## Uncertain Identity of Doxorubicin-Resistant MCF-7 Cell Lines Expressing Mutated p53

The doxorubicin (ADR)-selected human breast cancer cell line (MCF-7/ADR-RES) has been a useful model for a multidrug-resistant subline in cancer research. However, a recent letter to the Journal has alerted the scientific community about the finding that MCF-7/ADR-RES is not a MCF-7-derived cell line. This report led to a change of nomenclature from MCF-7/ADR-RES to NCI/ADR-RES *(1)*.

Another cell line (MCF-7 TH) has been independently selected by doxorubicin treatment at the National Cancer Institute (NCI) and demonstrated some of the same, interesting characteristics of NCI/ADR-RES, such as high levels of MDR-1 and P-glycoprotein expression and a mutated p53 tumor suppressor protein *(2)*. Of interest, both cell lines exhibit the same 21-base-pair deletion beginning at exon 5 spanning codons 126–133 *(3)*. We used this cell line to analyze the effect of the multidrug-resistant phenotype and p53 on the efficacy of anticancer drugs with different mechanisms of action. Various anticancer drugs were more effective in the MCF-7 TH subline than in the parental MCF-7 line, despite a dysfunctional p53 and multidrug-resistant phenotype. These agents induced apoptosis in MCF-7 TH cells as demonstrated by the characteristic morphologic features. Because the activity of specific caspases might affect the propensity of a cell to undergo apoptosis, we examined expression level and cleavage pattern of different caspases in drug-treated MCF-7 TH and parental cells. The MCF-7 TH cell line strongly expressed caspase-3 (Fig. 1, A) and cisplatin treatment led to a significant increase of caspase-3-like activity as measured by use of the fluorogenic caspase-3-specific substrate Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD-afc) (Fig. 1, B). The MCF-7 cell line has lost caspase-3 because of a 47-base-pair deletion within

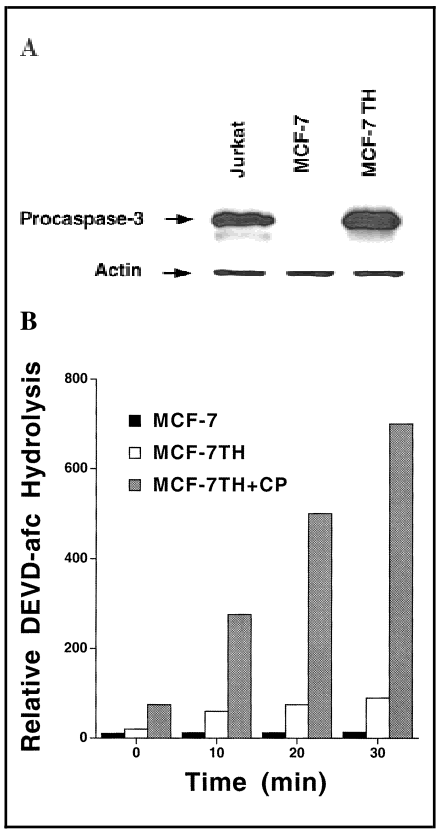


**Fig. 1.** Analysis of caspase-3 protein expression and activity in MCF-7 and MCF-7 TH cell lines. **A)** Immunoblot analysis of baseline caspase-3 expression in Jurkat (positive control), MCF-7, and MCF-7 TH cells using a caspase-3 polyclonal antibody *(6).* **B)** Activation of caspase-3 by cisplatin. MCF-7 TH cells were treated with 10 μg/mL cisplatin. At the indicated time points, caspase-3 activity was measured with the specific fluorogenic substrate Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD-afc) *(7).*

exon 3 of the CASP-3 gene *(4).* These findings thus suggest that MCF-7 TH also is not derived from MCF-7. This hypothesis was confirmed by DNA fingerprinting, which demonstrated that parental MCF-7 and MCF-7 TH cannot have the same donor, because they differ in all loci except the sex chromosome locus. The analyses were repeated with several different batches of parental MCF-7 and TH cells. Furthermore, MCF-7 parental cells from different sources were compared and all showed an identical allelic distribution profile of the short tandem repeat loci by DNA fingerprinting. The only cell line showing identity with MCF-7 TH by DNA fingerprinting was NCI/ADR-RES. Thus, MCF-7 TH, like NCI/ADR-RES, is not a MCF-7-related cell line.

This finding is important because doxorubicin-resistant cell lines are a popular tool for new drug development. In a recent article in the Journal, Leoni et al. *(5)* used NCI/ADR-RES to investigate a novel microtubule-binding indanone. Despite the fact that these authors mislabeled the cell line MCF-7/ADR-RES *(1),* they did not realize that their observation of caspase-3 activation by the drug would not have been possible in a MCF-7-derived cell line, which contains no caspase-3 *(4)* (Fig. 1, A). The use of a mislabeled cell line can clearly introduce a major source of misinterpretation and possibly wrong strategic decisions in drug development. We, thus, strongly suggest that the identity of cell lines be assessed regularly by DNA fingerprinting because this is a simple and reliable method for identity validation.

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postlabeling technique in which two cyclobutane pyrimidine dimers, T = T and T = C, and two 6-4 photoproducts, T–T and T–C, were quantified based on external standards. Many of the findings challenge results from earlier, published *in vitro* assays. Repair kinetics of dimers and 6-4 photoproducts encompassed fast and slow components, probably relating to repair of transcribed and nontranscribed sequences, respectively *(3).* Dimers were removed considerably more slowly than 6-4 photoproducts, with 50% removal times of approximately 15 and 5 hours, respectively; dimers at TT sites were repaired slower than those at TC sites *(3).* A 20-fold interindividual variation in repair rates was observed. Because we have applied this test to relatively small numbers of case subjects and matched control subjects, we need to refrain from making general conclusions. However, age does not cause a decline in repair of UVR damage *in situ,* in contrast to results from the host-mediated assay *(4).* As evidence of further contradictions, basal cell carcinoma patients have not displayed lower rates of DNA repair *(5),* and melanoma patients appear to repair DNA damage like healthy matched control subjects *(6).* The most recent development is a postlabeling assay for excreted urinary photoproducts, informative of the total-body UV damage *(7).*

Valid DNA repair tests will also be exceedingly valuable to analyze the functional effects of DNA repair gene polymorphisms, which is currently a popular research area. Here we concur with Berwick and Vineis who state, in reference to the bewildering literature on attempts to match metabolic genotypes to risk of any cancers: "It is not clear that conducting these studies without concomitant studies of expression and/or function will be fruitful." Conducting DNA repair tests in the relevant human organs will be fruitful and relevant.

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## RESPONSE

We are pleased that Hemminki et al. have taken seriously the challenge in our recent review *(1)* to develop definitive molecular assays for DNA repair. Their conclusions echo ours: neither they nor we really know what the studies in lymphocytes measure. Clearly, their research publications in 1999 and 2000 will be valuable in developing the techniques for studying DNA repair.

We find it interesting that the two studies using kinetic tests on skin biopsies, which Hemminki et al. believe fulfill the criterion of biologic relevance, had positive and statistically significant results (although carried out in only a few subjects). Roth et al. *(2)* showed that melanoma subjects had lower DNA repair rates than control subjects ($P < .001$), and Alcalay et al. *(3)* showed that dimers in basal cell carcinoma subjects were eliminated less rapidly than in control subjects ($P < .05$).

The overall message that we derive from our analysis of the literature is that a considerable number of studies consistently found an association between biologic tests, whose significance is still obscure, and cancer at several sites. Looking at it this way, we agree with Hemminki and colleagues that further clarification of the biologic background is crucial. However, at this time, we think that focusing our attention on the design of the studies, reproducibility, sample size, and selection of control subjects was necessary to draw valid conclusions at the population level. The issue of biologic relevance of studies of DNA repair in lymphocytes is critical for epidemiologic studies where associations are derived from numerous subjects to define small reproducible alterations that may be important. Most of the work Hemminki et al. cite has been conducted in very small groups and has not yet been reproduced.

In sum, the issues raised by Hemminki et al. are critical for the appropriate assessment of DNA repair. We hope that other eminent scientists will join their efforts to develop appropriate assays for DNA repair.

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