Resveratrol improves granulosa cell activity through mitochondrial biogenesis

Granulosa cells (GC) support the developing oocyte from its earliest primordial follicle stage, playing an essential role in oocyte maturation and human reproduction. These important cells provide energy for oocyte follicular maturation through carbohydrate, lipid, and protein metabolism (1). The energy needed by the oocyte is provided in the form of adenosine triphosphate, which is produced in the granulosa cell mitochondria and passed to the oocyte via gap junctions. Hence, the mitochondria serve a critical role in energy production for the growing oocyte. Mitochondrial dysfunction is implicated in cellular failures of aged oocytes, and one of the mechanisms for oocyte damage is chronic exposure to reactive oxygen species that damage mitochondrial DNA (2). In this study, the investigators impressively combine molecular biology approaches and imaging with electrophysiology to explore multiple aspects of GC function and energy metabolism. In doing so, they specifically describe the impact of resveratrol on the membrane potential of human GC, demonstrating that this polyphenol reduces potassium current conductance leading to membrane depolymerization and calcium influx and ultimately results in augmented GC metabolism and increased mitochondrial biogenesis. They then suggest that these downstream functions may render human GC more responsive to gonadotropins, including follicle-stimulating hormone, and may indicate a clinical application for resveratrol administration.

Voltage-gated potassium (Kv) channels are one of the most critical regulators of extracellular potassium levels and resting membrane potential in a variety of cell types (1, 3). Activated by characteristic changes in membrane potential, Kv channels can facilitate the influx of calcium (Ca^{2+}) or other ions and can trigger downstream signaling events. While the critical roles of Kv channels in signal conduction are well established in cardiac and muscle systems, the function of these channels in other cell types, such as the human GC profiled in this study, is less well understood. In porcine GC, however, two primary potassium currents have been identified, including "I_{KUR}," a fast activating, inactivating current discussed in this study (1, 4). Furthermore, avian GC have the capacity to produce action potentials, with Kv channels demonstrated to regulate their membrane potentials (4). In human GC, membrane depolarization and calcium influx via voltage-gated calcium channels may result in augmented mitochondrial biogenesis (1). In this study, the investigators confirm this hypothesis further by analyzing the role of Kv channels in calcium-dependent changes in GC energy metabolism and function, demonstrating a relationship between reduced I_{KUR} conductance, calcium influx, and enhanced mitochondrial function. While a few additional studies have elucidated a role for specific potassium channels in steroid hormone biosynthesis and have demonstrated that these channels may themselves be gonadotropin-responsive, how these channels may influence GC responses to gonadotropins and their downstream functions in ovulation remains to be elucidated (3).

Resveratrol is a naturally-occurring polyphenol compound, perhaps best known for its role as an antioxidant in a variety of systems and species. In contrast to its stereotyped role in inhibiting oxidative stress, the function of resveratrol in granulosa cell proliferation, energy metabolism, and gonadotropin-responsiveness varies widely between species. For example, resveratrol has been shown to inhibit proliferation in rodent theca cells, while promoting the proliferation of ovine GC (1). While folliculogenesis is analogous between the species and human follicle development, the disparate cell types as well as the difference between poly-ovulatory rodents versus mono-ovulatory sheep result in no clear hypothesis regarding the role of resveratrol in human GC proliferation and function. Interestingly, in bovine models, treatment with resveratrol can induce mitochondrial biogenesis and improved energy metabolism in aged oocytes (1). In this study, the investigators aim to address these conflicting data by elucidating the functional role of resveratrol in the proliferation, metabolism, and electrophysiological properties of human GC.

This study provides the first clear evidence for a functional role of resveratrol in many aspects of human GC dynamics and function. Importantly, in addition to immortalized human GC derived from a solid primary tumor, the investigators also test their hypotheses in isolated primary GC, collected from women undergoing oocyte retrieval as a result of assisted reproductive technology. While these two systems complement each other well and allow for robust conclusions from the immortalized cell line which can be tested in the primary cell lines, the small sample size of seven patients in the oocyte retrieval cohort is limiting, as the investigators readily acknowledge. In addition, while all of these patients were undergoing oocyte retrieval for the purpose of in vitro fertilization, they were pursuing these interventions for an array of clinical reasons, potentially introducing considerable variation among the GC populations obtained from these women. Indeed, the investigators do find that they can only validate the conclusions reached from the immortalized cell line in subsets of their primary cell lines. Overall, they showed that resveratrol increased mitochondrial number and activity in the immortalized granulosa cell line and the primary cell lines, which led to improved GC viability and proliferation at low resveratrol concentrations of 3 μM (1). At higher concentrations, however, a cytostatic effect was observed. Therefore, part of the disparity in GC growth between different studies and animal models could be related to a dose response. This study is a concrete advancement toward a better understanding of the ways in which resveratrol impacts GC function, but future studies should consider revisiting the question of primary cell lines with a greater cohort size, standardized for the cause of assisted reproductive technology intervention.

There is preliminary evidence from other human studies that resveratrol may improve human oocyte and embryo development by affecting the oocyte as well as GC via effects on mitochondria and angiogenesis pathways. Liu et al. (2)

studied the impact of resveratrol on human germinal vesicle oocytes. Seventy-five germinal vesicle-stage oocytes from aged patients were treated with 1.0 mM resveratrol for 24 and 36 hours. Formation rates of MII-stage oocytes in the culture medium containing 1.0 mM resveratrol (24 hours, 55.26%; 36 hours, 71.05%) were higher than those of the control group (24 hours, 37.84%; 36 hours, 51.35%) (1). In addition, the percentages of spindles with abnormal morphology and chromosomes with irregular arrangements were reduced in MII-stage oocytes treated with 1.0 mM resveratrol ($P<.05$) (2). Although this study did not evaluate GC directly, it provides indirect evidence that structural abnormalities related to mitochondrial function were improved in the resveratrol-treated group. The only randomized clinical trial using resveratrol was performed in a population with polycystic ovary syndrome (PCOS) (5). Sixty-two patients with PCOS were randomly assigned to two groups: resveratrol (800 mg/day) or placebo for 40 days from the beginning of their cycle until retrieval day. The high-quality oocyte rate and high-quality embryo rate were higher in the resveratrol group compared with that in the placebo group (82% vs 69% and 12% vs. 9%, respectively; $P<.05$) (5). They speculated that the mechanism was related to changing the serum levels of some sex hormones and the expression of *VEGF* and *HIF1* genes in the angiogenesis pathway of GC. Therefore, more work must be conducted to understand the different mechanisms in which resveratrol may beneficially impact follicular development and which patient population would benefit the most from this treatment (women of advanced maternal age versus those with PCOS).

The results presented in this intriguing study integrate many aspects of cell differentiation and function, inspiring further questions and potential future directions. A better understanding of the molecular mechanisms by which calcium signaling impacts functional outcomes in GC metabolism and mitochondrial biogenesis may help us better understand GC that fail to properly differentiate or potentiate follicle maturation. As the investigators describe, several signaling cascades have been implicated in downstream resveratrol responses; the ways in which these signaling pathways, including MAPK/ERK and Sirtuin, function downstream and cross-talk during GC differentiation will not only allow for a better understanding of the essential underlying mechanisms governing GC development but may also aid in the identification of small molecules to selectively target and promote GC differentiation. Finally, the mechanisms through which resveratrol-dependent effects interface with GC gonadotropin response remain to be revealed and may have profound clinical implications. Through these next critical studies, the complexities of GC differentiation may be illuminated further, along with potential clinical interventions to extend the reproductive lifespan and promote healthy folliculogenesis.

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