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The Role of the Extracellular Matrix in Ovarian Follicle Development

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Abstract

Regulation of ovarian follicle development depends on endocrine- and paracrine-acting hormones, the 3-dimensional architecture of the follicle, and the physical rigidity of the surrounding tissue. These 3 forces are integrated throughout the life cycle of the follicle to ensure appropriate hormone secretion, differentiation of the somatic cells, and maturation of the oocyte. The process of in-follicle maturation provides a new tool for understanding ovarian follicle development under the influence of these factors.

Keywords

Follicle; alginate; in-follicle maturation

Folliculogenesis is a highly regulated process in which various endocrine, paracrine, and autocrine factors act in a spatial and temporal manner to regulate and coordinate the growth and development of the oocyte and its surrounding granulosa and theca cell layers. Intracellular communication among the various cell types and the stroma is required for normal follicle development and oocyte maturation and is highly dependent on the architecture of the follicle. As such, the extracellular matrix (ECM) within the follicle is believed to play an essential role in regulating follicle development.

The ovary contains a large reserve of inactive primordial follicles that contain nongrowing oocytes and nondividing, flattened (squamous) pregranulosa cells surrounded by a basal lamina. Following entry into the growing follicle pool, squamous pregranulosa cells transform into a single layer of cuboidal granulosa cells surrounding a growing oocyte. Granulosa cells proliferate to form multiple layers, a process that requires expansion of the basal lamina. In multilayer follicles, the theca cell layer is recruited from the stroma to surround the basal lamina. A fluid-filled antral cavity, or antrum, also forms in the granulosa cell compartment of the follicle. Follicle-stimulating hormone stimulates the development of mature preovulatory follicles, characterized by a large antrum and an oocyte that is competent for ovulation in response to luteinizing hormone (LH). Upon ovulation, the basal lamina is degraded, and the remaining granulosa cells differentiate into luteal cells to form the corpus luteum.

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The extensive and intricate changes in the physical arrangement of cells within a developing follicle suggest that the ECM is an important and dynamic factor in folliculogenesis. Several studies have noted that the composition of the ECM undergoes significant changes as each follicle matures. The ECM plays various roles in cellular development in many systems, and in the ovary, the ECM influences a variety of cellular processes, including cell morphology, aggregation and communication, proliferation, survival, and steroidogenesis. Beyond providing structural support for follicle formation and maturation, the ECM acts as a reservoir for paracrine and endocrine signals within the ovary and permits or restricts their access to cells within the follicle. Furthermore, secreted ECM proteins can act in an autocrine and paracrine manner to regulate follicle fate.

Advances in in vitro follicle culture have allowed the role of ECM in follicle and ovarian development to be studied more closely. In turn, as we learn more about the ECM and the impact the 3-dimensional (3D) architecture of the ovary has on follicle development, more effective in vitro culture systems can be developed that more faithfully mimic in vivo conditions.

ECM Expression in the Ovary

The ECM in the ovary has been characterized for several species, including bovine,[1–4] human,[5,6] rat,[7,8] ovine,[9,10] and equine.[11] Our laboratory characterized the expression of ECM within the mouse ovary based on follicle stage and cellular compartment using immunohistochemistry and real-time polymerase chain reaction.[12] Collagen I was present throughout the ovary, with higher concentrations in the ovarian surface epithelium and follicular compartments. Collagen IV was abundant in the theca cell compartment, with low-level expression in the stroma and granulosa cells. The distribution of collagen was consistent throughout follicle maturation. Fibronectin staining in the stroma and theca cell compartment increased throughout follicle development, while staining in the granulosa cell compartment decreased. Heavy staining was also observed in the follicular fluid of antral follicles. Laminin was localized primarily to the theca cell compartment, with a defined ring at the exterior of the follicular granulosa cells marking the basement membrane. Low levels of laminin were also apparent in the stroma and granulosa cell compartment. Taken together, the ECM content of the mouse ovary changes during follicular development and reveals a distinct spatial and temporal pattern that may underlie the intricate intracellular and endocrine communication that is necessary for follicle growth and maturation.

Regulation of Ovarian Cell Morphology

The ECM is required for maintaining the cell-cell inter-actions and communication necessary for follicle formation, development, and migration within the ovary. Cell adhesion, supported by the ECM, leads to changes in cell shape and motility that are necessary for various cellular functions during folliculogenesis. Several studies have shown that the in vitro culture of granulosa cells plated on matrices of ECM proteins of various types and densities leads to changes in cell adhesion and shape. Normal granulosa cell morphology in vivo is round, yet granulosa cells seeded onto uncoated tissue culture plastic resemble fibroblasts, flattened in a monolayer and spread with little interaction between neighboring cells.[13–20] When plated on type I collagen gels, isolated basal lamina, or Matrigel, however, granulosa cells retain their spherical, epithelioid shape.[13,16,17,21,22] Granulosa cell morphology is also determined by its cytoskeleton, the composition of which is influenced by the surrounding ECM. Rat granulosa cells plated on ECM from bovine corneal epithelium have fewer actin filaments distributed in a diffuse manner and maintain their epithelioid shape when compared with cells cultured on uncoated plastic. Novel surfaces for optimizing in vitro granulosa cell culture have been developed by attaching distinct adhesive peptides to otherwise non-adhesive materials.

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Our laboratory has demonstrated the capacity of RDG-modified alginate, a polysaccharide isolated from algae, to promote granulosa cell adhesion and maintenance of cell morphology in vitro.[23]

Regulation of Cell Aggregation and Communication

Intracellular communication between the oocyte, granulosa, and theca cells within a follicle is required for normal follicle development and oocyte maturation. This communication is made possible by highly organized cellular aggregation into distinct functional layers and a network of gap junctions between each cell type that facilitates the transfer of small metabolites, ions, and second messengers.[24] Aggregation of granulosa cells in culture mimics the in vivo environment. Cell-to-cell communication can be disrupted in the flat architecture represented in 2-dimensional (2D) systems, unlike those cells that aggregate on particular ECM surfaces or within 3D hydrogels. Those cells maintaining a spherical shape in vitro have increased cytoplasmic processes and gap junctions[13,14,22,25] for proper communication between neighboring cells.

Survival and Proliferation

Cultured granulosa cells have a finite life span. Rat, ovine, and human granulosa cells plated on tissue culture plastic become apoptotic and/or undergo rapid cell death, whereas granulosa cells plated on ECM survive for several days in culture.[9,20,26] The presence and composition of the ECM influences granulosa cell survival,[27,28] and distinct changes in ECM composition occur as follicles shift from the growing pool to undergo atresia.[29–31] Numerous studies have characterized the ECM protein types necessary to support granulosa cell survival in vitro.[9, 20,26] Human ovarian tissue slices cultured on Matrigel have a larger number of viable follicles after 2 weeks of culture compared with tissues cultured in the absence of ECM proteins.[32]

The ECM is also important for granulosa cell proliferation throughout folliculogenesis. In vitro, the presence of ECM supports granulosa cell growth and proliferation. Bovine granulosa cells plated on bovine cornea epithelium ECM proliferate more rapidly than cells plated on tissue culture plastic.[33] Fibronectin and laminin increase proliferation of ovine granulosa cells.[9] The effect of ECM proteins on granulosa cell proliferation is dependent on follicle size, suggesting that the ECM composition within each follicle changes throughout folliculogenesis. In a 3D culture system for intact follicles, type I collagen mixed with alginate and RGD-modified alginate supported the growth of 2-layer follicles but had no significant effect on multilayered preantral follicles. Laminin and fibronectin mixed with the alginate actually inhibited the proliferation of granulosa cells in multilayered follicles.[34] Mouse ovaries cultured in the presence of either type IV collagen or laminin show increased follicle densities and initiation of primordial follicle growth compared with ovaries cultured on polylysine. ECM composition also influences the actions of growth factors on follicle development. The addition of activin A to ovaries cultured on laminin led to an increase in multilayer follicle development (an increase in the ratio of multilayered follicles to the total number of follicles). When ovaries were cultured on type IV collagen, however, multilayered follicle growth was suppressed in the presence of activin A.[35] Taken together, these studies suggest that the ECM has a profound impact on follicle growth and maturation and that changes in ECM composition are necessary for the regulation of growth factor and hormone access to the follicle and the oocyte in a stage-specific manner.

Steroidogenesis

Progesterone plays a central role in ovulation and establishing and maintaining pregnancy. In vitro, progesterone production is determined by follicle stage, composition of the ECM, the method by which the ECM is presented (liquid vs solid), and the dimensionality of the culture

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(2D vs 3D). The presence of specific ECM proteins has been shown to influence progesterone production by the follicle. Progesterone production in mouse granulosa cells was stimulated by plating on RGD-modified alginate but not on alginate alone.[36] In rat and human granulosa cells, progesterone levels increased in the presence of bovine corneal endothelial ECM, HR9 mouse endodermal ECM, fibronectin, laminin, and type I collagen but decreased in the presence of Matrigel.[13,14,18,22,26,37,38] Type I collagen coating, type I collagen gels, polyhema-modified plates, and heparin-coated plates lead to decreased progesterone production in ovine granulosa cells isolated from large follicles (4–7 mm).[9] Immature and mature chicken granulosa cells plated with the solubilized basal lamina showed increased progesterone production compared with cells plated on tissue culture plastic. However, when plated on solid basal lamina, chicken granulosa cells from mature follicles had decreased progesterone production, whereas the immature granulosa cells mimic the effect seen in liquid basal lamina.[39] Porcine granulosa cells showed increased progesterone production when plated on type I collagen, fibronectin, and laminin,[40] but when type I collagen gel was overlaid on the cells, progesterone production was decreased.[41]

Estradiol is essential for follicle growth, and as a paracrine factor within the ovary, estradiol stimulates LH receptor expression, antrum formation, gap junction development, and prevention of atresia.[42] Human granulosa cells plated on serum-coated tissue culture plastic did not produce estradiol unless androstenedione was present in the media.[17] However, human granulosa cells cultured on type I collagen gel have been shown to secrete estradiol without the addition of steroid,[14] suggesting that the ECM affects steroidogenic capacity, steroid hormone secretion, or both. The composition of the ECM also influences estrogen secretion. Mouse granulosa cells secreted estradiol when plated on RGD-modified alginate but not alginate alone.[36] Ovine granulosa cells isolated from large follicles had increased estradiol secretion on type I collagen–coated tissue culture plastic, type I collagen gels, pHEMA, and heparin-modified tissue culture plastic but decreased secretion on fibronectin and laminin surfaces.[9]

Several studies provide evidence that the ECM influences steroidogenic enzyme activity within follicle cells. Expression of cholesterol side-chain cleavage cytochrome P450 (P450scc) and the steroid acute regulatory protein was examined in rat granulosa cells plated on HR9 mouse endodermal ECM modified with basic fibroblast growth factor (bFGF) or plated on laminin alone.[26] Only those cells plated on the bFGF-modified ECM maintained expression, whereas those plated on laminin did not. Porcine granulosa cells plated between type I collagen gels had reduced P450scc gene expression when compared with those cells without the overlaid gel.[41] Bovine granulosa cells cultured in an anchorage-independent system had higher levels of P450scc and 3β-hydroxysteroid dehydrogenase in response to cyclic adenosine monophosphate.[43]

Differentiation

At the time of ovulation, granulosa cells differentiate into luteal cells, which produces progesterone required to sustain pregnancy until placental development. Human granulosa cells isolated when oocytes are removed for in vitro fertilization are competent to differentiate into luteal cells. These cells have unique characteristics, including mitochondria clustering, more lipid droplets, microvilli, and clustering to form lumen-like structures.[44] Human granulosa cells cultured on Matrigel or bovine corneal epithelium ECM share many of the characteristics of differentiated granulosa cells, while cells plated on plastic do not,[20,22] suggesting that the ECM influences granulosa cell differentiation. Similarly, rat granulosa cells isolated from pregnant mare serum gonadotropin–primed preovulatory follicles and plated on laminin become enlarged and are referred to as steroidogenic cells.[38]

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CONCLUSIONS AND FUTURE DIRECTIONS

The application of tissue-engineering principles to the study of structure-function relationships in ovarian follicle growth and maturation has led to the development of versatile in vitro culture systems in which follicles can be studied in conditions that mimic the complex 3D environment of the ovary.[34,45,46] The ECM is a significant contributor to establishing stage-specific follicle microenvironments that allow or restrict access of growth factors and hormones to the follicle. The exposure of follicles to ECM components within this 3D culture system has facilitated the characterization of the cell-cell interactions and intracellular signaling events that are required for follicle growth, maturation, and function. These culture systems have the potential to advance our understanding of the dynamic spatiotemporal regulation of folliculogenesis by providing a 3D context in which both soluble and insoluble signals can be manipulated.

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