the importance of integrating the phenotype analysis as a tool for identifying and understanding the biological pathways in tubal dysfunction.

Rodent models to study Fallopian tube function

The mammalian Fallopian tube is a dynamic, steroid-responsive tissue (Jansen, 1984). The hormone 17 β -estradiol (E₂) is implicated in the regulation of cell proliferation, differentiation and apoptosis in the Fallopian tube (Monroe *et al.*, 2002). The key to the biological effects of E₂ is the activation of intracellular estrogen receptors (ER), which are ligand-inducible transcription factors that regulate the expression of many genes that control physiological processes (Dougherty and Sanders, 2005). Withdrawal of E₂ has been shown to induce cell

death and increase levels of *caspase-1* mRNA in the hen Fallopian tube (Monroe *et al.*, 2002). Our preliminary results, obtained by western blot and immunofluorescence assay, indicate that caspase-1 is expressed in human and mouse Fallopian tubes (unpublished data). Since human and rodent Fallopian tube cells express both ER α and ER β (Shao *et al.*, 2007a, b; Horne *et al.*, 2009), it would be predicted that the transcriptional regulation of caspase-1 is ER-dependent in the Fallopian tube *in vivo*; however, the primary function of caspase-1 is to activate inflammatory cytokines rather than promote cell death in the Fallopian tube. When compared with wild-type mice, female mice with a targeted deletion of *caspase-1* (Cheng *et al.*, 2008) were less susceptible to tubal inflammatory pathologies, such as the inflammatory infiltration and hydrosalpinx formation by *Chlamydia trachomatis* infection, one of the common risk factors for the induction of tEP in humans (Farquhar, 2005; Barnhart, 2009). More recently, caspase-1 inhibitor (Z-YVAD-fmk, Biovision, Mountain View, CA, USA) has been shown to suppress the growth and development of *C. trachomatis* in infected cervical epithelial cells *in vitro* (Abdul-Sater *et al.*, 2009). Such an observation implies that the activation of caspase-1 may result in the epithelial cells undergoing an inflammatory process during chlamydial infection *in vivo*. Therefore, further investigations to determine how the activation of endogenous caspase-1 affects the severity of the pathological tubal phenotype are warranted. We have previously shown that the tubal transport of oocyte-cumulus complex in superovulated immature mice is significantly enhanced or blocked in the presence of E₂ or the ER antagonist ICI 162780, respectively, demonstrating effects on ER-mediated tubal transport (Shao *et al.*, 2009a). In agreement with the known role of estrogen in regulating pro- or anti-inflammatory dynamics depending on the tissue/cell type involved, we hypothesize that abrogated E₂/ER signalling and caspase-1-mediated inflammation in the Fallopian tube possibly contribute to infection-induced tEP. We can also expect that the use of caspase-1 knockout (KO) mice treated with E₂ and/or an inducer of inflammation will help us to elucidate novel genes and pathways that play a functional role in the process of tubal transport.

Endocannabinoids, a group of endogenous neuromodulatory lipids, are known to activate two G protein-coupled cannabinoid receptors CBI and CB2 (Taylor *et al.*, 2007), which are widely and differentially expressed in many tissues and organs. It has been shown that only CBI is expressed in the Fallopian tube, and the levels of tubal CBI in the follicular phase are lower than those in the luteal phase in humans (Horne *et al.*, 2008). Progesterone action, via binding to the progesterone receptor, is involved in cellular function in the Fallopian tube (Shao *et al.*, 2006; Wånggren *et al.*, 2008). Although the steroid hormone regulation of tubal CBI expression has not yet been reported, elevated progesterone levels in the luteal phase have been proposed as a regulator of Fallopian tube CBI expression (Horne *et al.*, 2008). The biological importance of CBI in the tubal transport process is illustrated by the presence of retained embryos in mice that lack either *CB1* or *CB1/2*, or mice that have been treated with synthetic CBI antagonist (Wang *et al.*, 2004). In contrast to CBI and *CB1/2* KO mice, tubal transport is not affected in *CB2* KO mice (Wang *et al.*, 2008): this difference in phenotype may reflect a differential distribution of CB receptors in the mouse Fallopian tube. Moreover, clinical validation of the close association between CBI expression and human tEP comes from the fact that a decrease in

CBI mRNA expression in the Fallopian tube is observed in women with tEP (Horne *et al.*, 2008). Previously, Paria *et al.* (1996) reported that the Fallopian tubes of pregnant mice are capable of synthesizing anandamide, a metabolic ligand for CB receptors. While smoking is one of the risk factors for the development of tEP (Farquhar, 2005), pre-clinical studies have shown that nicotine exposure results in changes in endocannabinoid content in the brain (Le Foll *et al.*, 2008). Moreover, it has been speculated that an imbalance in endocannabinoid levels in the circulation and reproductive tissues in humans may be associated with an increased incidence of ectopic pregnancy (Taylor *et al.*, 2007). These observations, although limited, provide compelling evidence that disturbances of endocannabinoid/CBI signalling during tubal transport could result in the development of tEP in humans. The success of future experiments to identify the significant loci of *CB1* that predispose individuals to the common form of human tEP will depend on the biological complexity of this disorder (Horne *et al.*, 2008). Interestingly, induction of inflammation has been shown to inhibit CBI-mediated uterine contraction in rats (Dmitrieva and Berkley, 2002). Therefore, it is tempting to hypothesize that the inhibition of tubal CBI signalling by the *in vivo* action of cannabinoids may also contribute to inflammation-induced tubal cell injury, and thus, influence tubal transport.

Dicer (encoded by *Dicer1*), a ribonuclease III enzyme required for micro-RNA processing, has been demonstrated to play an essential role in embryo implantation in mice and in the human endometrium under pathophysiological conditions, such as endometriosis (Hong *et al.*, 2008). While the complete loss of *Dicer1* in mice results in early embryonic lethality (Bernstein *et al.*, 2003), a considerable number of studies in female mice carrying a floxed allele of *Dicer1* under the control of antimüllerian hormone receptor 2 promoter-driven Cre recombinase has revealed that these mice exhibit tubal hypotrophy with the formation of prominent tubal cysts, leading to the disruption of tubal transport (Hong *et al.*, 2008; Nagaraja *et al.*, 2008; Gonzalez and Behringer, 2009), which is likely a result of abnormalities in the function of the uterotubal junction (Gonzalez and Behringer, 2009). Moreover, disorganization of epithelial cells and smooth muscle cells (Gonzalez and Behringer, 2009) was found to correlate with alterations in the expression of tubal epithelial and smooth muscle cell markers after the removal of Dicer (Nagaraja *et al.*, 2008); however, impaired ciliogenesis and ciliated function were not observed in tissue-specific *Dicer1* KO mouse Fallopian tubes (Gonzalez and Behringer, 2009). To date, there are no published reports regarding the expression and regulation of tubal *Dicer1* in humans during tubal transport and in tEP. A more conclusive understanding of the precise mechanisms of how aberrant *Dicer1* protein leads to human tEP will require further study; however, the results discussed herein support the notion that the Dicer-mediated integrity of normal tubal structure and function is necessary for tubal transport. Although there is no difference in circulating E₂ levels between *Dicer1* KO and wild-type mice (Hong *et al.*, 2008; Gonzalez and Behringer, 2009), it has recently been reported that E₂ is able to rapidly up-regulate *Dicer1* gene expression through ER α activation in luminal-type A/ER α -positive breast cancer cells *in vitro* (Bhat-Nakshatri *et al.*, 2009). The differences between the KO study *in vivo* and the ER agonist results *in vitro* may be accounted for by the differences in acute versus chronic effects of E₂ in tissues/cells. Thus, in the Fallopian tube, it cannot be completely ruled out that

time-dependent E₂ action regulates the micro-RNA pathway by affecting Dicer expression.

Summary and perspective

There is a gap in our knowledge about the precise reasons for and the mechanisms underlying the implantation of an embryo in the Fallopian tube (Farquhar, 2005). Fallopian tube function is regulated by complex physiological and molecular processes, and therefore, the development of genetically modified animal models provides a powerful means to specifically investigate the impact of particular molecules and their discreet pathways in tubal function, as well as pathologies arising from the dysregulation of these molecules. The deletion of CBI or Dicer1 in mice results in the retention of an oocyte/embryo within the Fallopian tube and may provide clues for tubal abnormality-induced tEP in humans. It is important to note that while mice are genetically similar to humans and both share features of tubal cell physiology, tEP is present in humans but is absent or rare in rodents (Corpa, 2006). It is possible that the molecular mechanisms involved in tubal transport may be different in mice and humans, thus, not all of the information gained from mouse models may be applicable to human disease. The mammalian Fallopian tube is composed of heterogeneous cell types: ciliated and secretory epithelial cells as well as smooth muscle cells, all of which appear to be specialized to perform different functions (Shao *et al.*, 2007a, b). Although the intricate manner by which numerous factors regulate the process of tubal transport is clear, available studies indicate that ciliary beating and smooth muscle activity are the major factors responsible for propelling the gametes and embryo through the Fallopian tube (Jansen, 1984). Mechanisms regulating ciliary beating and smooth muscle activity are complex, and they can be either coordinately or independently responsible for different stages of tubal transport. In tubal cells, altered gene expression must presumably underlie the process of tubal transport. Therefore, extensive studies of temporal changes in the gene expression and in cell-specific signalling pathways in the Fallopian tube will be essential in order to understand the contribution of each of these factors to tubal function. Genetically modified mouse models could help us to elucidate the underlying molecular mechanisms that are responsible for ciliary beating and muscular activity in the Fallopian tube *in vivo* under physiological conditions and in diseased states. A first step would be to develop an *in vitro* tubal cell/organ culture system in which gene programmes/pathways could be manipulated and possibly scrutinized by microarray procedures. The net results of these future studies combined with information from KO mouse models will add further important insight into the biology of tubal transport processes. Moreover, the key issue becomes one of whether CBI or Dicer1 levels are regulated during tubal transport under physiological conditions, thus, the contribution of CBI and Dicer1 to the process of tubal transport in wild-type mice *in vivo* needs to be evaluated. Finally, experiments designed to rescue CBI or Dicer1 KO mice from the disruption of tubal transport observed in these models are needed to test the role of potential therapeutic targets. The coordination of oocyte/embryo–tubal epithelial cell interaction plays a role in this process (Jansen, 1984), and abnormalities in this interaction may also alter the timely homing of the oocyte/embryo in the Fallopian tube (Shao *et al.*, 2009b); hence, a combination of different strategies and clinical studies

(Barnhart, 2009) is necessary to dissect the mechanisms underlying tEP, still a poorly understood tubal disorder.

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