

Prostate Cancer, Prostate-Specific Antigen, and the Polymerase Chain Reaction

The detection of malignant cells in the blood or lymph nodes has important clinical implications. Recently, the polymerase chain reaction (PCR) has been used to detect malignant cells for diagnosis or monitoring of various malignancies. PCR was invented by Kary Mullis in 1985 (1, 2), for which he won the 1993 Nobel prize in Chemistry. The original PCR protocol was cumbersome and not very specific. Subsequent significant improvements, especially the introduction of thermostable DNA polymerases, not only made the technique very specific but also allowed for its complete automation (3). PCR is now a standard molecular biology procedure used extensively in research and more recently for routine clinical applications. PCR has revolutionized the way molecular experiments are performed and has created a new discipline, PCR diagnostics, which is expected to dominate routine testing for genetic, infectious, and neoplastic disease in the next 5-15 years (4).

Why has PCR enjoyed such enormous success? First, PCR is a surprisingly simple technique, within a few hours amplifying a segment of a DNA sample by a factor of 10^6 or more. Thus, PCR can give the molecular biologist as much DNA as is necessary for detection and characterization of a given target. Second, PCR, under appropriate conditions, is highly specific. One can start with a complex mixture of DNA sequences in which the target DNA of interest is embedded, and after amplification, the sequence of interest will become the overwhelmingly dominant component and can be purified. Third, PCR is highly sensitive, capable of successfully amplifying a target that is present in a complex mixture as a single molecule (4). Books (5, 6) and numerous reviews (7, 8) have been published on PCR. It is outside of the scope of this editorial to cover the applications of PCR and the large number of variations that have been introduced since the original report; however, we wish to call attention to one of the most important variations of PCR, the so-called reverse transcription-PCR (RT-PCR), which is used to amplify mRNA sequences. In RT-PCR, an mRNA species is first reverse-transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in the standard PCR reaction. RT-PCR is a powerful technique, capable of revealing the presence of a specific mRNA population in a complex mixture containing thousands of other mRNA species. Depending on the differentiation of the cell of interest, one cell may contain thousands of different mRNAs, some of which may be present in only minute amounts. RT-PCR can reveal by amplification

the presence of a single species of mRNA. If this mRNA is highly specific for the cell that produces it, RT-PCR can identify the presence of a specific type of cell. Because one cell usually expresses many molecules of a specific mRNA population, we can now detect one cell in the presence of thousands or millions of other cells that do not produce the mRNA of interest.

The ability to spot the presence of one or more cells that produce a unique mRNA species in the presence of large numbers of other cells offers opportunities for disease diagnosis or monitoring. For example, if the unique mRNA species is produced by neoplastic cells, RT-PCR can be used to monitor the presence of the neoplastic cell population for diagnosis or during therapy.

Two general approaches have been extensively studied. First, neoplastic cells could be spotted in the blood as a means of verifying the presence of a hematogenous cancer cell spread. Second, neoplastic cells could be detected in the regional lymph nodes as a means of verifying the presence of local cancer cell spread. RT-PCR has been used to detect neoplastic cells in blood or lymph nodes in leukemia (9-11), melanoma (12), neuroblastoma (13, 14), breast cancer (15, 16), and prostate cancer (17, 18). In general, these studies have converged to the conclusion that RT-PCR is more sensitive than immunohistochemistry for detecting neoplastic cells present at minute amounts in mixtures with normal cells. These findings are important because they allow more accurate identification of the patients who have higher chances of relapse. These patients are candidates for treatment with adjuvant therapy after surgery.

Prostate-specific antigen (PSA), a 33-kDa protein originally thought to be produced exclusively by prostatic epithelial cells (19), has been used extensively as a tumor marker to aid the detection and monitoring of prostate cancer. From studies in our laboratory, the absolute specificity of PSA for prostatic tissue is open to question. We have found that PSA is present in 30% of female breast tumors (20, 21), more rarely in many other tumors (22), in normal breast tissue (23), and in biological fluids such as breast milk (24), breast cystic fluid, and amniotic fluid (25). Others have demonstrated the presence of PSA in endometrial tissue (26). PSA is a powerful marker for disease monitoring, especially when ultrasensitive PSA methods are used (27).

Prostate cancer cells can be identified by RT-PCR of mRNA encoding for PSA. Deguchi et al. (17) detected PSA mRNA in regional lymph nodes from patients with

prostate cancer. Their RT-PCR method could detect PSA mRNA in the equivalent of 0.1 cell, i.e., ~500–5000 copies of mRNA (28, 29). For this experiment they used the prostate carcinoma cell line LNCaP. They also showed that they could detect one LNCaP cell in a mixture with 10^6 non-PSA-expressing cells. Using such an extraordinarily sensitive and specific method, they could detect prostate cancer cells in all immunohistochemically positive lymph nodes from prostate cancer patients—and also, in a fraction of lymph nodes that were immunohistochemically negative, suggesting that RT-PCR allows more sensitive detection of metastasis of prostate cancer cells into the lymphatic stream than do immunohistochemical methods.

Moreno et al. addressed a different question (18). They examined whether patients with advanced prostate cancer (stage D) have prostate cancer cells in their general circulation, a question also raised previously by Hamdy et al. (30). Moreno et al. developed an RT-PCR method for PSA mRNA but its exact sensitivity was not evaluated (18). From the 12 patients' blood samples examined, they found 4 that contained cancer cells positive for PSA mRNA by RT-PCR: 2 with stage D1 and 1 each with D2 and D3. All four patients staged as D0 were negative for PSA, as were two patients with D1 and two patients with D3. The reason why the patients in D3, who all have bone metastasis, were negative for PSA mRNA by RT-PCR is not clear. Perhaps a methodological artifact was involved since prostate cancer cells migrate preferentially into the buffy coat during the initial isolation (31). Alternatively, perhaps poorly differentiated prostate cancer cells lose their ability to produce PSA. Third, the circulating tumor cells may total <10 000 so the chance of finding 1 or 2 cells in 2 mL of blood may not be very high. Fourth, perhaps the PCR assay used was not sensitive enough to reveal PSA mRNA present in one tumor cell. Nevertheless, Moreno et al. clearly demonstrated the existence of circulating prostate cancer cells in some patients with prostate cancer, offering strong evidence that lymphatic spread is not the only route for prostate cancer metastasis.

In this issue of *Clinical Chemistry*, Jaakkola et al. (29) examine a similar question. Although they also used RT-PCR for PSA mRNA to detect tumor cells in the circulation, the details of their method differ from that of Moreno et al. (18); e.g., in the method for RNA isolation, the sequences of the PCR primers, and the PCR protocol. Jaakkola et al. used a nested-primer technique (8); Moreno et al., a classical PCR protocol. Both methods, however, were designed to be free of interference by genomic DNA and by mRNA from the human glandular kallikrein gene (*hGK-1*), which has extensive homology with the PSA gene (19). Moreover, Moreno et al. chose untreated patients, whereas Jaakkola et al. selected patients with bone metastasis who had been previously treated; blood was drawn from these patients 1 month to 2 years after prostatec-

tomy. Almost all patients had also received hormonal therapy or had been orchiectomized.

The method of Jaakkola et al. is capable of detecting ~2 LNCaP cells per 10^6 blood leukocytes, being slightly less sensitive than the method of Deguchi et al. (17). Using this method, the authors found PSA mRNA in 9 of the 18 patients studied. None of the patients with benign prostatic hyperplasia or other malignancies were positive for PSA mRNA. Those patients who were positive for PSA mRNA tended to have higher serum concentrations of PSA than patients who were negative.

What is the significance of these data? The newly available technology allows detection of specific mRNA produced by only one cell. When working with a 5-mL blood sample, one would need at least 1000 tumor cells present in the circulation to have a 70% chance (by Poisson distribution calculations) of obtaining a positive result. When the tumor cells are naturally circulating in blood, as is the case with hematologic malignancies, RT-PCR is a very effective, specific, and sensitive diagnostic and monitoring tool, the value of which has already been demonstrated (9–11).

In light of the data presented by Moreno et al. (18) and Jaakkola et al. (29), which show that patients with early prostate cancer and (or) well-differentiated or localized disease were found negative for PSA mRNA by PCR and that 50–70% of patients with advanced disease with bone metastasis were also negative, we can safely predict that RT-PCR for PSA mRNA would not be a very useful tool for either diagnosis or monitoring of this cancer. However, the data have value because they contribute to our further understanding of the mechanisms associated with prostate cancer metastasis. A different issue relates to the detection of prostate cancer cells in the regional lymph nodes. The data by Deguchi et al. (17) and others for different cancer types (15, 16) clearly show that RT-PCR is a valuable tool, superior to immunohistopathology for detecting lymph node metastasis. Patients who have PSA-positive nodes by RT-PCR should be classified into the high-risk groups eligible for close monitoring and early and aggressive treatment.

RT-PCR is a powerful method with diverse practical applications; however, current evidence suggests that it will not play a major role in prostate cancer diagnosis or monitoring patients after radical prostatectomy. Apparently, diagnosis and monitoring of this cancer will continue to be done with digital rectal examination, ultrasonography, and assays for PSA in serum.

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