To the Editor:

Tissue polypeptide antigen (TPA) is a keratin-derived product containing fragments of cytokeratins (CKs) 8, 18, and 19 (1). The determination of serum TPA has been used in the management of cancer patients (2, 3). TPA has also been measured in tissue extracts (4, 5). In our experience, high TPA contents in the cytosols of breast cancer specimens were associated with longer relapse-free periods and overall survival rates (4). This was an unexpected relationship and seemed not to be supported by a clear biological rationale.

It has been assumed that during mitosis the mother cell expels its cytokeratin in a degraded form to the cellular environment. This behavior may explain both the high serum cytokeratins in more aggressive tumors and the lower cytosolic TPA found in tumors with a worse prognosis. Because the reconstruction of cytoskeleton takes place soon after cell division, the latter hypothesis regarding tissue cytokeratin distribution was considered poorly tenable (6). Moreover, the release of TPA from MCF7 cells seems to be unrelated to proliferation (7), at least in the experimental conditions used by the authors.

Cytokeratin filaments are insoluble or poorly soluble in aqueous systems unless they are first processed to soluble fragments (8). We hypothesized that the fragmentation of the cytokeratin filaments could occur during cell necrosis, often occurring in rapidly growing tumors, or otherwise during cell apoptosis, a protective phenomenon against uncontrolled cellular growth.

If this were true, the soluble fragments of CKs produced in rapidly growing and necrotic tumors should be very quickly eliminated in the bloodstream. On the other hand, in tumors with active apoptosis, the CK fragments should be produced, kept in the apoptotic cells, and therefore be detectable in the cytosolic fraction and undetectable in the serum. This hypothesis could explain why high cytosolic concentrations of TPA indicate a good prognosis. In addition, it could also support the finding of high TPA in serum of patients with more rapidly growing tumors. Therefore, we were prompted to evaluate the relationship between TPA concentrations and the apoptotic phenomenon in cell lines.

TPA concentrations were evaluated...
in the cytosol of MCF7 cells incubated for 1, 2, and 3 days in control conditions and in the presence of sodium butyrate or camptothecin, two apoptosis-inducing drugs. TPA was measured in the cell extracts and in the corresponding cell culture, using a commercially available immunoradiometric assay (kindly supplied by Sangtec Medical, Bromma, Sweden) whose validation and performance characteristics have been reported previously (4).

Fig. 1 shows that in control conditions the TPA concentration remains stable, whereas in the cytosol of sodium butyrate-treated cells it increases progressively from 7.9 units/10,000 cells on the first day to 32 units/10,000 cells on the third day, when apoptosis can be detected in the floating cells by agarose gel electrophoresis-DNA fragmentation. In the case of camptothecin, both apoptosis and an increase of TPA are detectable on the second day.

Recently published studies support this preliminary finding. In apoptotic cells, CK18 and CK19 are processed, probably by a caspase cleavage, generating soluble fragments found inside the cells (9, 10).

Moreover, indirect support came from the relationship between cytosolic TPA and p53 measured in the cytosol of 220 node-negative breast cancer tissues by a chemiluminescent immunoassay (LIA-p53, kindly supplied by Sangtec Medical, Bromma, Sweden). The p53 was a significant independent prognostic factor (11), although the TPA was more powerful as a predictor of relapse-free survival (unpublished data). In this study TPA and p53 were inversely correlated ($r_{\text{Spearman}} = -0.220; P = 0.001$), as expected if TPA was in fact directly associated with apoptosis. The pathway leading to apoptosis for sodium butyrate and camptothecin does not require p53 expression. This could suggest that TPA expression could indicate the presence of apoptosis independently of the origin of the phenomenon.

Therefore, cytosolic TPA may be an indicator, although nonspecific, of apoptosis. It could be interesting to reconsider the clinical significance of TPA, investigating more thoroughly both its prognostic role in the breast cancer cytosol and its role as a serum tumor marker, given the possibility of its use as a putative indicator of susceptibility to chemical and physical agents.

References

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