Prostate-specific Antigen Expression in Neuroblastoma Cell Lines

To the Editor:

We read with particular interest the article of Melegos et al. *(1)* on the prostate-specific antigen (PSA) immunoreactivity of cerebrospinal fluids, in which the authors report positive results for this kallikrein-like serine protease in ~7% of patients affected by various neurological disorders, suggesting that PSA could originate from the brain tissue. In light of this hypothesis, we undertook to study the expression, subfraction distribution, and secretion of PSA from two neuroblastoma cell lines: the SK-N-BE cell line, established in culture from a bone marrow biopsy specimen *(2)*; and the SK-N-MC cell line, derived from a metastatic tumor mass *(3)*. Both cell lines have tumor-producing capacity as well as the morphological patterns of malignant neuronal cells *(4)*. These cell lines, all *Mycoplasma* free, were cultured in Eagle's minimum essential medium supplemented with nonessential amino acids, 15% fetal bovine serum, and antibiotics (10^5 IU/L penicillin and 100 mg/L streptomycin) and maintained according to conditions reported previously *(2, 3)*. After growing, the cells were scraped and resuspended in the culture media. After a centrifugation at 5000*g* for 15 min at 4 °C, the supernatants were stored at −80 °C until assayed, while the cell pellets were lysed according to a described previously *(5)*.

Total and free PSA determinations on culture media and supernatants of cell extracts, expressed in μg/L, were carried out in triplicate by using an automated microparticle enzyme immunoassay with a mouse anti-human monoclonal antibody (AxSYM®, Abbott Labs). The PSA determination procedure, performed according to manufacturer's recommendations, was described in detail elsewhere *(5)*. The detection limit of the AxSYM PSA assay, defined as the concentration at two SDs above the zero calibrator, was reported to be 0.02 and 0.01 μg/L for total and free PSA, respectively. To exclude the possibility of "matrix" artifacts due to interfering substances present in cell lysate, neuroblastoma cell extracts were serially diluted in PSA-negative healthy female serum and reanalyzed for the response linearity. The analytical recovery of at least two concentrations of purified human seminal fluid PSA (Sigma Chemical Co.) added to the neuroblastoma cellular extracts was tested, then the recovered amount of PSA was calculated by subtracting the concentration initially present from the measured concentration after the addition.

Table 1. PSA distribution in cytosolic neuroblastoma cell extracts.

	PSA, μg/L (mean ± SE)	
Cell lines	**Total**	**Free uncomplexed**
SK-N-BE		
Cell extract	3.95 ± 0.08	0.03 ± 0.01
Culture medium	0.09 ± 0.01	ND[a]
SK-N-MC		
Cell extract	42.16 ± 7.56	12.82 ± 3.53
Culture medium	0.54 ± 0.03	0.11 ± 0.01

[a] ND, not detectable.

The linearity and interference studies revealed good linear correlation between PSA concentration and dilution performed ($Y = -0.183 + 68.9x$, $r^2 = 0.987$), thus demonstrating that neuroblastoma cell matrix extract did not affect the PSA analysis with the widely used immunoassay for serum samples. The mean analytical recovery of purified seminal fluid PSA added to cytosolic extracts from neuroblastoma cell lines was 95 ± 3%. Imprecision (CV) was 3.9% within-run and 5.4% between-run.

The cellular extract of the SK-N-BE cell line showed a markedly lower total PSA content than that found in the SK-N-MC cell line extract, with a different proportion of the PSA subfractions as well. In fact, the cellular extract of the SK-N-MC neuroblastoma cell line showed a predominant complexed form of PSA with a minor fraction (~30% of the total PSA) of free uncomplexed form (Table 1). Moreover, the higher amount of both the PSA fractions in culture media of the SK-N-MC cells is consistent with the capability of this cell line to more actively secrete this kallikrein-like serine protease in respect to the other neuroblastoma cell line, after a period 3–5 days of in vitro culture.

In addition to neuroectodermal tumor-derived cell lines, SK-N-MC and SK-N-BE are typically composed of heterogeneous cellular subpopulations, including neuroblastic and nonneuronal cells with different neuronal enzyme and protein expression *(4)*. This preliminary report adds further experimental data to the notion that PSA is an ubiquitous kallikrein-like serine protease with biological functions much more complex than recently thought *(6, 7)*. Although the role of PSA in neuroblastoma cells is still unknown, our data are consistent with the proposal that the brain tumor tissue can actively produce and secrete this kallikrein-like serine protease.

A more detailed study, performed through additional methodological approaches (biochemical characterization and ultrastructural immunocytochemical localization of immunoreactive PSA protein) is currently in progress.

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Hybrid Capture

To the Editor:

Readers of *Clinical Chemistry* may be interested in an alternative view from that offered in the recent review, "Molecular diagnostics of infectious diseases" by Tang et al. *(1)*. I specifically refer to the dismissal of Digene's Hybrid Capture® test as of "limited utility owing to poor sensitivity".

Hybrid Capture was classified as "nucleic acid analysis without amplification". In fact, Hybrid Capture is a quantitative nucleic acid test that uses an efficient signal amplification strategy with a chemiluminescent readout. The second generation Hybrid Capture II test, launched in the summer of 1997, has a detection limit one-fifth to one-tenth that of branched DNA, as measured by cut-off analyses with carefully calibrated clinical specimen dilution series. This latter commercial test was given its own DNA signal amplification paragraph in the review by Tang et al. *(1)*.

There are nearly 50 recent papers in the last two years alone that demonstrate the value of Hybrid Capture to detect targets such as cytomegalovirus *(2)*, human papillomavirus *(3)*, and herpesvirus *(4)*.

Interested readers may peruse these selected peer-reviewed papers or contact me to obtain a full list of references.

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Two of the authors respond to the Scientific Director of Digene Corporation:

To the Editor:

The letter by Dr. Lörincz states that we somehow implied that the Digene product itself lacks sensitivity. The section referred to is a paragraph on page 2024, in which we collectively describe conventional nucleic acid probe techniques as being of "limited utility owing to poor sensitivity". We stand by this statement. Signal-amplified probe techniques such as Hybrid Capture and branched DNA still require relatively large numbers of targets to be present in the clinical sample, as is the case for human papillomavirus. For most organisms, including human papillomavirus, substantially higher sensitivity can be attained by using target amplification methods. Many publications have described these sensitivity differences *(1–7)*. Whether these differences are clinically significant is another matter. Nevertheless, the reason a Hybrid Capture test for, say, HIV RNA is not commercially available is most likely related to its lower sensitivity.

The reference to modification of the Hybrid Capture System II is interesting; once data on the system are published, the data may need to be cited in future articles.

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