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Letter to the Editor

Genomic profiling of enzastaurin-treated B cell lymphoma RL cells

To the Editor,

Follicular lymphoma (FL) is an indolent lymphoma associated with follicular centre B cells and typically contains the Bcl-2 chromosomal translocation t(14;18). FLs are sensitive to chemotherapy; however, the majority of patients eventually die from the disease. Thus, there is a need for new, less toxic and more active treatments. Enzastaurin (LY317615.HCl), an acyclic bisindolylmaleimide, was initially developed as an ATP-competitive selective inhibitor of PKC. Enzastaurin was shown to target the phosphoinositide 3-kinase (PI3K)/AKT pathway and to inactivate BAD, a pro-apoptotic member of the Bcl-2 family proteins. We recently investigated the effect of enzastaurin on proliferation and survival of myeloma and lymphoma cell lines[1,2]. We found that enzastaurin inhibits cell proliferation and induces apoptosis. These results are consistent with decreased phosphorylation of AKT pathway and its downstream targets as the glycogen synthase kinase-3 beta. To provide new insights into the molecular mechanisms of the anti-tumour action of enzastaurin in Non-Hodgkin lymphoma, we investigated its effects on the gene expression profiles (GEP) of the B cell lymphoma RL cell line, carrying t(14;18), by microarray analysis. Enzastaurin was a gift from Eli Lilly & Co. (Indianapolis, IN, USA). Total cell lysates were prepared and analysed by Western Blot analysis. The antibodies used for immunoblotting included anti-caspase 9, anti-caspase 8, anti-PARP, anti-AKT, anti–Cyclin I, anti–Stat, anti–Myc and p44/MAPK. The RL cell line was treated with enzastaurin at the IC50 concentration (2.5 μM) for 48 h. Total RNA was isolated from three independent replicas of RL cells, either treated or untreated, using the TRIzol reagent (Life Technologies Inc., Rockville, MD, USA) and then purified using the RNeasy® total RNA Isolation Kit (Qiagen, Valencia, CA). The Whole Transcript (WT) Assay (Affymetrix Inc., Santa Clara, CA) was used on 100 ng of purified total RNA to generate a single-stranded DNA sense target. Hybridization of the fragmented and labeled DNA targets on the Gene 1.0 ST Arrays (Affymetrix) and scanning of the chips (GeneChip® Scanner 3000 7G, Affymetrix) were performed accordingly to the manufacturer's protocols. Data were acquired using the GeneChip® Operating Software (GCOS v1.4, Affymetrix Inc., Santa Clara, CA, USA), quality evaluation and RNA normalization were performed with the Expression Console Software (EC v1.1, Affymetrix Inc., SantaClara, CA, USA). The supervised gene expression analysis and the functional annotation study of the selected probe list were performed as previously described[3,4], using the Gene@Work software platform and DAVID 6.7 tool. The ethical background to our study was approved by institutional review board.

In a previous study, we examined the effects of enzastaurin on B cell lymphoma cell lines. Enzastaurin was shown to target the AKT pathway and to induce apoptosis through the activation of intrinsic and extrinsic pathways, partially inhibited by Z-VAD[1,2]. To confirm these data, the effects of enzastaurin on caspase 9, caspase 8, PARP and p-AKT were re-evaluated, at IC50 concentration (2.5 μM) after 48 h. Then we decided to investigate the RL cell line transcriptional profile after enzastaurin treatment, at the IC50 concentration (2.5 μM) for 48 h. A total of 43 differentially expressed probe sets, corresponding to 12 upregulated and 30 downregulated genes, were identified. As shown in Figure 1, genes modulated by enzastaurin are related to several important biological processes. Regarding the proapoptotic and anti proliferative effects of the drug, a considerable fraction of modulated genes are associated with apoptosis and cell proliferation signalling pathways (MME, INSIG1, CCNI, STAT, BCL-6, MYC and HBP1). Further, enzastaurin induced deregulation of several genes associated to immune response (CXCR4, IFIT1, MX1, PLEKHA2, HCST). Among genes upregulated after enzastaurin treatment, several belonged to IFN family (IFI6, IFI1, IFI44 and MX1). To confirm up or down regulation of cyclin I, Myc, STAT and p44 we performed Western Blot analysis. Our results are consistent with GEP analysis results, showing that protein expression correspond to up or down regulation of respective genes (Figure 2). In conclusion, we identified enzastaurin-responsive genes in RL cell line by oligonucleotide microarray analysis, providing insight into the molecular pathways that are affected by this drug[1]. Recently, we and others have shown that treatment with enzastaurin was associated with reduced phosphorylation of AKT in several tumour cell lines, although the exact mechanisms underlying enzastaurin-dependent cell death induction have remained unclear[5]. By GEP analysis we showed interesting effects of enzastaurin on some genes involved in important signalling pathways, as STAT, IFNs, MYC and p44. It is very interesting to note that enzastaurin seems to induce alterations in gene expression in the IFN-regulated JAK/STAT pathways[6,7]. In addition, we

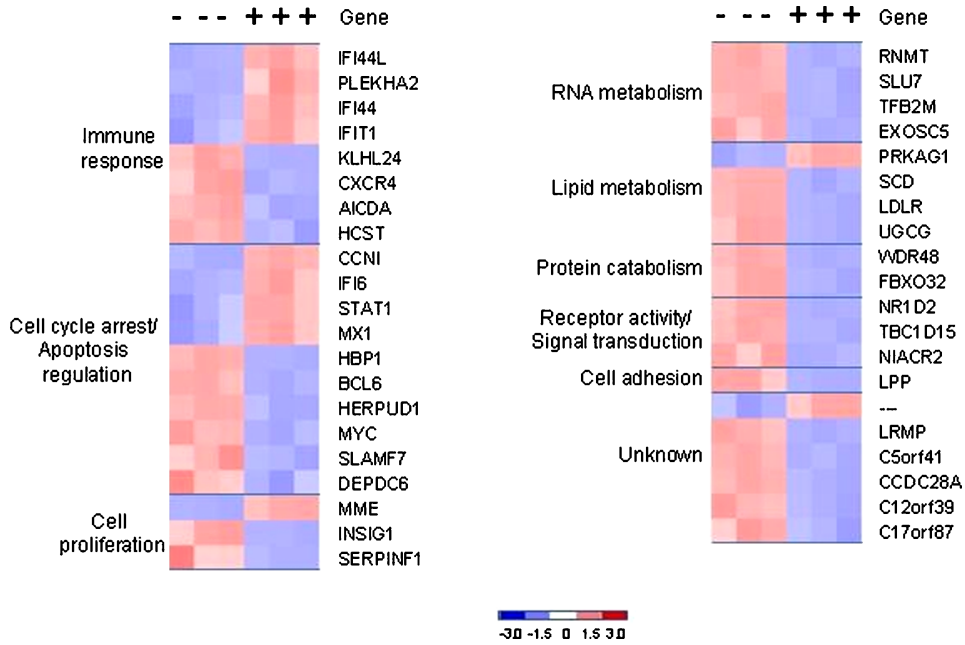

Figure 1. Identification of the differentially expressed genes in RL cell line after treatment with enzastaurin (48 h, IC50) by Gene@Work supervised analysis. The genes differentially expressed in treated (+) versus untreated (−) samples are grouped according to their functional categories and ranked within each category according to their zg score, using the untreated group as the baseline. The colour changes in each row represent the gene expression relative to the mean across the samples.

found that up regulation of p44/MAPK after enzastaurin treatment could support an activation of the MAPK pathway as reported by others[8]. The MAPK signalling pathways have multiple roles in natural processes in particular on the apoptosis. These observations provide new insights into the mechanisms involved in the induction of apoptosis by enzastaurin in B cell lymphoma cell lines and identify possible pathways which could significantly contribute to the induction of apoptosis process.

The data discussed in this paper have been deposited in the National Centre for Biotechnology Information's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.mih.gov/geo) and are accessible through GEO Series accession number GSE 24183.

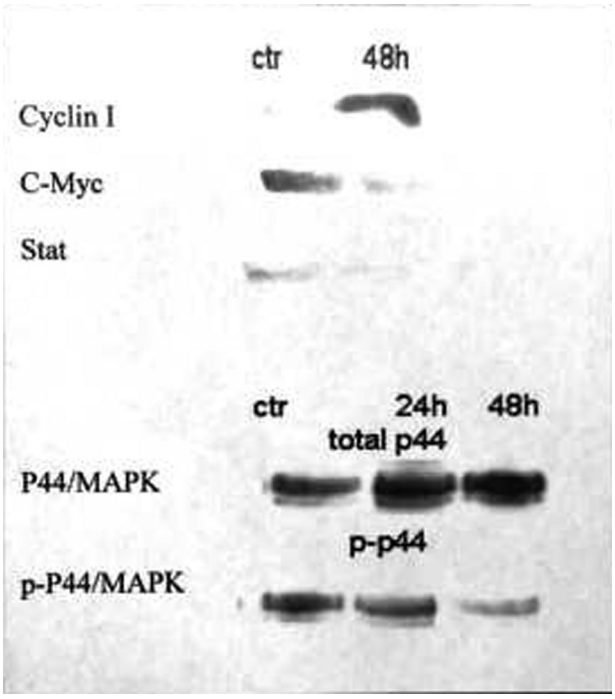

Figure 2. Immunoblot of extracts from RL cells treated with 2.5 μM enzastaurin for 48 h. Cellular extracts were probed with antibodies against cyclin I, c-Myc, and STAT and p44/MAPK.

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References

1. Civallero M, Cosenza M, Grisendi G, Marcheselli L, Todoerti K, Sacchi S. Effects of enzastaurin, alone or in combination, on signaling pathway controlling growth and survival of B-cell lymphoma cell lines. *Leukemia & lymphoma* 2010; **51**: 671–679.
2. Neri A, Marmiroli S, Tassone P, *et al*. The oral protein-kinase C beta inhibitor enzastaurin (LY317615) suppresses signalling through the AKT pathway, inhibits proliferation and induces apoptosis in multiple myeloma cell lines. *Leukemia & lymphoma* 2008; **49**: 1374–1383.
3. Mattioli M, Agnelli L, Fabris S, *et al*. Gene expression profiling of plasma cell dyscrasias reveals molecular patterns associated with distinct IGH translocations in multiple myeloma. *Oncogene* 2005; **24**: 2461–2473.
4. Verdelli D, Nobili L, Todoerti K, *et al*. Molecular targeting of the PKC-beta inhibitor enzastaurin (LY317615) in multiple myeloma involves a coordinated downregulation of MYC and IRF4 expression. *Hematological Oncology* 2009; **27**: 23–30.

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5. Lee KW, Kim SG, Kim HP, *et al*. Enzastaurin, a protein kinase C beta inhibitor, suppresses signaling through the ribosomal S6 kinase and bad pathways and induces apoptosis in human gastric cancer cells. *Cancer Research* 2008; **68**: 1916–1926.
6. Calò V, Migliavacca M, Bazan V, *et al*. STAT proteins: from normal control of cellular events to tumorigenesis. *Journal of Cellular Physiology* 2003; **197**: 157–168.
7. Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. *Journal of Biological Chemistry* 2007; **282**: 20059–20063.
8. Jane EP, Pollack IF. Enzastaurin induces H2AX phosphorylation to regulate apoptosis via MAPK signalling in malignant glioma cells. *European Journal of Cancer* 2010; **46**: 412–419.

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