Multidrug-Resistant MCF-7 Cells: An Identity Crisis?

Two recent correspondences published in the Journal *(1,2)* labeled the MCF-7/ADR cell line—a multidrug-resistant (MDR) human breast cancer MCF-7 subline—as having a non-MCF-7 origin, which led to a change in the nomenclature of this cell line to NCI/ADR. We believe the original nomenclature of MCF-7/ADR should be retained.

Although the two MDR MCF-7 sublines (MCF-7/ADR and MCF-7 TH) used by the investigators whose work prompted the nomenclature change were independently established, they showed several genotypic and phenotypic similarities. Both contained a full-length functional caspase-3 protein *(2),* despite complete loss of this protein in the parental MCF-7 cells because of a 47-base-pair deletion in exon 3 of the CASP-3 gene *(3).* Interestingly, several features of the MCF-7/DOX subline established in our laboratory several years ago *(4)* were identical to those of the MCF-7/ADR and MCF-7 TH cells but different from those of the parental MCF-7 cells *(5).* For example, similar to MCF-7/ADR and MCF-7 TH cells, the MCF-7/DOX cells showed high expression levels of P-glycoprotein (P-gp) and of a protein cross-linking enzyme, tissue transglutaminase; they also contained the full-length caspase-3 protein *(6).* We thus sought to determine whether the development of drug resistance in MCF-7 cells represents selective selection and expansion of an inherently resistant

clone in the parental MCF-7 cell population. We purchased MCF-7 cells from the American Type Culture Collection (lot 2015862; Manassas, VA) and treated them with doxorubicin at 1 μg/mL. More than 99% of the MCF-7 cells died within 1 week, but a few colonies (an average of two colonies per T-75 flask were observed to grow in the presence of doxorubicin) of cells survived about 3 weeks of continuous culture in the presence of the drug. We expanded these colonies and, to our surprise, the newly established MCF-7 cell subline (MCF-7/WT/DOX) exhibited several biochemical features similar to those of the MCF-7/DOX and MCF-7/ADR cells but different from those of the parental MCF-7 cells. The MCF-7/WT/DOX cells showed high expression levels of both P-gp and tissue transglutaminase and contained full-length functional caspase-3 protein (Fig. 1, A). Karyotypic analysis of the MCF-7, MCF-7/DOX, and MCF-7/WT/DOX cell lines revealed unique features that were highly conserved in the drug-resistant sublines but were quite distinct in the parental MCF-7 cells *(5)*.

MCF-7 cells express a truncated isoform of caspase-3 transcript, whereas drug-resistant sublines express full-length caspase-3 transcript. Thus, we amplified the complementary DNA (cDNA) from MCF-7 and MCF-7/WT/DOX cells by polymerase chain reaction (PCR) using primers specific for full-length caspase-3 cDNA to further validate the presence of a drug-resistant clone in the parental MCF-7 cells. The resulting PCR products were analyzed for the presence of caspase-3 transcripts by agarose gel electrophoresis and ethidium bromide staining. The PCR products of the MCF-7 cells contained a predominant band corresponding to truncated caspase-3 transcript (Fig. 1, B); a minor band corresponding to full-length caspase-3 transcript became evident after many PCR cycles on the amplified cDNA (Fig. 1, B, arrow). The PCR-amplified products from the MCF-7/DOX cells showed only a single band, corresponding to the full-length caspase-3 transcript. These results confirmed the presence of one or more inherently resistant subclones in the parental MCF-7 cells that harbor the full-length CASP-3 gene and are likely to propagate into drug-resistant cell lines in the presence of MDR-related drugs. This possibility was further supported by our inability to establish any doxorubicin-resistant cell lines from two MCF-7 single-cell clones. These results appeared to be consistent with the findings that caspase-3-deficient MCF-7 cells, when reconstituted with caspase-3 cDNA, become more susceptible to chemotherapy-induced apoptosis *(6)*. It is likely that caspase-3-adequate MCF-7 subclones that propagate into drug-resistant sublines have additional pathways that confer selective resistance of these cells to chemotherapeutic agents. Despite their high resistance to doxorubicin, these cells are exquisitely sensitive to certain apoptosis-inducing agents. For example, Leoni et al. *(7)* observed that MCF-7/ADR cells were more sensitive to indanone-induced apoptosis than were the drug-sensitive MCF-7 cells. In our experience, the MCF-7/DOX cells were much more sensitive to a staurosporine-induced apoptosis than were the parental MCF-7 cells *(5)*.

Our research has demonstrated that drug-resistant MCF-7 cell lines result from parental MCF-7 cells that harbor the full-length CASP-3 gene. In view of these results, we suggest that the original nomenclature of MCF-7/ADR for MCF-7-derived drug-resistant sublines be retained to reveal the fact that various clones in a given tumor population can be extremely diverse in terms of their genotype and phenotypic characteristics.

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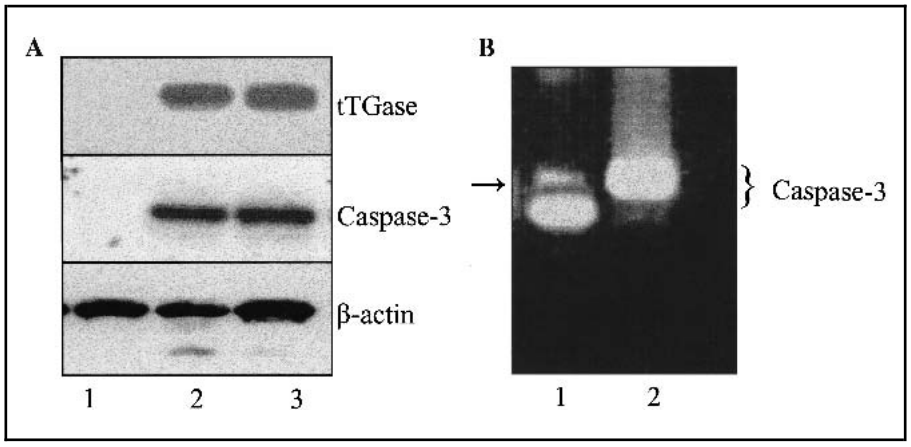

Fig. 1. Tissue transglutaminase (tTGase) and caspase-3 expression in MCF-7, MCF-7/WT/DOX, and MCF-7/DOX cell lines. **A**) tTGase and caspase-3 protein expression levels were examined by western blot analysis in the parental MCF-7 cells (purchased from American Type Culture Collection; **lane 1**), in drug-resistant MCF-7/WT/DOX cells (obtained by continuous culture of the parental MCF-7 cells in doxorubicin at 1 μg/mL; **lane 2**), and in MCF-7/DOX cells (obtained from Dr. Ken Cowan, National Cancer Institute; **lane 3**). **B**) Caspase-3 transcripts in MCF-7 cells (**lane 1**) and MCF-7/WT/DOX cells (**lane 2**), as determined after many polymerase chain reaction cycles of the complementary DNA from the respective cell lines. **Arrow** indicates the presence of full-length caspase-3 transcript in MCF-7 cells.

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Editor's note: Dr. Scudiero declined to comment.

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RESPONSE

We read with great interest the correspondence by Mehta et al. These authors propose the hypothesis that the various doxorubicin (ADR)-selected MCF-7 sublines may result from the selective expansion of inherently resistant subclones that contaminate the parental MCF-7 cell line and that harbor the full-length CASP-3 gene. Although this theory is quite interesting, we have some caveats *(1).* Because the CASP-3 gene is deleted in parental MCF-7 *(1),* it is difficult to understand how this genotype could have been reverted in the hypothetically inherently resistant subclones *(2).* In our original description, we assessed the nonidentity of MCF-7 and MCF-7 TH by DNA fingerprinting *(2).* This method is more reliable than phenotypic karyotyping *(3).* When they claim that an inherently resistant subclone contains functional caspase-3, Mehta et al. somehow contradict the finding that caspase-3 restores chemotherapy sensitivity in MCF-7 cells *(3).*

Most importantly, Mehta et al. explain their hypothesis with a contamination of the parental MCF-7 cell line. Thus, we strongly caution against retaining the original nomenclature—MCF-7/ADR or MCF-7 TH—for these sublines of unknown origin. Good research cannot be performed with tools of uncertain identity.

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