INDUCTION OF DRUG RESISTANCE AND DIFFERENTIATION IN HUMAN LEUKAEMIA CELL LINES

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Acknowledgements

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Most of all I wish to dedicate this Thesis to Murray.
ABSTRACT

The ability of low, clinically relevant levels of the chemotherapeutic drugs epirubicin and vinblastine to induce drug resistance was examined in the K562, U937, KG-1a and HEL human leukaemia cell lines. Treatment with epirubicin and vinblastine induced the MDR phenotype and P-glycoprotein expression in K562 and U937 cells. However this treatment had no effect on drug resistance in the P-glycoprotein expressing KG-1a and HEL cells. In the U937 cells, drug resistant cells were not only MDR but were also resistant to other drugs including cisplatinum and chlorambucil which are not normally associated with MDR. The drug resistant U937 sublines were also sensitised to doxorubicin, cisplatinum and chlorambucil by buthionine sulphoximine (BSO), suggesting that glutathione-related mechanisms also contributed to resistance in these sublines. The U937 sublines also had an increased DNA content and an increased ability to recover from DNA damage, as determined by cell cycle analysis, indicating that the broad cross-resistance exhibited by these cells was due to the co-existence of multiple resistance mechanisms. Drug treatment induced changes in expression of differentiation associated antigens in all four cell lines.

Treatment with inducers of differentiation (TPA, sodium butyrate, granulocyte-macrophage colony-stimulating factor; GM-CSF). Treatment of K562 and K562/E15B cells with TPA induced megakaryocytic differentiation, with increases in drug resistance, and increased P-glycoprotein expression in the K562/E15B subline. TPA induced monocyctic differentiation in the U937 cells but not the U937/E15 subline, with increased P-glycoprotein expression and function in the U937/E15 cells but not the U937 cells. Staurosporine, an inhibitor of PKC, inhibited differentiation in these cell lines, but did not inhibit increases in P-glycoprotein expression, suggesting drug resistance was not mediated by PKC.

Sodium butyrate induced erythroid differentiation, and increased P-glycoprotein expression in the K562/E15B cells. However at a higher concentration (15 mM) this was not accompanied by increased drug resistance. Granulocyte monocyte colony stimulating factor (GM-CSF) did not induce differentiation in the K562 cells or K562/E15B subline, although the K562/E15B cells became more drug resistant after treatment with GM-CSF. Treatment with GM-CSF induced differentiation in the U937/E15 subline but did not change drug resistance in either the U937 cells or the U937/E15 subline.

Therefore the P-glycoprotein expressing K562/E15B and U937/E15 sublines were more responsive to inducers of differentiation than the parental cell lines. Induction of differentiation therefore induced increases in P-glycoprotein expression and drug resistance, suggesting that expression of P-glycoprotein or a multidrug resistance phenotype was associated with differentiation.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>AML</td>
<td>acute myeloid leukaemia</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphocytic leukaemia</td>
</tr>
<tr>
<td>APAAP</td>
<td>alkaline phosphatase anti-alkaline phosphatase</td>
</tr>
<tr>
<td>BCIP</td>
<td>5-bromo,4-chloro,3-indolyphosphate</td>
</tr>
<tr>
<td>BSO</td>
<td>buthionine sulfoximine</td>
</tr>
<tr>
<td>CML</td>
<td>chronic myeloid leukaemia</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukaemia</td>
</tr>
<tr>
<td>COL</td>
<td>colchicine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNR</td>
<td>daunorubicin</td>
</tr>
<tr>
<td>DOX</td>
<td>doxorubicin</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine triacetic acid</td>
</tr>
<tr>
<td>EPR</td>
<td>epirubicin</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione (reduced)</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione-S-Transferase</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>IDA</td>
<td>idarubicin</td>
</tr>
<tr>
<td>MDR</td>
<td>multidrug resistance</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>MTT</td>
<td>3-4,5-dimethylthiazol-2,5 diphenyl tetrazolium bromide</td>
</tr>
<tr>
<td>NBT</td>
<td>nitro blue-tetrazolium</td>
</tr>
<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PI</td>
<td>propidium iodide</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>Rh123</td>
<td>rhodamine 123</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>STP</td>
<td>staurosporine</td>
</tr>
<tr>
<td>TBS</td>
<td>tris buffered saline</td>
</tr>
<tr>
<td>TEMED</td>
<td>tetramethylethylenediamine</td>
</tr>
<tr>
<td>topo II</td>
<td>topoisomerase II</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>TPA</td>
<td>12-O-tetradecanoylphorbol-13-acetate</td>
</tr>
<tr>
<td>VCR</td>
<td>vincristine</td>
</tr>
<tr>
<td>VER</td>
<td>verapamil</td>
</tr>
<tr>
<td>VLB</td>
<td>vinblastine</td>
</tr>
<tr>
<td>VP-16</td>
<td>etoposide</td>
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