Induction of insulin resistance by phosphatidylinositol-3-kinase inhibitor in porcine granulosa cells

Insulin resistance on porcine granulosa cells was induced by wortmannin, the phosphatidylinositol-3-kinase inhibitor, and insulin signaling key molecules were investigated including GLUT4 and mitogen-activated protein kinase. Granulosa insulin resistance decreased GLUT4 expression but increased mitogen-activated protein kinase, indicating the cross talk between the metabolic and mitogenic pathways of insulin signaling in the ovary. (Fertil Steril® 2009;92:2119–21. ©2009 by American Society for Reproductive Medicine.)

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The sequences of MAPK were 5'-AACCCAAACAAGCGCATCACAGT-3' and 5'-GGAGCAGGACCA GATCCAA AAGG-3'. The sequences of β-actin were 5'-GATGAAGATT GGCATGGCTTT-3' and 5'-CACGAAGGCTCATTCAA-3'. We did an analysis of variance for group comparisons with Dunnett as a post hoc test with the DMSO group as the control by the SPSS 11.5 system software (SPSS, Inc., Chicago, IL). A value of \( P < .05 \) was considered statistically significant.

As a result, wortmannin-treated cells at 1.5 \( \mu \)mol/L with well morphology and viability had nearly 40% of glucose uptake by the vehicle-treated control cells, leading to 13.76% higher glucose level left (15.55/C6 1.62 mmol/L) in the medium than the control (13.27/C6 1.29 mmol/L, \( P < .05 \)). Although the uptake of glucose by granulosa cells treated with 3.0 \( \mu \)mol/L or 5.0 \( \mu \)mol/L wortmannin was reduced, their morphology and viability were damaged significantly.

Therefore, the optimal concentration of wortmannin-induced IR in granulosa cells for the following studies was determined to be 1.5 \( \mu \)mol/L.

In the fluorescence microscopy test, both glut4 (Fig. 1A) and MAPK (Fig. 1B) expressed at the cytoplasm and presented light flavovirens, whereas they were not detected in either nucleus or cell membrane. However, there was no expression in the negative control group. The porcine granulosa cells were treated with medium (control), 0.1% DMSO, or 1.5 \( \mu \)mol/L DMSO and 1.5 \( \mu \)mol/L wortmannin (IR) for another 48 hours. Messenger RNA expression was detected by reverse transcriptase-polymerase chain reaction for glut4 (C, a), MAPK (C, b), and β-actin (C, c) in granulosa cells. The target genes were determined by the intensity of ethidium bromide \( \times \) Area/β-actin \( \times \) Area with arbitrary unit (D). \(* P < .01 \) and \(** P < .05 \) versus DMSO.

Recently some reports showed that the insulin signal also exists in the microenvironment of the ovary, and impairment of the metabolic pathway in granulosa cells in women with PCOS possibly was due to defective local insulin action (6). Moran et al. (7) isolated plasma membrane fraction of ovarian tissue. They showed defective insulin signaling in the ovaries of women with PCOS, which was similar to the findings in insulin classic target tissues. Therefore, we examined the effects of experimentally induced IR on the cultured ovarian granulosa cells.

In our experiment, we confirmed the method of wortmannin induction with regard to IR (8) and clearly demonstrated for the first...
time that wortmannin can induce the granulosa cells to be IR by the inhibition of the PI-3 K activity in vitro. The model success was supported further by impaired [3-3H]glucose uptake by granulosa cells but increased the medium glucose levels. Moreover, a study shows that wortmannin not only blocks the PI-3 K activity but also consequently reduces insulin action (4). We found that IR in granulosa cells resulted in decreased glut4 expression but enhanced MAPK. This is in accordance with the reports by Gaster et al. (9) that slow-twitch fibers in subjects with type 2 diabetes have reduced glut4 content. Our results also are consistent with the conclusion of Montagnani et al. (4) that inhibition of the metabolic branch of insulin signaling leads to an enhanced mitogenic action of insulin in vascular endothelial cells. Inhibiting PI-3 K pathway can induce IR as shown in our study, but the expression of extracellular regulated protein kinases (ERK) 1/2 (a number of the MAPK family) is enhanced, which may serve as a physical feedback of insulin signaling and contribute to a compensatory mechanism to rescue resistance to insulin’s metabolic actions in the PCOS ovary (10). The reduction of glut4 of the metabolic signaling pathway and the enhancement of MAPK with respect to mitogenic potential suggest a cross talk between multiple intracellular insulin signaling pathways in ovarian cells.

It is not entirely clear whether the postreceptor signaling pathway is used in insulin-mediated steriodogenesis, but signaling by both the mitogen-activated kinase and the PI-3 K pathways are thought to be involved. One study proposed that inhibition of ERK activity, by its inhibitor U0126, significantly decreased the expression of P450c17 with concomitant reduction of androstenedione production. It suggests that ERK activity plays a positive role in promotion of steroidogenesis in thecal cells (11). However, another study found that P450c17 activity increased after specific inhibition of mitogen-activated ERK kinase (MEK)/ERK phosphorylation in normal human theca cells (12).

As for granulosa cells, one study found that the addition of PI-3 K inhibitor significantly decreased insulin-stimulated aromatase mRNA levels and E2 accumulation, whereas inhibition of the MAPK pathway significantly increased aromatase mRNA abundance (13). Our study showed that inhibition of PI-3 K contributed to the reduction of glut4 and enhancement of MAPK. In addition, our recent work found that IR could directly exaggerate androgenic potential within theca cells, suggesting a possible involvement of this ovarian metabolic phenotype in PCOS hyperandrogenemia (14). Therefore, insulin-induced granulosa steroidogenesis may be mediated by both PI-3 K and MAPK pathway activation. Such an association is further evidence that insulin sensitizer, such as troglitazone and metformin, effects via the MAPK pathway. Troglitazone could divergently alter expression of various insulin-receptor substrate molecules and insulin actions and could be used as an ovarian insulin sensitizer and mitogen/steroiodogenic inhibitor in PCOS (3). Furthermore, metformin has a direct effect on granulosa cells and makes phosphorylated protein kinase B (AKT) and MAPK expressions enhanced or decreased respectively in these cells (15).

However, the MAPK pathway is more complicated. We cannot rule out the possibility that the granulosa steroidogenesis also is modulated by an alternative signaling mechanism of MAPK pathway such as activation of mitogen-activated protein kinase kinase (MKK)3/p38 or Jun N-terminal kinase (JNK)1/2. Therefore, our study only offered a concomitant change of MAPK and steroidogenesis, both of which resulted from the inhibition of PI-3 K, other than the casual association between MAPK and androgenesis. A weakness of this study is that protein quantification has not been done because of insufficient cell availability, but semiquantification of the mRNA of the insulin signaling molecules was detected instead. The glut4 translation by confocal microscopy as a future direction deserves to be investigated to understand its significance to altered insulin signaling. In summary, IR induced by wortmannin could directly reduce the expression of glut4 but enhance MAPK expression, indicating the cross talk between two pathways of insulin signaling in ovarian cells.

REFERENCES