

## Multiple Drug Resistance in Variants of a Human Lung Line Exposed to Adriamycin and VP-16

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Multiple drug resistance (MDR) places a serious limitation on chemotherapy of cancer. Cell lines which have become cross-resistant to a variety of anticancer drugs following prolonged exposure to adriamycin, VP-16 or vinca alkaloids *in vitro* exhibit molecular mechanisms relevant to some aspects of resistance *in vivo* (Clynes, 1994) although other aspects relating to tissue organisation and vascularisation, drug delivery and tumour dormancy may also be important in clinical resistance. This paper describes different MDR lines with different patterns of cross-resistance and different mechanisms of resistance, derived by adaptation of a poorly differentiated human lung squamous cell carcinoma line, DLKP, by exposure to adriamycin or to VP-16. Clonal variation within one of these lines (DLKP-A) is also described.

### Methods

Details of the cell line DLKP, and adaptation methods used in generating the adriamycin-resistant variant, DLKP-A, as well as immunocharacterisation methods, have been described (Clynes, et al., 1992). Toxicity was assessed using the acid phosphatase method (Martin and Clynes, 1991). Adriamycin uptake was assessed fluorimetrically essentially as described by Ganapathi & Grabowski (1983). (see also Cleary, et al., in this issue). Specific mRNA levels were estimated as described by O'Driscoll, et al. (1993). P-170 overexpression was assessed using C219 antibody, as described in Clynes et al. (1992).

Topoisomerase II Western Blots used polyclonal and monoclonal antibodies kindly supplied by Dr. W. Beck and Dr. G. Astaldi-Ricotti, respectively.

### Results and Discussion

Two new cell lines were selected in the presence of VP-16: DLKP/VP-3 and DLKP/VP-8 were adapted to grow in the presence of 3  $\mu\text{g/ml}$  and 8  $\mu\text{g/ml}$  respectively of VP-16. Using the acid phosphatase method, DLKP/VP-3 was found to be approximately 90-, 110-, and 58-fold resistant to adriamycin, vincristine and VP-16 respectively; the corresponding figures for DLKP/VP-8 were 170-, 1700-, and 100-fold. The lines showed unaltered sensitivity to 5-fluorouracil, but were more sensitive to cis-platin (0.3- and 0.2-fold resistant for DLKP/VP-3 and DLKP/VP-8 respectively). Resistance was stable in both lines after 3 months' subculture in the

absence of any selective agent. DLKP/VP-3 (but not DLKP/VP-8) has a shorter doubling time than the parental line DLKP. Both DLKP/VP lines show reduced levels of mRNA for topoisomerase II $\alpha$  and elevated levels of *mdr-1* mRNA, as determined by RT-PCR. Western blot analysis indicates overexpression of P-glycoprotein, especially in DLKP/VP-3, and reduced levels of topoisomerase II $\alpha$  (the levels in DLKP/VP-8 were particularly low).

Adriamycin accumulation is greatly reduced in both DLKP/VP-variants, but can be restored to levels similar to those in the parental DLKP line by Verapamil or Cyclosporin A. In DLKP/VP-8, fluorescent microscopy indicates that adriamycin is excluded from the nucleus even after long incubation times in high concentrations of adriamycin, when drug is clearly detectable elsewhere within the cell. The molecular basis for this observation is unknown. The operation of an outwardly-directed efflux pump on the nuclear membrane is difficult to reconcile with current ideas on nuclear pore size and function. Both Verapamil and Cyclosporin A (in the concentration range 0.2-3  $\mu\text{g/ml}$ ) can circumvent resistance to adriamycin or vincristine (and to a lesser extent to VP-16) in both cell lines. The increased resistance to adriamycin and to VP-16 in DLKP/VP-8 in comparison to DLKP/VP-3 could correlate with the reduced levels of topoisomerase II $\alpha$ , even though P-170 levels may be lower than in DLKP/VP-3. The increased level of resistance to vincristine is less readily explained; possibly altered phosphorylation levels of P-170 may be relevant. The molecular basis for the hypersensitivity to cis-platin is not known.

Nine independent clones have been isolated from the adriamycin-resistant variant of DLKP, DLKP-A. They span a range of 37- to 330-fold resistance to adriamycin, 220- to 2000-fold to vincristine and 18- to 100-fold to VP-16. DNA fingerprinting confirms genetic identity of the clones with DLKP and DLKP-A. Resistance of the clones is stable in the absence of drug for at least 3 months. An appropriate mixture of the clonal populations results in a cell line with toxicity profiles similar to those of the parental DLKP cell line. Preliminary experiments indicate that cellular interactions [but not interactions involving gap junctions (Meda, 1990)] possibly involving soluble mediators may be involved in promoting survival of the subpopulations with lower resistance levels, which should be killed in the levels of drug in which DLKP-A is normally maintained. Miller et al. (1981) have reported an interaction between subpopulations of a tumour line resulting in altered drug sensitivity.

We have also isolated 3 morphologically distinct clonal populations from the parental cell line, DLKP. These clones

also have marked differences in modal chromosome number and colony forming efficiency in agar. One of the clonal populations showed twofold enhanced sensitivity to adriamycin and to vincristine, as compared to the parental line; sensitivity to VP-16 is however only slightly increased.

Clonal variation may be a widespread property of MDR cell lines, whether cloned or uncloned populations should be used in experiments depends on the question being investigated. If similar clonal variations exists in resistant tumours *in vivo*, it may be relevant to design of therapeutic strategies.

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