Molecular aspects linking insulin resistance to breast cancer by activation of cell signalling pathways.

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Molecular aspects linking insulin resistance to breast cancer by activation of cell signalling pathways

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Abstract

Recent findings suggest a connection between obesity and breast cancer. Obesity is linked with higher incidences of insulin resistance as part of the metabolic syndrome, resulting in chronically elevated insulin plasma levels. We examined the effect of high insulin concentrations (100 nM) on estrogen-receptor (ER) negative breast cancer cells (MDA-MB-231) and normal breast epithelial cells (MCF-10a).

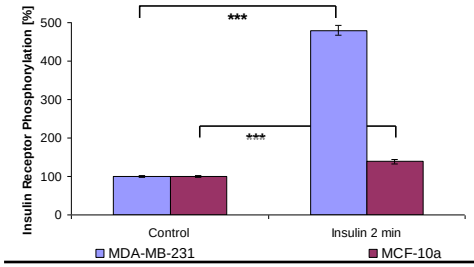
Treatment with high insulin concentrations increased insulin receptor (IR) phosphorylation significantly in both cell lines. Phosphorylation of protein kinase B (Akt), representative of PI3-kinase cell signalling pathway activation was increased by 101% (p=0.0112) in MDA-MB-231 cells and by 81% (p=0.0031) in MCF-10a cells after 10 min insulin treatment. Phosphorylation of extracellular regulated kinase 1/2 (ERK1/2), representative of MAP-kinase cell signalling pathway activation did not change in both cell lines after 10 min of insulin treatment. Cell proliferation did not change in MDA-MB-231 cells and increased by 75% (p=0.0067) in MCF-10a cells after 24 h insulin treatment. Cell proliferation was decreased in MDA-MB-231 cells by 15% (p=0.0083) after 24 h treatment with PD98059, a MAP-kinase inhibitor. In MCF-10a cells cell proliferation was decreased by 51% (p<0.0001) after 24 h treatment with wortmannin, a PI3-kinase inhibitor and by 56% (p=0.236) after 24 h treatment with PD98059.

These preliminary findings suggest that insulin resistance may increase carcinogenesis of breast epithelial cells but may not increase cancer progression in ER-negative breast tumours. Both the PI3-kinase and MAP-kinase pathway may be responsible for mediating insulin induced cell proliferation. Further studies are needed to verify the involvement of the MAP-kinase pathway in cell proliferation in MDA-MB-231 cells.

Results

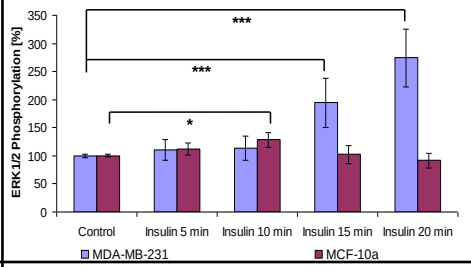
High insulin levels and Insulin Receptor phosphorylation

ELISA kits were used to measure IR phosphorylation after 2 min treatment with 100 nM Insulin



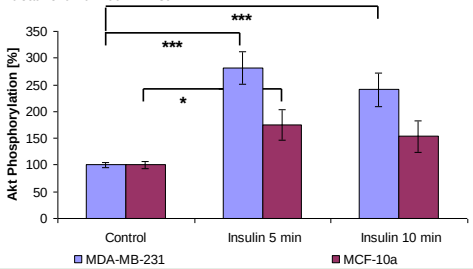
High insulin levels and MAP-kinase cell signalling pathway activation

ELISA kits were used to measure ERK1/2 phosphorylation after 5-20 min treatment with 100 nM Insulin



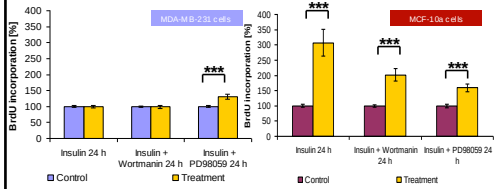
High insulin levels and PI3-kinase cell signalling pathway activation

ELISA kits were used to measure protein kinase B (Akt) phosphorylation after 5-10 min treatment with 100 nM Insulin



High insulin levels and cell proliferation

BrdU-incorporation was used to measure cell proliferation after treatment for 24 h with 100 nM Insulin alone or in combination with either 100 nM wortmannin or 50 µM PD98059.



All experiments are expressed as mean ± sem of three experiments with two replicates (n≥6), except BrdU (n≥18). T-test has been used to compare control to treatment.
* p<0.05; *** p<0.001

Objectives

1. **To examine the effects of high insulin levels (100 nM) on phosphorylation of Insulin Receptor in cancerous MDA-MB-231 and normal breast epithelial MCF-10a cells.**
2. **To examine activation of PI3-kinase and MAP-kinase cell signalling pathways after insulin treatment.**
3. **To examine cell proliferation in cancerous MDA-MB-231 and normal breast epithelial MCF-10a cells after insulin treatment.**
4. **To examine the role of PI3-kinase and MAP-kinase cell signalling pathways in cell proliferation.**

Conclusion

1. **High insulin levels affect both cancerous MDA-MB-231 cells and normal breast epithelial MCF-10a cells.**
2. **Activation of Insulin Receptor, PI3-kinase and MAP-kinase cell signalling pathways is higher in cancerous MDA-MB-231 cells than in normal breast epithelial MCF-10a cells.**
3. **But only normal breast epithelial MCF-10a cells respond to high insulin stimulation with increased cell proliferation, pointing to a role of insulin resistance in breast cancer initiation.**
4. **High insulin levels stimulate cancerous MDA-MB-231 cells, but the physiological effects need to be investigated.**

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