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Insulin-induced gene expression changes in breast cancer cells and normal breast epithelial cells

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Introduction

Breast cancer is prevailing as the most diagnosed cancer in women. Obesity and its co-morbidities, including type-II diabetes, are increasing to epidemic proportions.

A pathological link between obesity, breast cancer risk and mortality has been established recently.

Insulin resistance has been closely associated with obesity. It is considered a pre-stage of type 2 diabetes and is characterised by chronic high circulating levels of insulin.

Previously we have demonstrated the ability of high insulin levels to differentially activate insulin receptor, PI3- kinase and MAP-kinase cell signalling pathways in MDA-MB 231 human breast cancer cells and in MCF-10a human normal breast epithelial cells in addition to increase cell proliferation in MCF-10a cells.

We here demonstrate changes in gene expression profiles after treatment of both cell lines with 100 nM insulin for 1 h.

Objective

To examine the effects of high insulin levels (100 nM) on gene expression in MDA-MB 231 cells and MCF-10a cells.

Method

Oligo GEArray® Human Cancer PathwayFinder™ Microarray from SABiosciences was used to detect gene expression changes.

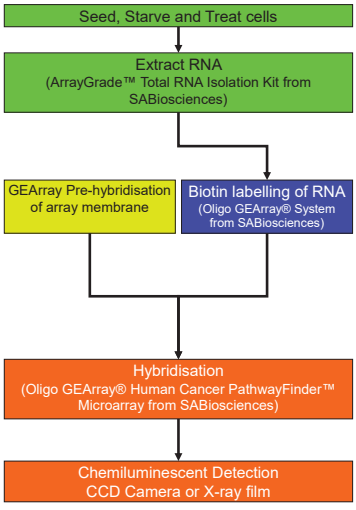
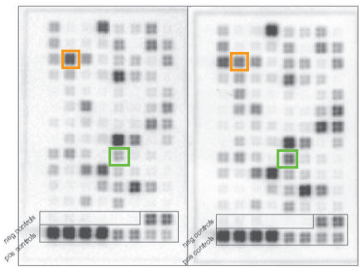


Figure 1: Flowchart of Microarray-analysis

Results



Control 100 nM Insulin

Gene expression increased after treatment

Gene expression decreased after treatment

Figure 2: Representative image of microarray result

Summary and Conclusion

High insulin levels increased expression of genes involved in cell cycle control (e.g. cyclin E1) and DNA damage repair (e.g. ATM) in MDA-MB 231 cells and in MCF-10a cells (e.g. CDC25a).

Expression of genes responsible for mediating apoptosis and cell senescence (e.g. APAF, BAD, bcl-X) was decreased after insulin treatment in MDA-MB 231 cells but the expression of the same group of genes did not change in MCF-10a cells.

High insulin levels increased expression of genes encoding for signal transduction molecules (e.g. AKT1) and transcription factors (e.g. FOS, JUN, MYC), and of genes responsible for invasion and metastasis (e.g. MMP2) in MCF-10a cells whereas gene expression of the same groups of genes did not change or was decreased in MDA-MB 231 cells.

These results suggest a role for insulin resistance in breast cancer initiation and progression, aggravating the potential of breast cancer cells to evade apoptosis, to metastasise and may promote carcinogenesis of healthy epithelial cells.

Detailed Results

Gene description	MDA-MB-231	MCF-10a
Ribosomal protein S27a	1.1	8.3
V-akt murine thymoma viral oncogene homolog 1	0.6	UP
Angiopoietin 1	0.2	ND
Angiopoietin 2	0.9	ND
Apoptotic peptidase activating factor 1	0.3	ND
Ataxia telangiectasia mutated	0.8	ND
BCL2-antagonist of cell death	0.7	ND
Brain-specific angiogenesis inhibitor 1	0.7	ND
BCL2-associated X protein	1.2	UP
B-cell CLL/lymphoma 2	6.1	UP
BCL2-like 1	2.5	ND
Baculoviral IAP repeat-containing 5 (survivin)	1.3	2.9
Breast cancer 1, early onset	2.8	ND
Breast cancer 2, early onset	0.6	UP
Caspase 8, apoptosis-related cysteine peptidase	0.7	ND
Caspase 9, apoptosis-related cysteine peptidase	0.9	UP
Cyclin D1	UP	ND
Cyclin E1	4.0	UP
CD44 molecule (Indian blood group)	3.7	UP
Cell division cycle 25 homolog A (S. pombe)	1.3	4.1
Cadherin 1, type 1, E-cadherin (epithelial)	DOWN	ND
Cyclin-dependent kinase 2	0.5	UP
Cyclin-dependent kinase 4	1.1	2.4
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	0.9	1.4
Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	UP	ND
Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	ND	ND
CASP8 and FADD-like apoptosis regulator	ND	ND
CHK2 checkpoint homolog (S. pombe)	ND	ND
Collagen, type XVIII, alpha 1	1.7	ND
Catenin (cadherin-associated protein), beta 1, 88kDa	DOWN	ND
E2F transcription factor 1	0.9	ND
Epidermal growth factor (beta-urogastrone)	0.3	ND
Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	2.6	4.6
V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	1.2	2.5
V-Ets erythroblastosis virus E26 oncogene homolog 2 (avian)	1.7	ND
Fibroblast growth factor 2 (basic)	ND	ND

Gene description	MDA-MB-231	MCF-10a
Jun oncogene	1.5	3.8
CD82 molecule	ND	ND
KiSS-1 metastasis-suppressor	ND	UP
Mitogen-activated protein kinase kinase 1	0.7	1.2
Mitogen-activated protein kinase 14	DOWN	ND
Melanoma cell adhesion molecule	0.4	ND
Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	DOWN	ND
Met proto-oncogene (hepatocyte growth factor receptor)	UP	ND
MHC class I polypeptide-related sequence A	UP	ND
Matrix metallopeptidase 1 (interstitial collagenase)	0.8	ND
Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	0.4	1.5
Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	DOWN	ND
Metastasis associated 1	DOWN	ND
Metastasis associated 1 family, member 2	DOWN	ND
Metastasis suppressor 1	ND	ND
V-myc myelocytomatosis viral oncogene homolog (avian)	ND	UP
Neural cell adhesion molecule 1	ND	ND
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	0.8	1.4
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	1.1	1.0
Non-metastatic cells 1, protein (NM23A) expressed in	1.0	1.9
Non-metastatic cells 4, protein expressed in	0.4	0.7
Platelet-derived growth factor alpha polypeptide	DOWN	ND
Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	0.3	ND
Phosphoinositide-3-kinase, catalytic, beta polypeptide	UP	UP
Phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	ND	1.8
Plasminogen activator, urokinase	1.0	1.2
Plasminogen activator, urokinase receptor	1.1	1.7
Pinin, desmosome associated protein	1.5	1.2
Protein kinase, DNA-activated, catalytic polypeptide	ND	ND
Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	DOWN	ND
V-raf-1 murine leukemia viral oncogene homolog 1	DOWN	ND
RAS p21 protein activator (GTPase activating protein) 1	ND	ND
Retinoblastoma 1 (including osteosarcoma)	2.4	2.6
S100 calcium binding protein A4	0.9	0.9
Serpin peptidase inhibitor, clade B (ovalbumin), member 2	1.2	2.0
Serpin peptidase inhibitor, clade B (ovalbumin), member 5	0.9	1.0

Table 1: Changes in gene expression expressed as fold of expression in control cells, i.e. expression in control cells is 1.0

≥1.5 gene expression increased with treatment (green)
≤0.7 gene expression decreased with treatment (orange)

"UP": expression only detected in treated cells
"DOWN": expression only detected in control cells
"ND": No expression detected in either cells

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breast cancer CAMPAIGN
researching the cure

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