Editorial

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From Tissue Polypeptide Antigen to Specific Cytokeratin Assays

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In this issue of Tumor Biology, Bonfrer et al. [1] demonstrate that the tissue polypep-tide-specific antigen (TPS) assay, which is marketed as a specific assay for tissue poly-peptide antigen (TPA) in serum, is in fact measuring fragments of cytokeratin 18 (CK 18). The TPA assay routinely used during several years measures fragments of CKs 8, 18 and 19, as now also declared by the manufacturer of these kits. Recently, CK 8 and 18 polypeptides were found and characterized in sera from non-small cell lung cancer patients [2]. Furthermore, an assay specific for CK 19 fragments in serum (CYFRA 21-1) appears to be of value in the management of these lung cancer patients [3]. Such findings, combined with new knowledge regarding the cytokeratins as part of the cytoskeleton of epithelial cells, give us an improved background for the understanding of TPA, TPS and other CK fragments as tumor markers, and also of the original work done by Björklund et al. [4, 5] in the fifties.

The cytoskeleton is usually said to consist of filamentous structures of three types: the microfilaments, 5-7 nm in diameter; micro-tubuli, about 25 nm in width and the intermediate sized intermediate filaments (IFs), with
a diameter around 10 nm. Of these, the IF system is by far the most complex, made up of a large family of more or less related protein subunits. The expresseion of these, and their formation into the cellular filaments, are dependent on cell type: vimentin filaments are found in mesenchymal cells, desmin in muscle cells, neurofilaments in nerve cells, glial filaments in astrocytes, while the cytokeratins are found in epithelial cells.

Twenty different types of cytokeratin protein subunits are known at present. Based mainly on amino acid sequence relationships, these can be subdivided into two groups: CKs 9-20 form the type I group, with relatively small (40-56 kD) and acidic subunits, while the type II group (CKs 1-8) have slightly larger (53-67 kD) and more basic protein sub-units.

These subunits polymerize, apparently following certain simple laws: stoichiometric amounts of one protein subunit of type I and one of type II assemble into a heterodimer. Two such dimers align side by side to form a tetramer, and further lateral and end-to-end associations and formation of a ‘coiled coil’ produces the cytokeratin filament, which can be up to 40 µm long [6].

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Any epithelial cell exhibits a characteristic, differentiation-dependent combination of two or more cytokeratin proteins [7, 8]. Typically, stratified squamous epithelia express CKs 1-6 and 9-

17, while CKs 7, 8, and 18-20 are found in simple columnar epithelia. The function of the IFs are largely unknown, but the marked insolubility of the cytokeratin filaments points to a role in preserving the mechanical integrity of the epithelial cells.
Thus, the cytokeratins are epithelial markers with some organ system specificity. Importantly, the cells usually retain their pattern of expression of CKs and other IFs after malignant transformation. This is useful for the histopathologist, allowing him or her to classify tumors of uncertain origin by the use of specific antibodies.
But how can fragments of these apparently insoluble filaments appear in serum? And how can they be used as tumor markers, as long as they are in fact found in all normal epithelia? And, finally, can the serum level be a measure of tumor cell proliferation rather than cell mass, as advocated by the vendors of the TPA and TPS assays?
Normal epithelial tissues are delineated by a basal membrane (except in the liver), and their products and by-products are secreted to internal or external surfaces. In contrast, malignant growth by definition infiltrates through the basal membrane, allowing a more direct release of cell products into the circulation. Cytokeratin fragments may be released from the insoluble filaments by proteolytic degradation in dead and dying cells in a growing tumor. Alternatively, the appearance of fragments may represent a spillover of monomer subunits from the quite complicated synthesis of cytokeratin filaments in proliferating cells. This mechanism could possibly also explain a role for the fragments as ‘proliferation markers’.
Thus, the mechanisms behind the use of cytokeratin fragments as tumor markers are essentially the same as for several other markers in routine use. However, due to the occurrence of CKs in a multitude of organs, ‘unspe-cific’ increases will be an even greater nuisance than with other tumor marker assays, for example for carcinoembryonic antigen. This in itself is not a serious objection, as the assays are intended for monitoring patients who have already received a diagnosis of cancer. What can we
expect in the future? As shown by the histopathologists, there is some organ specificity in the expression of cytokeratins in normal and neoplastic tissues [8]. One could therefore hope that assays specific for single CK fragment types will give an improved organ specificity, as possibly demonstrated by the CYFRA 21-1 assay in lung cancer [9]. Such improvement may also reduce the incidence of unspecific increases during follow-up of a cancer patient.
In the meantime, those using the CK fragment assays in their daily routine or in research should be aware that the clinical documentation for an assay is not necessarily valid if the manufacturer changes the antibodies used in the kit. This problem is presumably worse here than with most other tumor marker assays, due to the heterogeneity of the ana-lyte with respect to its origin and epitope structure.

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