American Journal of Pathology, Vol. 153, No. 6, December 1998

Correspondence

TRAF-4 Expression in Breast Carcinomas

To the Editor-in-Chief:

I read with interest the recent paper by Krajewska et al[1] concerning the pattern of TRAF-4 expression, notably in breast carcinomas. This constitutes the second study in this field, the first one having been performed in our laboratory.[2] To examine the TRAF-4 expression, Krajewska et al used immunohistochemistry, whereas we performed Northern blotting and *in situ* hybridization for TRAF-4 mRNA. They conclude that "breast cancers down-regulate expression of TRAF-4 as part of the malignant transformation processes." In contrast, we found that TRAF-4 mRNA can be overexpressed in some breast carcinomas. In order to account for this discrepancy, Krajewska et al proposed two explanations.

The first is that "contaminating nonmalignant TRAF-4 expressing cells may have contributed to the presence of TRAF-4 mRNA in (our) breast cancer specimens." To our knowledge, *in situ* hybridization permits, as efficiently as immunohistochemistry, discernment between normal and cancerous cells. In breast carcinomas, cells that are overexpressing TRAF-4 mRNA are malignant cells, as also shown by Regnier et al.[2] In addition, TRAF-4 was first identified by overexpression in breast cancer metastatic axillary lymph node,[3] a tissue in which contamination by normal breast cells would appear extremely unlikely.

For their second explanation, Krajewska et al referred to Tomasetto et al[3] to argue that we failed to observe TRAF-4 gene amplification in tumors. This is misleading because the paper reports TRAF-4 gene amplification in breast cancer cell lines. However, it seems the authors are not aware of our study[4] including 98 human breast carcinomas and showing that *in vivo* overexpression of TRAF-4 mRNA is always associated with TRAF-4 gene amplification.

We have no doubt that the TRAF-4 gene is amplified and overexpressed in some breast carcinomas, at least at the transcriptional level. Why this overexpression was not observed by Krajewska et al using immunohistochemistry remains unexplained.

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Authors' Reply:

We thank Drs. Tomasetto, Regnier, and Rio for their thoughtful comments about our paper on TRAF-4 expression in normal tissues and cancers.[1] Some of the points they have raised appeared in the draft Discussion section of our paper but were removed during revision in an effort to shorten it.

The principal issue is the discrepancy between levels of TRAF-4 mRNA and protein in breast cancers. Though the Rio group has found abnormally high levels of TRAF-4 mRNA in about one-quarter of adenocarcinomas of the breast,[2] we failed to find evidence of TRAF-4 protein up-regulation by immunostaining in our analysis of 84 cases of primary breast cancer.

Several potential explanations can be imagined for the apparent discrepancy between the Rio group's TRAF-4 mRNA data and our TRAF-4 immunostaining results.

First, because both groups have not analyzed the same breast cancer, we do not know whether differences in patient sample selection may explain the differences in our findings. Dr. Rio and colleagues have verified amplification of the chromosomal locus where the TRAF-4 gene resides in their specimens by Southern blotting. We, in contrast, have no information about the status of the 17q11–21 region in the tumors analyzed for our paper.

Second, it is possible that translation of the TRAF-4 mRNA is highly regulated or that the stability (half-life) of the TRAF-4 protein is a point of regulation, resulting in TRAF-4 mRNA production without accumulation of TRAF-4 protein. Certainly, many samples exist in which the presence of mRNA does not guarantee the presence of the corresponding protein.

Third, as we mentioned in our paper, our anti-TRAF-4 antiserum was generated using a synthetic peptide corresponding to first 18 amino acids of the predicted TRAF-4 protein. Therefore, we cannot exclude the possibility that other forms of TRAF-4 protein could be produced that are undetectable with our antiserum through such means as alternative mRNA splicing mechanisms or proteolytic processing. In this regard, Dr. Rio and col-

leagues have reported that TRAF-4 protein is nuclear.[3] In contrast, we observe exclusively cytosolic immunostaining, sometimes with a vesicular/organellar pattern typical of other TRAF-4 family proteins.[4,5] Thus, although unusual for TRAF-family proteins, we cannot exclude the possibility that alternative forms of the TRAF-4 protein exist that are targeted to the nucleus.

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Interstitial Cells of Cajal: Pacemaker Cells?

To the Editor-in-Chief:

Interstitial cells of Cajal have recently been identified as pacemaker cells for gastrointestinal motor activity[1] and have been implicated in motility disorders such as pseudo-obstruction.[2] Maturation of interstitial cells of Cajal is linked to interaction between Steel factor and its Kit tyrosine kinase membrane receptor.[3] This has led to the use of anti-Kit antibodies to characterize ICC in normal and pathological tissue. Evidence now suggests that CD34-positive stromal tumors, which have been shown to be Kit-positive, may differentiate into ICC-like cells.[4,5] Kindblom and coworkers[5] extend the identification of tumors by ultrastructural examinations and suggest using the term "pacemaker tumors."

The structural identification of cells as interstitial cells of Cajal has taken almost 100 years of painstaking work with the notable efforts in recent years of Thuneberg, Rumessen, and Faussone-Pellegrini.[6,7] In fact, in the human GI tract it is still difficult to recognize ICC by electron microscopy.[8] The general consensus is that ICC do not have unique characteristics or a specific ultrastructural marker but that a list of electron microscopy features together with their relationship to smooth muscle cells and nerves have to be used to make a positive identification. The list of features usually includes the presence of caveolae, smooth endoplasmic reticulum, an abundance of mitochondria and an abundance of intermediate and thin filaments, but absence of thick filaments. However, it is recognized that there are differences among species and according to location within the same musculature. In the paper by Kindblom and coworkers, the strategy used was to identify individual features in various tumors cells such as the presence of rough endoplasmic reticulum or caveolae or prominent Golgi zones. Then the suggestion is made that these cells might be ICC. However, this is extremely risky because it is the combination of all the features in a single cell, in addition to its location, that identifies an ICC. Obviously the location as well as natural contacts with other cells are lost in tumor cells; for this reason alone, identification will be difficult. It would be misleading to identify ICC in tumors or pathological specimens using only a few ICC features, because none of these features are unique to ICC.

ICC have been identified as pacemaker cells; however, it is clearly recognized that only a few subsets of ICC function in this capacity. Other subsets of ICC have an as yet unidentified function or possibly are involved in inhibitory neurotransmission.[9,10] At the moment it appears very likely that all intestinal organs have ICC that are not involved in pacemaking activity, such as the ICC associated with the deep muscular plexus in the small intestine and the ICC dispersed within the circular muscle layer of the stomach. Hence, it seems inappropriate to call tumors that might be differentiated into ICC-like cells as pacemaker tumors if the cells cannot be linked to a specific ICC subtype. In the small intestine, for example, one could call a tumor a pacemaker tumor (if the term has any validity at all) only if the tumor is shown to have developed from or into ICC from the myenteric plexus area.

Kindblom and coworkers identify GANT tumors as a subgroup of stromal tumors with prominent axon-like cytoplasmic processes, loosely organized intermediate filaments, scattered microtubules, and occasional dense-core granules that may be found in bulbous synapse-like structures. We agree with this. Kindblom and coworkers remark that tumors with GANT-like features did not differ from other stromal tumors and that "the ultrastructural features described as being diagnostic of GANT are, in fact, characteristics of gastrointestinal pacemaker cells." Of course, interstitial cells of Cajal do not have dense core granules or synapse-like structures. However, ICC are indeed often associated with neural structures. Since Kindblom and coworkers also noted that 55 out of 78 Kit-positive tumors were immunopositive for PGP9.5, a nonspecific neuronal marker, their conclusion that the close association between ICC and nerves may be reflected in the ICC tumors seems logical. The focal GANT-like features, which can be present in CD34- and Kit-positive stromal tumors, will then be one reason for the cellular heterogeneity of these tumors.[11] At present, however, it appears judicious to classify tumors as GANT when they have predominant immunopositivity to PGP9.5 and neuron-specific enolase as well as the ultrastructural