Genes Involved in the Destruction of Leukaemic Cells by Induced Photosensitivity

(HL-60 / HEL / 5-aminolaevulinic acid / photodynamic process / gene expression / array technology)

M. BELIČKOVÁ, H. BRUCHOVÁ, H. CAJTHAMLOVÁ, Z. HRKAL, R. BRDIČKA

Institute of Haematology and Blood Transfusion, Prague, Czech Republic

Abstract. Gene expression changes were observed in the HEL and HL-60 cell lines after the stimulation of protoporphyrin IX synthesis by ALA administration and photodynamic process induction. Isolated ribonucleic acids were radiolabelled by reverse transcription, and the cDNA obtained was hybridized to membrane macroarrays (Clontech 7742-1) containing 588 gene probes. Besides changes in the activity of genes supposed to be involved in the programmed cell death and DNA reparation processes, increased or diminished transcription activity was also observed in several other genes; the reason for this phenomenon was not clear. The activation of programmed-cell-death genes appeared after the ALA load application, indicating the toxic effect of ALA. The gene expression changes observed in the two cell lines differed substantially, only a few of them were common for both cell lines.

One of the approaches to the virtually historical concept of the selective destruction of cancer cells without causing damage to normal cells is to provoke oxidative stress resulting from the high photosensitivity of cells accumulating protoporphyrin IX after induction with 5-aminolaevulinic acid (ALA). Although neither radiotherapy nor chemotherapy – the tools used in this regard – have achieved this goal perfectly, both are commonly and sometimes successfully used. The best results are achieved by means of the "additive" or "multiplicative" effect, which presumes that a selective combination of the different tools may increase their effectiveness and result in the resistance of cancer cells being more easily broken down. In each case, the processes leading to cell death are not yet fully understood, and further studies may still yield surprising results. The increasing number of known genes and the technological progress made in the development of membrane arrays, which allows the activity of hundreds of genes to be monitored simultaneously, makes the goal of finding a successful treatment for neoplasia more attainable. The authors' *in vitro* studies of promyelocytic leukaemia (HL-60) and human erythroleukaemia (HEL) cell lines, which have revealed a set of genes whose activity has been influenced by induction of the photodynamic process, are among the most recent steps that have been taken towards its better understanding.

Material and Methods

HL-60 (Collins, 1987) and HEL (Martin and Papayannopoulou, 1982) were cultured under the same conditions as given in the paper by Grebeňová et al. (1998). Likewise, the stimulation of internal protoporphyrin IX synthesis by incubation with ALA and light induction of the photodynamic process was carried out using the procedure set out by Grebeňová et al. (1998). Briefly, the transcription activity of leukaemic HL-60 and HEL cells was manipulated by their induction with 1 mM ALA for 4 h. Cells were then harvested, resuspended in a fresh RPMI-1640 medium without Phenol Red, adjusted to a density of 1×10^6/ml and either kept in the dark or immediately irradiated with an 18 J/cm^2 dose of blue light (illumination provided by two Osram Dulux DS9/71 discharge lamps from a distance of 25 cm for 60 min). The cell viability was checked by the Trypan blue exclusion method and by the flow cytometry/propidium iodide assay.

The number of viable cells always exceeded 90%. After harvesting the cells, total RNA was isolated by the method of Chomczynsky and Sacchi (1987), and 2 μg of total RNA was reverse-transcribed into cDNA (Clontech kit 7742-1, MMLV RT, Palo Alto, CA); this procedure also included labelling with ^{32}P (Amersham PB10204, α^{32}PdATP10Ci/l:3000Ci/mmol, Amersham, Buckinghamshire, England).

^{32}P-labelled cDNAs were hybridized to Atlas Human Cancer Array (Clontech 7742-1) and autoradiographed. The gene activity was evaluated with AtlasImage 1,01 (Clontech) software, used in accordance with the manufacturer's instructions.

The utility of Atlas Arrays for accurately assessing gene expression in a reproducible manner is well established (CLONTECHniques, 1997). In addition, the authors conducted their own control experiment to confirm the reproducibility of the Atlas technology. The same (control)

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Corresponding author: Radim Brdička, Institute of Hematology and Blood Transfusion, U Nemocnice 1, 128 20 Prague 2, Czech Republic. Fax: 420 (2) 21977371; e-mail: molgen@uhkt.cz.

Abbreviations: ALA – 5 aminolaevulinic acid, HEL – human erythroleukaemia cells, HL-60 – promyelocytic leukaemia cells.

Table 1. Gene expression changes in HL-60 cells.

Protein (gene)	Gene expression		
	A	B	C
DVL1 (dishevelled homolog)	++	++	++
EGR1 (early growth response 1, transcription factor ETR 103)	++	++	++
FAU	++	u	++
G22P1 (Ku protein subunit, ATP-depend. DNA helicase II70 kDa subunit)	++	u	++
NOTCH 1 (*notch1*)	++	u	++
HDGF (hepatoma-derived growth factor)	++	−−	+
MFNG (manic fringe)	++	−−	+
IL6R (interleukin-6 receptor)	++	−−	u
TOP2A (DNA topoisomerase II alpha)	++	−−	u
TRK-T3, P68 TRK-T3 oncoprotein (*trk*-fused gene)	++	−−	−−
MIF (macrophage migration inhibitory factor)	++	−−	−−
PDGF-associated protein	++	−	+
DDIT3 (*gadd153*-growth arrest and DNA-damage-inducible transcript 3)	+	+	++
xeroderma pigmentosum group C repair protein HHR23A (*rad23A*)	+	+	++
PDCD2 (programmed cell death-2)	−−	−	−−
VEGF, VPF (vascular endothelial growth factor)	−−	+	−
induced myeloid leukaemia cell differentiation protein MCL-1	−	++	u
CCNNA (cyclin A)	−	−	−−
CCNNB1 (cyclin B1)	−	−	−−
PLK (serine/threonine-protein kinase, *plk-1, stpk13*)	−	−	−−
CCNND2 (cyclin D2)	−	−−	−−
DNA-PK+DNA-PK catalytic subunit, XRCC7	u	−−	−−
CDC42 (*cdc42* homolog)	u	−−	−−
EB1 protein	u	−−	−−
CD59 glycoprotein	u	−−	−−
TRIP6, zyxin-related protein ZRP-1	u	−−	−−

Comparisons:
A: HL-60 influenced with ALA – non-influenced HL-60
B: HL-60 influenced with ALA and after illumination – HL-60 influenced with ALA
C: HL-60 influenced with ALA and after illumination – non-influenced HL-60
Letters in **bold** type indicate genes mentioned in the discussion. The gene expression symbols ++ or −− indicate a significant increase or decrease (more than twice as much or twice as less), + and − indicate changes of a lesser extent, u = unchanged (i.e. there was no difference in the compared expression), 0 = no expression in both cases.

sample was hybridized to two identical arrays, and the expression patterns obtained were, remarkably, identical.

Three samples of labelled cDNA from each cell line (1, 2, 3) were hybridized separately to identical membranes, thereby facilitating mutual comparisons (A, B, C):

1. "Standard" or "control" cDNA from cells cultivated under normal conditions
2. cDNA from cells influenced with ALA
3. cDNA from cells illuminated to induce the photodynamic process

A. 2 minus 1
B. 3 minus 2
C. 3 minus 1

Results

The results of evaluation of the transcription activity, conducted as described in the Material and Methods section above, have been summarized in Table 1 (HL-60 cell line), and Table 2 (HEL cell line).

Only the most distinct differences were taken into account, and the values obtained were converted into symbols indicating an increase or decrease in activity. This simplification naturally results in a substantial reduction in the information content of the results, but also allows the qualification of activity changes in genes with a very low standard transcription. In these cases, the calculation of a relative change is obviously impossible.