

Pgp Immunochemistry: Use with Caution

Correspondence re: C-H. Kao *et al.*, P-Glycoprotein and Multidrug Resistance-related Protein Expressions in Relation to Technetium-99m Methoxyisobutylisonitrile Scintimammography Findings. *Cancer Res.*, 61: 1412–1414, 2001.

Letter

In the February 15th issue of *Cancer Research*, Kao *et al.* (1) report findings on Pgp and MRP expression in relation to sestamibi scintimammography. As “it is difficult to extract RNA from surgical breast specimen embedded and maintained in Tissue-Tek OCT,” they analyzed Pgp and MRP expression only by IHC.¹

We have conducted a similar study on 30 patients with large breast cancer before neoadjuvant chemotherapy. Our data confirmed that sestamibi uptake was inversely correlated to expression of Pgp, MRP, and LRP. Unlike Kao *et al.*, in our hands, IHC failed to detect Pgp in breast carcinoma cells using JSB1 antibody, whereas internal controls (interstitial mononuclear cells) and external controls (adrenal gland and KB-A1 cells) showed a characteristic membrane staining. Beck *et al.* (2), in their consensus recommendations, listed among requirements for optimizing the detection of MDR1/Pgp, the use of two antibodies for IHC and combination of RNA and protein based assays, aiming at validation of results. Therefore, we confirmed these results with C494 antibody. Moreover, reverse transcription-PCR with 42 cycles of PCR amplification also failed to detect mRNA *MDR1*. Only a more sensitive technique, as real-time fluorescent PCR, allowed us to detect a low level of *MDR1* expression. These results were in accordance with Yang *et al.* (3), who showed that Pgp was not expressed in breast carcinoma cells at significant levels before chemotherapy. Like these authors, we found that the level of *MDR1* gene expression was not correlated to response to chemotherapy. On the other hand, the meta-analysis of Trock *et al.* (4) in 1997 showed that 41.2% of breast cancer expressed MDR1, but the range was from 0 to 100%, and detection was not always realized before chemotherapy. These data raise a question about Fig. 1 of the article published by Kao *et al.* in *Cancer Research*. Staining appears to be cytoplasmic. Pgp is a membrane protein, and JSB1 is directed against an epitope

localized in the intramembranar domain of the protein. Should not a positive staining be a membrane-bound Pgp staining? Otherwise, how do you interpret a cytoplasmic staining? In our positive controls, a strong and homogeneous membrane-bound staining was seen, and only a weak cytoplasmic staining was observed in some breast cancer samples. A very recent article confirms our results and shows that in breast cancer chemotherapy naive patient samples, basal expression of MDR1/Pgp is too low to be detected by IHC (5). However, there was no correlation between cytoplasmic staining and MDR1/Pgp mRNA level, whereas membranous staining in controls was correlated with higher expression. These authors suggest that the cytoplasmic reactivity detected with JSB1 could therefore reflect an artifact, rather than specific Pgp staining. Our data lead to the same observation.

In conclusion, we think that the results of Pgp expression related by Kao *et al.* should be interpreted with caution.

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¹ The abbreviations used are: IHC, immunohistochemistry; MDR, multidrug resistance.

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