Table 2. Gene expression changes in HEL cells

Protein (gene)	Gene expression		
	A	B	C
MFNG (manic fringe)	++	+	++
TIE (tyrosine kinase receptor tie-1 precursor)	++	u	++
RAC2 (*ras*-related C3 botulinum toxin substrate 1, *p21-rac2*)	++	u	++
TIMP-1, *EPA* (erythroid potentiating activity)	++	–	+
PDCD2 (programmed cell death-2)	++	–	+
CTNNA 1 (alpha catenin, cadherin-associated protein, alpha E-catenin)	++	–	+
ZYX (zyxin + zyxin-2)	++	– –	++
MET (met proto-oncogene)	++	– –	0
xeroderma pigmentosum group C repair complementing protein HHR23A (*radd23A*)	++	– –	0
MGST1L1 (*pig12*, microsomal glutathione-S transferase 1-like 1)	++	– –	0
NRPB (*pig10*, nuclear restricted protein)	++	– –	0
NOTCH 1 (*notch1*)	++	– –	0
TOP1 (DNA topoisomerase I)	++	– –	0
DDIT3 (*gadd153*-growth arrest and DNA-damage-inducible transcript 3)	++	– –	0
PIN 1	++	– –	u
IGFBP2 (insulin-like growth factor-binding protein 2)	++	– –	u
STAT5B (signal transducer and transcription activator 5B)	+	++	++
ITGB3 (integrin beta3)	+	+	++
CD9 antigen (p24)	+	+	++
BTK (tyrosine-protein kinase BTK, Bruton's tyrosine kinase gene)	+	+	++
RAC1, RAS-like protein TC25 (*ras*-related C3 botulinum toxin substrate 1)	+	+	++
DNA excision repair protein ERCC1	+	+	++
CTNNB1 (beta-catenin)	+	+	++
GARP	+	+	++
FAU	+	+	++
ARHC (rhoC, small GTPase)	+	+	++
JUP (plakoglobin), DP3 (desmoplakin III)	+	+	++
PLK (serine/threonine-protein kinase, *plk-1, stpk13*)	+	– –	u
IRF1 (interferon regulatory factor 1)	+	– –	u
IL-10 (interleukin-10)	– –	++	+
ITGAE (integrin alpha E)	– –	++	+
RFC (replication factor C38-kDa subunit)	– –	++	u
PRKM9, stress-activated protein kinase JNK2 (C-JUN N-terminal kinase 2)	– –	++	u
CDK10, PISSLRE (*cdc2*-related protein kinase)	0	++	++
DLK (delta-like protein precursor, contains foetal antigen 1)	0	+	++
VEGF, VPF (vascular endothelial growth factor)	u	+	++

Comparisons:
A: HEL influenced with ALA – non-influenced HEL
B: HEL influenced with ALA and after illumination – HEL influenced with ALA
C: HEL influenced with ALA and after illumination – non-influenced HEL

Discussion

Influence of ALA

In this study, the authors did not concern themselves with the genes controlling the metabolic pathway of protoporphyrin IX synthesis, or further metabolic steps taking protoporhyrin IX as a substrate. This subject has been reviewed by Peng et al. (1997). ALA itself induces changes in the activity of several genes lying outside the metabolic pathway of protoporphyrin IX synthesis in both cell lines, and influences the transcription of some genes in both cell lines (*notch1*, Manic fringe and DDIT3). All of these are known to be involved in the differentiation processes (Carlesso et al., 1999), and in growth regulation (Kim et al., 1999). The Notch signalling pathway, when activated, for example, by cytokines, inhibits myeloid differentiation (Bigas, 1998). HEL cells seem to be more fragile and more sensitive to ALA load, as indicated by the increased transcription of, e.g., *gadd153*, *rad23A*, and the *pig10* and *pig12* representatives of *pig* group genes (Polyak et al., 1997; Jakobsson et al., 1999). This might result from DNA damage leading to apoptosis initiation. Surprisingly, a decline of PDCD2 transcription (programmed cell death-2), a gene known since 1995 (Kawakami et al., 1995), was observed in HL-60 cells, whereas in the HEL line it was increased. The *fau* gene also increased its activity in both cell lines after ALA administration, albeit to a lesser extent in HEL, and it also remained active after illumination. This may be a non-specific reaction resulting from its supposed housekeeping function.

Changes caused by the photodynamic effect provoked by illumination of cells with a high content of protoporhyrin IX

The first question to which the authors attempted to find an answer was whether apoptosis plays a principal role in the death of cells suffering from oxidative stress. More precisely, how much are apoptotic genes involved in this process (Polyak et al., 1997; Adams and Cory, 1998; Ashkenazi and Dixit, 1998; Miller and Marx, 1998; Piwnica-Worms, 1999)? Of the few hundred genes presumed to be involved in programmed cell death, only slightly more than a hundred probes were spotted on the membrane used, and conspicuous increases in activity were observed in few of them. This was evident in both, HL-60 and HEL cells after the application of ALA, as indicated in tables and mentioned above.

A slight reduction in *mcl-1* transcription after ALA administration, followed by a distinct increase after illumination, was observed in the HL-60 cell line. *Mcl-1* is a member of the gene family related to *bcl-2*, with an antiapoptotic function (Ohta et al., 1995). Thus, the reduction of *mcl-1* would agree with apoptosis involvement, stimulated, in contrast to the high transcription, by illumination. The activation of EGR1 (ETR103) – which acts in growth regulation and suppression of transformation by transactivation of the transforming growth factor β-1 (TGF-β1) gene and in co-operation with *sp1*, *jun-B*, *p21waf1/cip1* - also stimulates apoptosis by transactivation of the *p53* gene (Liu et al., 1998). It is appropriate to mention here the specific role of DNA topoisomerase II α, which interacts with Rb as well as with p53 (HL-60) (Hochhauser et al., 1999); this also accords with the reduced activity of cyclin genes A, B1 and D2, which was particularly clear when the illuminated cells were compared to the norm.

C-Jun N-terminal kinase gene activation should correlate with GTPase *cdc42* and with *dvl* increase (Li et al., 1999), and with further signal transduction via *bcl-2* (*mcl-1*) (Yamamoto et al., 1999). An increase of kinase activity was observed in HEL after illumination only, whereas *dvl* increased in HL-60 after ALA induction, and increased further after illumination. The transcription level remained higher even when compared to non-influenced cells. Transcription of DNA-PK, and of its catalytic subunit recombination repair gene *XRCC7*, increased in HL-60 after illumination only (Sturgis et al., 1999), as was the case for replication factor *C3* expressed in HEL (Mossi and Hubscher, 1998).

The early expression of manic fringe and *notch1* in both cell lines is consistent with their role in cell cycle kinetics, as noted by Carlesso et al. (1999).

The effect of illumination on activity changes seems to be more expressed in HL-60, and to better fit the supposed pathways of apoptosis. Activation of the signalling pathways differed considerably between the two cell lines, as had already been indicated by the observations of Hrkal et al. (2000), who proved a much higher sensitivity of HL-60 cells to photodynamic destructive effects. On the basis of the results of this project, it is possible to speculate and attempt to disclose the cause of these differences – devastation on the one hand and relative resistance on the other. The opposing reactions of PDCD2 – repressed in HL-60 and stimulated in HEL – are worthy of note.

Gadd153 activation after ALA administration, increasing after illumination in HL-60, but decreasing in HEL, and the repression of cyclins in HL-60 only, indicate that the high sensitivity resulting in cell death may originate in differences in the reaction to DNA damage caused by ALA administration.

It is difficult to elucidate the role of individual genes influenced directly or indirectly by ALA administration and illumination when only two observations were carried out. Monitoring at different intervals would be necessary to better understand the dynamics of the processes involved. The authors, at the very beginning of their study, have been able to separate genes into two groups: one set which seems to fit with preconceptions as to the anticipated effects, and for which it is felt that increases or decreases in their activity can be explained,