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Transcriptional profiling validates involvement of extracellular matrix and proteinases genes in mouse gonad development

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ABSTRACT

Extracellular matrix (ECM) plays an important scaffolding role in the establishment of organs structure during development. A great number of ECM components and enzymes (proteinases) regulating formation/degradation of ECM during organ remodeling have been identified. In order to study the role of ECM in the mouse gonad development, especially during sexual differentiation of the gonads when the structure of the testis and ovary becomes established, we performed a global analysis of transcriptome in three main cell types of developing gonad (supporting, interstitial/stromal and germ cells) using transgenic mice, cell sorting and microarray. The genes coding for ECM components were mostly expressed in two gonadal cell lines: supporting and interstitial/stromal cells. These two cell lines differed in the expression pattern of ECM components, which suggests that ECM components might be crucial for differentiation of gonad compartments (for example testis cords vs. interstitium in XY gonads). Collagens and proteoglycans coding genes were mainly expressed in the interstitium/stromal cells, while non-collagen glycoproteins and matricellular coding genes were expressed in both cell lines. We also analyzed the expression of genes encoding ECM enzymes that are secreted to the ECM where they remodel the scaffolding of developing organs. We found that the ECM enzyme genes were also mostly expressed in supporting and interstitial/stromal cells. In contrast to the somatic cells, the germ cells expressed only limited number of ECM components and enzymes. This suggests that the germ line cells do not participate, or play only a minor role, in the sculpting of the gonad structure via ECM synthesis and remodeling. Importantly, the supporting cells showed the sex-specific pattern of expression of ECM components. However, the pattern of expression of most ECM enzymes in the somatic and germ cells is independent on the sex of the gonad. Further studies are required to elucidate the exact roles of identified genes in sexual differentiation of the gonads.

1. Introduction

The structure of testes and ovaries emerges from the common bipotential anlagen – the genital ridges. During sexual differentiation the cells of the genital ridges gather in the sex specific arrangements, which leads to the development of elongated testis cords in the male gonads or spherical ovarian cysts in the female gonads (Fig. 1).

In the mouse, the genital ridges appear at the ventromedial surface of mesonephroi as the longitudinal thickenings of the coelomic epithelium at about 9.5 dpc (*dies post coitum*). In this location, the cells of monolayer coelomic epithelium proliferate forming multilayer cluster of coelomic-epithelium derived cells, under which the basement membrane disintegrates (Hu et al., 2013; Tanaka and Nishinakamura, 2014). The primordial germ cells (PGCs) settle in these thickenings of coelomic epithelium. At about 10.5 dpc, the expression of the male sex determining *Sry* gene is initiated in the coelomic-derived XY somatic cells, while this gene is absent in XX fetuses (Hacker et al., 1995). At 11.5 dpc, SRY upregulates the expression of *Sox9* – another male sex determining gene. Subsequently, at about 11.5 dpc, the SOX9-positive cells gather and enclose the germ cells and differentiate into the pre-Sertoli cells (XY supporting cells). At about 12.5 dpc the pre-Sertoli cells enclosing the germ cells form the primitive testis cords, which clearly distinguish the male gonads from the ovaries (Karl and Capel, 1995). At 13.5 dpc, solid and elongated definitive testis cords are present in the developing

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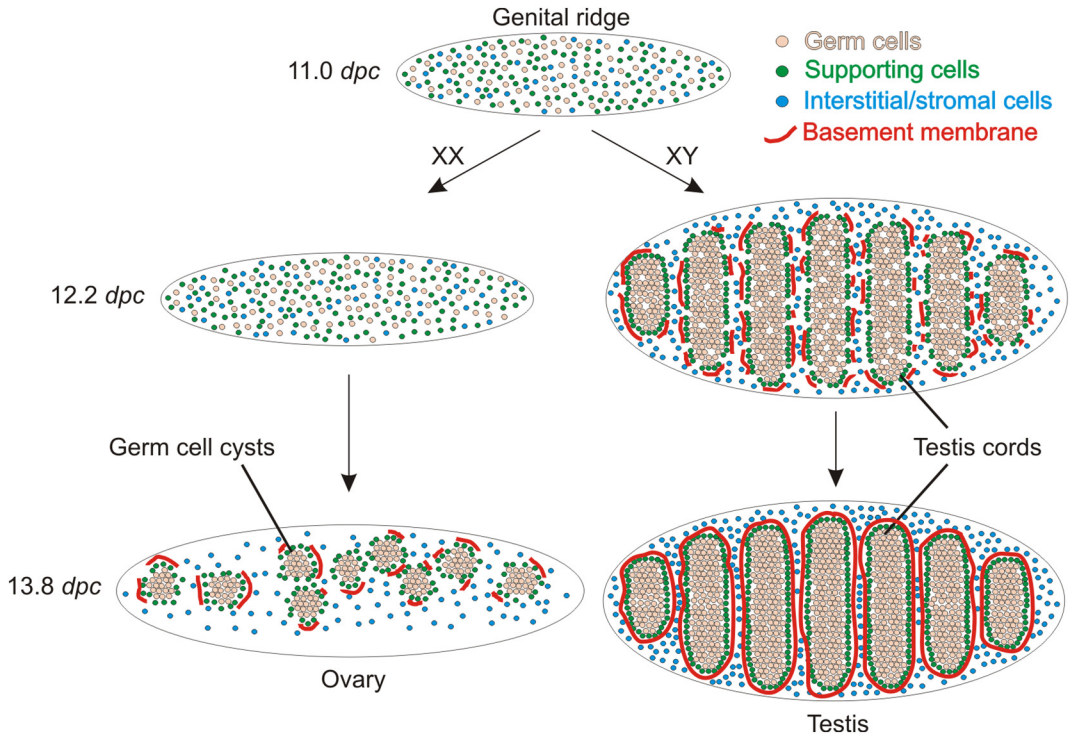

Fig. 1. Diagram of the changes in gonad structure and the basement membrane formation during sexual differentiation of mouse gonads. The basement membrane forms around 12.2 dpc in XY gonads when the first signs of testis formation occur. In XX gonads the basement membrane forms around developing germ cysts at 13.8 dpc.

male gonads (Combes et al., 2009). In contrast, in the XX mouse gonads the *Sry gene* is absent, *Sox9* is not upregulated, and the coelomic epithelium-derived cells do not form solid cords. The pre-follicular cells (XX supporting cells) enclose the XX germ cells and form the germ cell cysts, which at birth transform into the ovarian follicles (Wilhelm et al., 2007) (Fig. 1).

The cellular mechanisms driving the formation of testicular vs. ovarian structure still remain obscure. Our recent study indicated a role of cell adhesion in establishing the gonadal structure (Piprek et al., 2017). Here we focused on the role of the extracellular matrix (ECM) and the basement membrane in the gonadogenesis because it is known that the ECM undergoes specific changes during the process of sexual differentiation of the gonads. The formation of elongated, solid testis cords in the developing male gonads is associated with deposition of basement membranes around these structures (Skinner et al., 1985). The testis cords are composed of the germ cells enclosed by differentiating Sertoli cells. Between the testis cords interstitium accumulates. The developing mouse ovaries do not form elongated, tubular structures enclosed by the basement membranes, and instead, at about 13.5 dpc the female germ cells aggregate into small accumulations termed the ovarian cysts. The ovarian cysts that are separated by the stromal cells (stroma) contain germ cells surrounded by differentiating follicular cells. After 13.5 dpc the basement membrane is clearly visible around ovarian cysts. Thus, developing gonads contain three main cell types: the supporting cells (Sertoli and follicular), interstitial/stromal, and the germ cells (Fig. 1). Because the development of male gonad relies on the early deposition of components of the basement membrane, which contributes to the formation of solid testis cords and separates them from the interstitium we hypothesized that the male sex-determining pathway, in contrast to the female sex-determining pathway, may upregulate expression of the components of the basement membrane. We also supposed that differential expression of genes coding for the components of ECM and for the enzymes (proteinases) responsible for ECM remodeling (deposition and disintegration) in three main cell types in XY and XX should play an important role in establishing testis or ovarian structure, and thus drives the sexual differentiation. In order to elucidate this issue, first the expression of these genes must be identified in these three cell types in the gonads of both sexes during their during sexual differentiation.

The ECM consists of an intercellular space filled with gels of polysaccharides and fibrous proteins. The basement membrane is a layer of concentrated ECM components, which in developing gonads encloses testis cords and germ cells cysts. The main components of basement membrane are the laminin, type IV and VII collagens, fibronectin and several proteoglycans such as perlecan. The molecular machinery involved in the basement membrane remodeling has been well studied in other developmental systems (Clark et al., 2008). The formation of a new basement membrane occurs through the exocytosis of components from the basal surface of the epithelial cells. The assembly of ECM is mediated by integrins also located at the basal surface of epithelial cells. The remodeling of the basement membrane is more complex and involves the expression and activation of metalloproteinases, MMPs – the proteolytic enzymes that digest components of ECM. Considering a great number of ECM components (about 50 genes in the mouse genome) and enzymes involved in EMC remodeling (23 MMPs and 4 inhibitors of MMPs, 29 ADAM, 19 ADAMTS and 17 other proteases in the mouse), we decided to perform a global profiling of gene expression using microarray in three XX and XY cell types at three different stages of mouse gonad development to show the evolution and the dynamics of the expression of this group of genes.

Three cell types (supporting, interstitial/stromal, and germ cells) were isolated from XX and XY gonads at three stages as previously described (Piprek et al., 2017). Our previous studies showed that in developing mouse gonads the cell gathering and segregation occurs at 11.4 dpc (days *post coitium*) (Piprek et al., 2017). Thus, in order to study gene expression before the onset of the sexual differentiation we isolated gonads at 11.0 dpc (13 tail somites; TS13), i.e. when gonads are filled with the mass of uniformly distributed somatic and germ cells (Fig. 1). The second time point selected for this study was 12.2 dpc (TS24), when a clear separation of interstitium and supporting cells appears in XY gonads (Fig. 1). The third time point was 13.8 dpc (TS34), when the testis cords had been already formed, and there are the first