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The Search for New Prostate Cancer Biomarkers Continues

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Reports published in top-ranked scientific journals frequently draw public media attention. A recent report in *Nature* by a stellar group of investigators, led by Arul M. Chinnaiyan from the University of Michigan, raises hopes that their discovery may provide a better understanding of prostate cancer progression and may contribute to noninvasive detection or prognostic evaluation of prostate cancer *(1)*. Interestingly, although the authors convey in their title that sarcosine may play a role in prostate cancer progression, published news and commentaries on this report have focused more on sarcosine's diagnostic and prognostic potential *(2, 3)*. Here we analyze this report in some detail and comment primarily on the potential value of sarcosine as a urinary prostate cancer diagnostic/prognostic test, a topic that should be of interest to the readers of *Clinical Chemistry*.

Being the most frequently diagnosed cancer in males, prostate cancer is a major health problem. Despite the fact that its mortality rate has been decreasing by about 4% per year since 1992 *(4)*, this cancer still kills 30 000 men annually in the US alone. Prostate cancer is rather unique among solid tumors in that it presents in 2 distinct forms. One is a latent form that occurs at some time in almost half of men older than 60 years and poses no threat to the patient's life. The second is an aggressive form that metastasizes quickly and eventually kills the patient. The disadvantages of the frequently used prostate-specific antigen (PSA) serum test are that this test detects indiscriminately both types of prostate cancer and that PSA can also be increased in nonmalignant prostatic diseases, such as benign prostatic hyperplasia and prostatitis. It is thus not surprising that by using the serum PSA test, we end up with overdiagnosis (of latent forms) followed by overtreatment, with the associated side effects. Clearly, markers that enable us to distinguish aggressive from nonaggressive disease are urgently needed, and the results reported by Sreekumar et al. *(1)* may contribute to the solution to this problem. On the other hand, the confusion on how to best use the PSA test has been increased recently by the results of 2 large, prospective clinical trials on the effectiveness of prostate cancer screening using PSA in reducing prostate cancer mortality *(5, 6)*. It appears that PSA screening for prostate cancer may slightly reduce prostate cancer-related mortality, but at a high cost of overdiagnosis and overtreatment *(4–6)*.

A large metabolomic profile study by Sreekumar et al., published in *Nature*, had 26 authors, 4 figures (19 panels), 26 supplementary figures with multiple panels, and 10 supplementary tables *(1)*. So what did the authors of this report do? They attempted to identify metabolites that could be used to distinguish normal (benign) prostatic tissues and fluids from both localized and metastatic prostate cancer tissues and fluids. These investigators used GC-MS and LC-MS to interrogate the concentrations of various metabolites across 262 prostate-related biospecimens. These specimens included 42 tissue samples and 110 matched plasma and post–digital rectal examination urine specimens from biopsy-positive prostate cancer patients (n = 59) and biopsy-negative individuals (n = 51). Of the tissue samples, 16 were derived from benign adjacent prostatic tissue, 12 from clinically localized prostate cancer, and 14 from metastatic prostate cancer. In total, the authors identified 1126 metabolites in these samples. Comparison of the metabolomic profiles of plasma or urine from biopsy-positive and negative individuals did not identify any robust differences. For this reason, the authors focused on the metabolomic profiles of tissues.

In tissues, the investigators identified 626 metabolites, of which the vast majority (515 of 626) were shared by sample donors from the 3 diagnostic classes. However, 60 metabolites were detected in prostate cancer but not in the benign tissue. Among these 60 metabolites, 6 caught the attention of the investigators because they were significantly increased during disease progression from benign disease to prostate cancer to metastatic prostate cancer. One of the 6 metabolites, sarcosine, was selected for further examination to provide clues and serve as a biomarker of progressive disease.

Sarcosine is a derivative of the amino acid glycine and is generated by the enzymatic transfer of a methyl group from S-adenosylmethionine to gly-

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cine. The reaction is catalyzed by the enzyme glycine-N-methyltransferase, which is expressed in the prostate, among other tissues. Glycine-N-methyltransferase is a major player in modulating circulating concentrations of S-adenosylmethionine, an important methyl donor for many essential reactions regulating gene expression and protein activity, including cystosine methylation of DNA, lysine methylation of histone proteins, and arginine methylation of histones and other proteins *(2)*. Sreekumar et al. went to great lengths to show that sarcosine may play important roles in prostate cancer progression. For example, they demonstrated that sarcosine supplementation of benign prostatic epithelial cells promoted invasion, whereas attenuation of glycine-N-methyltransferase in prostate cancer cell lines reduced their invasiveness. Other notable findings relating sarcosine to prostate cancer progression included the highly increased concentrations of sarcosine in metastatic samples and in prostate cancer cell lines. Additionally, RNA interference studies showed that knockdown of genes promoting sarcosine synthesis decreased invasion, whereas knockdown of genes involved in sarcosine degradation increased invasion. Furthermore, a link was established between androgen and v-ets erythroblastosis virus E26 oncogene homolog (*ETS*) gene family signaling and genes associated with sarcosine synthesis (upregulation) and degradation (downregulation). The take-home message is that the master transcriptional regulators of prostate cancer progression (androgen receptor and *ETS* gene fusions) *(7)* seem to directly regulate sarcosine concentrations by transcriptional control of its regulatory enzymes. Sarcosine may then accumulate in prostate cancer tissues and increase their tumorigenic potential.

If these findings can be reproduced and shown to be driving forces in prostate cancer progression, they may contribute to the future development of new therapeutic interventions.

What about the value of sarcosine as a diagnostic marker? In the study reported by Sreekumar et al., sarcosine had discriminatory value in tissues because it was almost invariably increased from benign tissue, to prostate cancer tissue, to metastatic tissue [see Fig. 3 in the report by Sreekumar et al. *(1)*] and could serve as a prognostic marker in biopsy specimens. However, the need for a biopsy makes this candidate marker less attractive for diagnostic purposes. The data comparing urine sediments between biopsy-negative and -positive patients are not impressive [see Fig. 3B in the report by Sreekumar et al. *(1)*] for 3 reasons: (*a*) the range of values is enormous in both groups (4 orders of magnitude), (*b*) the overlap of sarcosine values between the 2 groups is very high, and (*c*) the area under the ROC curve is only 0.71. The data for sarcosine in urine supernatants are even weaker, with an area under the ROC curve of 0.67. From the scattergram provided [see Figure 14A in the report by Sreekumar et al. *(1)*], it can be calculated that at 100% specificity, the sensitivity would be <7%.

The report by Sreekumar et al. suggests that urinary sarcosine concentrations in sediments or urine supernatants may be a better test for prostate cancer than serum PSA alone [see Supplementary Fig. 15 in the report by Sreekumar et al. *(1)*].We believe that the comparison of the 2 tests in this setting is not a fair one. Although not explicitly mentioned in the report, it is likely that those patients who were biopsy negative or were biopsy positive for prostate cancer in the PSA diagnostic clinical grey zone (PSA 2–10 μg/L) very likely had been selected from a larger group of men who had undergone PSA testing. Consequently, serum PSA would not be a discriminatory parameter between these 2 groups (biopsy negative and biopsy positive) but would have high diagnostic value in the cohort from which these men were selected. We believe that a fair comparison between urinary sarcosine and serum PSA would be one in which, for example, 1000 asymptomatic men age 50–70 years were screened by each method, followed by prostatic biopsy of the men with positive screening results, and calculation of diagnostic sensitivities and specificities. In this scenario the PSA diagnostic sensitivity and specificity are approximately 90% and 25%, respectively. It would be interesting to see the diagnostic sensitivity of sarcosine at a cutoff yielding approximately 25% specificity and the correlation of sarcosine concentrations with indices of aggressiveness such as Gleason score.

What about the role of metabolomics? Recently, the human metabolome has been published *(8)*, along with a commentary on its potential for diagnostic purposes *(9)*. The human metabolome consists of approximately 2500 metabolites (www.hmdb.ca). Will the metabolome be a good source of biomarkers for diagnostics? We will see, but concerns have been raised already about the concept, because one person's profile of metabolites will likely be dramatically different from another person's, and each one could fluctuate markedly depending on the time of day, the time of food consumption, and other aspects of lifestyle *(9)*. Indeed, Holmes et al., who studied large numbers of urine samples from many ethnic groups around the world, found that each group was remarkably different *(10)*. Another study has shown that the metabolic profiles of meat eaters are different from those of vegetarians *(11)*.

We conclude that this remarkable study by Sreekumar et al. has shed light on a metabolite that is found at much higher concentrations in cancer tissues (especially metastatic tissues) than in normal prostatic tis-

sues. Some clues to the mechanism of this increase have also been provided. Any claim that these findings demonstrate that sarcosine offers a promising, noninvasive diagnostic and prognostic test for prostate cancer diagnosis or progression seems premature. Although the limitations of the serum PSA test are well known, the data do not support sarcosine having any advantage or superiority over serum PSA for noninvasive prostate cancer diagnosis. Sarcosine could be added to the long list of candidate prostate cancer biomarkers that await more validation before they reach the clinic *(12)*.

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