Developmental Biology 401 (2015) 287–298

Contents lists available at ScienceDirect

Developmental Biology

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

Oocyte glycoproteins regulate the form and function of the follicle basal lamina and theca cells

Alice P Christensen [a], Saloni H Patel [a], Patricia Grasa [a], Helen C Christian [b], Suzannah A Williams [a,*]

[a] Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Women's Centre, Level 3, John Radcliffe Hospital, Oxford OX3 9DU, UK
[b] Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK

ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form
28 October 2014
Accepted 20 December 2014
Available online 31 December 2014

Keywords:
Oocyte
Ovary
Follicle
Core 1 *O*-glycans
C1galt1
T-synthase
Extracellular matrix
Electron microscopy
Basal lamina
Multiple-oocyte follicle
Collagen
Laminin
ZP3 Cre recombinase
Floxed
Lox*P*

ABSTRACT

Maintaining follicle integrity during development, whereby each follicle is a functional unit containing a single oocyte, is essential for the generation of healthy oocytes. However, the mechanisms that regulate this critical function have not been determined. In this paper we investigate the role of the oocyte in maintaining follicle development. To investigate this role, we use a mouse model with oocyte-specific deletion of *C1galt1* which is required for the generation of core 1-derived *O*-glycans. The loss of oocyte-generated *O*-glycans results in the joining of follicles and the generation of Multiple-Oocyte Follicles (MOFs). The aim was to determine how Mutant follicle development is modified thus enabling follicles to join. Extracellular matrix and follicle permeability were studied using histology, immunohistochemistry and electron microscopy (EM). In ovaries containing Mutant Oocytes, the Follicle basal lamina (FBL) is altered both functionally and structurally from the primary stage onwards with Mutant follicles possessing unexpectedly thicker FBL. In Mutant ovaries, the theca cell layer is also modified with intermingling of theca between adjacent follicles. MOF function was analysed but despite increased numbers of preantral MOFs in Mutants, these do not reach the preovulatory stage after gonadotrophin stimulation. We propose a model describing how oocyte initiated changes in FBL and theca cells result in follicles joining. These data reveal new and important roles for the oocyte in follicle development and follicle integrity.

Introduction

Ovarian follicle development is intricately regulated resulting in the generation of numerous functional follicles from which the appropriate number for each species is selected to ovulate. The role of the oocyte in follicle development is complex and has been gradually unravelled during the last 20–30 years. Initial studies were able to determine that the oocyte had a role in cumulus expansion (Buccione et al., 1990; Vanderhyden et al., 1990) and granulosa cells proliferation, differentiation and steroidogensis (Vanderhyden, 1996; Vanderhyden et al., 1990, 1993; Vanderhyden and Macdonald, 1998). However, in 1996, the oocyte was revealed as critical to follicle development since follicles containing oocytes lacking GDF-9 failed to develop beyond the primary stage (Dong et al., 1996). Furthermore, elegant studies by Eppig et al. (2002) using reaggregated ovaries revealed that the rate of follicle development was oocyte regulated. The oocyte also has many other important and potentially subtle influences on follicle development including regulation of the growth and differentiation of granulosa cells in multiple species (McNatty et al., 2005a, 2005b). Thus the oocyte has a critical role in follicle development and can affect its own destiny.

More recently it has been shown that glycans of glycoproteins generated by the oocyte also have important roles in follicle development. These glycans not only alter follicle development but also ovulation rate. Glycoproteins are proteins with carbohydrate molecules attached at specific motifs in a process known as glycosylation (Marino et al., 2010). Glycosylation is a highly organised process that requires specific enzymes for the addition of specific sugars in a stepwise manner to generate specific structures (reviewed in Stanley, 2011); complex *O*-glycans are one such type of structure (Fig. 1). Glycosylation is the most common form of post-translational modification and various

* Corresponding author.
E-mail address: suzannah.williams@obs-gyn.ox.ac.uk (S. Williams).

http://dx.doi.org/10.1016/j.ydbio.2014.12.024
0012-1606/

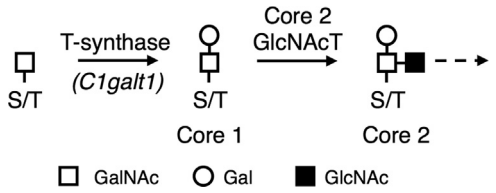

Fig. 1. Generation of core 1-derived *O*-glycans. The glycosyltransferase T-synthase encoded by *C1galt1* is required for the generation of the core 1 *O*-glycan by transferring galactose (Gal) to *N*-acetylgalactosamine (GalNAc) on serine/threonine residues (S/T). This core 1 structure can be further extended by other specified glycosyltransferases.

glycoproteins are produced by many different cell types including secreted proteins, receptor proteins, and binding proteins. Glycosylation not only modifies the structure of the protein but can also affect protein function such as secretion, half-life, and receptor binding (Jiang et al., 2014; Marino et al., 2010). Many molecules critical for female reproduction are glycoproteins including FSH and LH from the pituitary and their receptors (Jiang et al., 2014), granulosa cell secreted AMH (Pankhurst and McLennan, 2013), oocyte-secreted GDF-9 (Hayashi et al., 1999) and the zona pellucida proteins (Bleil and Wassarman, 1980).

Important roles for oocyte generated *O*- and *N*-glycans in follicle development and ovarian function have been revealed in more recent years regulating many aspects including zona morphology (Shi et al., 2004; Williams et al., 2007), transzonal process (TZP) quantity (Williams and Stanley, 2009b), granulosa cell luteinisation (Williams and Stanley, 2009b), follicle development and selection (Grasa et al., 2012, 2014; Williams and Stanley, 2008), apoptosis (Grasa et al., 2014) and the maintenance of follicle integrity (Williams and Stanley, 2008). These discoveries have been achieved thought the advent of *Cre loxP* technology (Lewandoski et al., 1997) since ubiquitous ablation of these glycans causes embryonic lethality (Shi et al., 2004; Williams et al., 2007).

Follicles exist and function within the ovary as individual units. Follicle integrity, and thus basal lamina function, is altered in mice with oocyte-specific ablation of core 1-derived *O*-glycans because follicles containing oocytes deficient in *O*-glycans can join to form Multiple-Oocyte Follicles (MOFs; follicles containing two or more oocytes) (Williams and Stanley, 2008). Oocyte-specific ablation of core 1-derived *O*-glycans is achieved using mice homozygous for the floxed *C1galt1* gene (previously referred to as *T-syn*) carrying a ZP3*Cre* recombinase transgene (Grasa et al., 2012, 2014; Shi et al., 2004; Williams and Stanley, 2008, 2009a, 2009b, 2011; Williams et al., 2007). *C1galt1* encodes core 1 β1,3-galactosyltransferase (T-synthase) responsible for the formation of the core 1-derived *O*-glycans (Fig. 1). The oocytes of *C1galt1*$^{F/F}$:ZP3*Cre* (*C1galt1* Mutant) mice are thus unable to generate core 1-derived *O*-glycans and all oocyte-generated glycoproteins that contain the core 1 glycan structure are modified leading to the generation of MOFs. The mechanism of MOF formation in *C1galt1* Mutant mice is distinct from MOF generation formed by incomplete breakdown of the germ cell nest at birth which occurs sporadically in wild-type mice (Pepling, 2012). An alternative mechanism for MOF generation must exist in the *C1galt1* Mutant since oocyte-specific deletion of *C1galt1* is initiated by a ZP3*Cre* transgene, which is only active from the primary follicle stage onwards (Philpott et al., 1987). The numbers of MOFs are greater at preantral and antral stages in Mutant mice and they appear to form by the fusing of two adjacent follicles (Williams and Stanley, 2008). Mutant mice also have increased fertility (30–50% more pups than Controls) and an increased number of healthy follicles in the ovary (Williams and Stanley, 2008). The ovulation of multiple eggs from MOFs does not cause the increase in fertility since the number of corpora lutea (CL), eggs ovulated, and embryos implanted were equivalent (Williams and Stanley, 2008). It remains unclear whether MOFs are able to progress to the ovulatory stage with one, if not more, of their oocytes remaining healthy.

For follicles to join, follicle integrity must be breached implying that the follicle basal lamina (FBL), a specialised barrier of extracellular matrix between the granulosa and theca cells enclosing the follicle (Irving-Rodgers et al., 2010) is altered in this mouse model. The generation of the FBL begins at the primordial stage and is continually remodelled throughout follicle development to allow the follicle to expand and eventually rupture during ovulation (Irving-Rodgers et al., 2010). The FBL in the mouse ovary is comprised of numerous proteoglycans and glycoproteins, which include collagen type IV, laminin, perlecan, nidogen, fibronectin and usherin (Irving-Rodgers and Rodgers, 2006; Pearsall et al., 2002). The most abundant molecules are laminin and type IV collagen and are stabilised by forming a network with nidogen (Paulsson, 1992; Yurchenco and Schittny, 1990). However, the origin of these molecules still remains unclear, as do the signals that regulate the construction and development of the FBL. The FBL enables the development of a localized microenvironment within the follicle, essential for follicular function and development. The granulosa cells adjacent to the basal lamina are polarised and thus distinct from the inner granulosa cells. Additionally, as a consequence of its location and structure, the FBL regulates follicular growth by controlling entry of molecules into the follicle. In mice, molecules less that 100 kDa travel freely through the FBL, those between 100 kDa and 500 kDa selectively pass across, and those greater than 500 kDa do not cross the FBL (Perloff et al., 1954; Shalgi et al., 1973). The charge of molecules is also a determinant in their FBL permeability as more negatively charged molecules cross the membrane more readily (Hess et al., 1998). Since the FBL has such a critical role in the regulation of molecules entering the follicle, it is clear that changes to the composition of the FBL are likely to alter its form and also its function.

Previously, we have proposed that changes in the FBL in the *C1galt1* Mutant are involved in the generation of MOFs by either altered FBL synthesis or enhanced degradation during the course of normal remodelling (Williams and Stanley, 2008). Therefore investigating the role for oocyte-generated *O*-glycans in maintaining follicle integrity is important. The aim of this paper was to establish if and how the FBL is modified in *C1galt1* Mutant ovaries, and the consequence of these changes on follicle development. We show here that although MOFs can become preovulatory, the vast majority of preantral MOFs and antral MOFs present in Mutant ovaries do not progress to the preovulatory stage. We also demonstrate that glycoproteins generated by Mutant oocytes have altered not only the structure and function of the FBL but also the theca cells that surround the FBL in Mutant ovaries. These results reveal a novel role for the oocyte in FBL generation and theca function and we propose a new model for MOF formation.

Materials and methods

Mice

All animal studies using mice (*Mus musculus*) were carried out with approval of the Local Ethical Review Panel at the University of Oxford under licence in accordance with the UK Animals (Scientific Procedures) Act 1986. The mice carrying the *C1galt1* floxed allele with the ZP3*Cre* transgene were maintained in a mixed genetic background of 129/SvlmJ and C57BL/6 J (Williams et al., 2007). Female mice were homozygous floxed *C1galt1* with Mutants also containing one copy of the ZP3*Cre recombinase* transgene (Mutant: *C1galt1*$^{F/F}$:ZP3*Cre*, Control: *C1galt1*$^{F/F}$) as the