Agilent 2100 Bioanalyzer replaces gel electrophoresis in prostate cancer research

Application

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Our work suggests that one of the underling reasons that chromosomes break, rejoin, and rearrange in cancer cells is the disruption in the capacity to methylate DNA at cytosine residues that occurs during tumorigenesis. These studies suggest that there may be a link between the disruption of methylation patterns and the expression of telomerase. In studying this link we have begun to look closely at the telomerase itself since it seems to play an important role in chromosome stability.

Telomerase is a ribonucleoprotein with enzymatic activity. Much like the ribosome, it has a strand of RNA with several protein cofactors bound to it that together are able to carry out an important biological process, in this case the addition of hexameric DNA segments to the ends of chromosomes via reverse transcriptase activity.

Telomerase functions by synthesizing six nucleotide segments of DNA at the 3' ends of chromosomes, thus stabilizing chromosomes against incomplete replication, nuclease degradation and end-to-end fusion during replication. Formally speaking, telomerase is a reverse transcriptase. It brings its own RNA template and connects to the end of the telomere (specialized nucleoprotein structure at the end of the chromosome) and then extends the chromosome.

Although telomerase activity is detected in almost all tissues during human development, it is downregulated in most normal adult human somatic tissues except those of the germline (testes/ovaries) and stem cells of renewing tissues, which express very low levels of telomerase. This makes it a tumor marker with significant clinical utility, since there is a growing body of evidence that telomerase enzymatic activity, as well as the presence of individual telomerase subunits like the RNA moiety hTR, are present in 80 to 90 % of malignant tissues tested.

Recently, we and other members of the Division of Surgery at the City of Hope National Medical Center in Duarte, California (www.cityofhope.org), have been working together to simplify the use of existing tests for the presence of telomerase in biofluids so that they may be adapted for use

in a variety of clinical settings. In particular, we have been exploring the utility of a new microfluidics based detection system, the Agilent 2100 bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA.), to quickly detect the presence of telomerase in the Expressed Prostatic Secretions of patients being evaluated for prostate cancer.

We had been studying telomerase activity and its potential as a diagnostic marker using standard agarose gel electrophoresis and analysis. Recently, we learned about a new microfluidics based detection system, the Agilent 2100 bioanalyzer. The Agilent 2100 bioanalyzer replaces the current analytical gel electrophoresis step used in most telomerase tests with a more rapid and reliable detection system. Typically the gel electrophoresis step uses a slab of chemically modified agarose about the size of a CD box. The investigator places the gels into a buffer solution, hooks up two electrodes and places an electric potential across the gel after the samples are loaded. This electrophoresis process separates mixtures of the DNA or RNA fragments by molecular weight. Once the separation is complete, the gels are transferred to an imaging station where an image is captured either electronically or photographically.

Dyes that stain DNA by intercollating between the bases of the DNA, are used by the gel electrophoresis system to produce a pattern of lines associated with the different sizes of fragment which are in the sample. The density or intensity of the signal that is obtained is proportional to the amount of material that is present. Moreover, the standard staining methods are quite sensitive because the fluorescence of the dye is enhanced by the intercolation process which in turn enhances signal to noise. Thus, the investigator is able to obtain information about the size, the quantity, and the concentration of the fragments. Gel electrophoresis, staining and quantification of gels takes 4-5 hours to perform in addition to the gel preparation time. A photographic, video or digital camera systems can be used for image analysis. In each system the investigator must manually adjust the light intensity and the focus. That means the investigators own bias may come into play casting a shadow of subjectivity to the interpretation of results. This subjectivity makes the estimates of quantity and concentration less exact. A typical result obtained with a video camera is shown in figure 1 (left panel).

Fundamentally, the Agilent 2100 bioanalyzer does the same thing as gel electrophoresis in a more efficient way. The system works with a chip, in our case a DNA 500 assay (DNA 500 LabChip® kit) that works with DNA from 20 base pairs to 500 base pairs. The chip holds up to 12 samples for processing. The investigator selects the desired assay and loads samples and reagents on to the chip. The chip is placed in the instrument and the lid closed. The lid contains electrodes that sit down into the wells of the chip. Automated regulation of voltage between these electrodes controls the speed and direction of fluid movement. The sample moves through the channel and is separated electrophoretically. The sample fragments are detected by their fluorescence and translated into gel-like images (bands) and electrophoerograms (peaks). The system then reads the intensity that is generated which gives a direct indication of the concentration of the separated fragments. The Agilent 2100 bioanalyzer is able to present the raw data and analysis in multiple formats. It displays a simulated gel view or prints out an electropherogram, which looks similar to a chromatogram. There is also a data table that labels each of the peaks and furnishes information about the size and concentration of each fragment.

The advantages of the Agilent 2100 bioanalyzer are a dramatic reduction in sample consumption (only 1 µl is required), faster analysis time (12 samples can be analyzed in less than one hour), and integration of materials handling for improved accuracy (the system provides an accurate and reproducible determination of both fragment sizes and concentrations). Most importantly, the computer generates an image that visually communicates the size and quantity of the bands, thereby eliminating the need for an investigator to make qualitative and subjective calls from a camera based image. Figure 1 is a comparison of data derived from the Agilent 2100 bioanalyzer and the gel electrophoresis equipment. The point of the comparison is that the Agilent 2100 bioanalyzer generates the same results as the gel but in a more rapid fashion with no user variability.

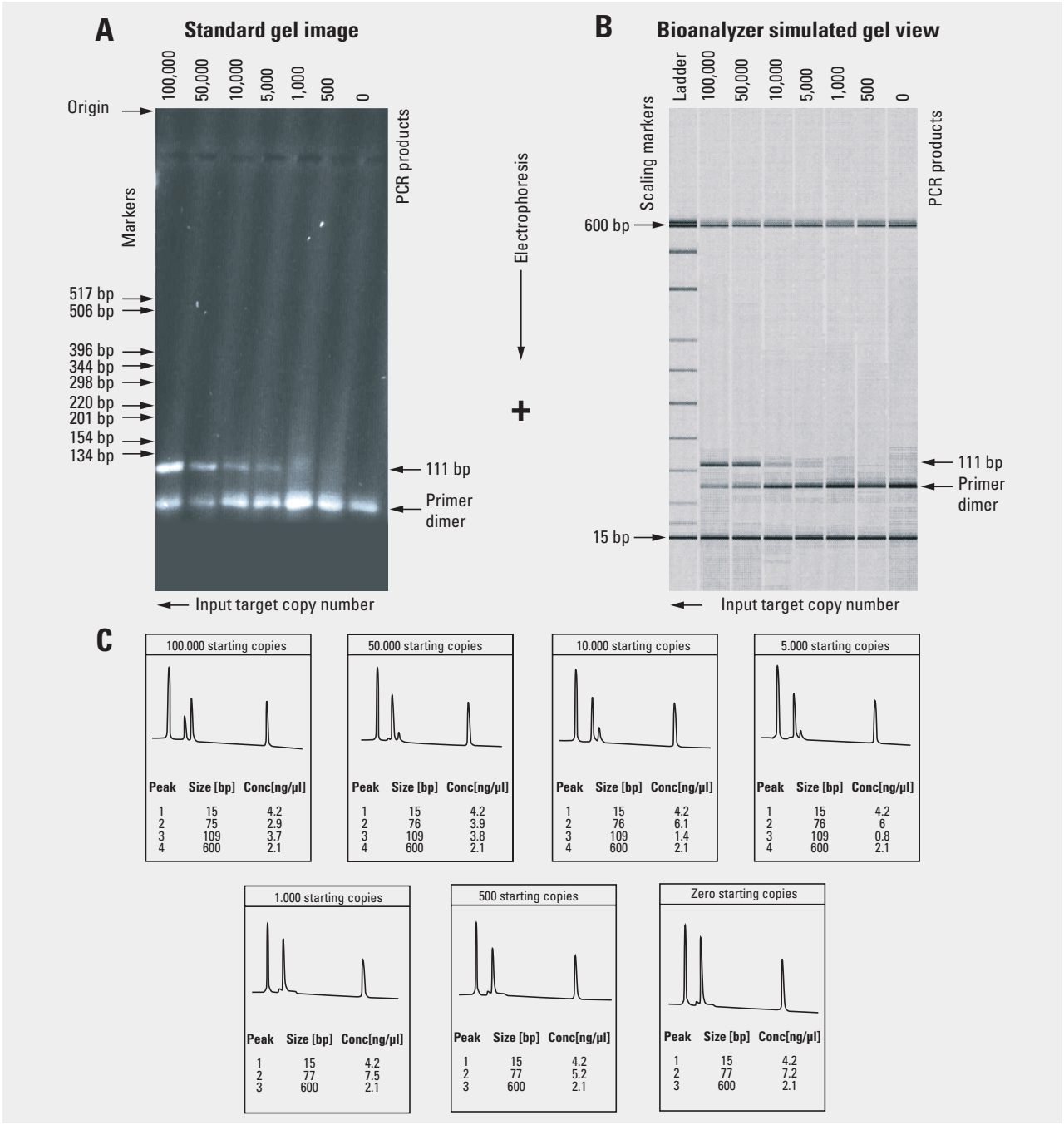

Figure 1

PCR data analysis. hTR-RNA was serially diuted and placed in a final volume of 50 µL containing the indicated number of copies of hTR-RNA. PCR detection with the 25 cycle amplification scheme permitted the detection of hTR-RNA as a 111 bp DNA fragment at all levels above 5000 copies. Since the average tumor cell should contain on the order of 100 copies of hTR-RNA, the system is capable of detecting hTR-RNA when about 10 exfoliated tumor cells are present in the specimen. An image of the data obtained with standard gel electrophoretic analysis is shown in the upper left (A, black panel). For comparison, data obtained with automated capillary electrophoresis using the Agilent 2100 bioanalyzer is shown in the upper right panel (B) plotted as though it were obtained with slab gel electrophoresis. The same data plotted as scanned fluorescence intensity (C).

Analysis is enabled in another way. Instead of having to perform discrete operations to do an analysis, a chip is used, saving several steps. Only 1 µl of sample is required which allows the sample to be used for many different assays. More significantly, the chip is disposable. Chips are equal in cost to the materials we use to run the gel. Further, the technology itself is flexible so that new chip-based assays can be developed in the future.

Currently, we are using the Agilent 2100 bioanalyzer to detect telomerase in expressed prostatic fluid of patients being evaluated for prostate cancer. We hypothesize that this test will be more sensitive and specific than current screening modalities. At this time, only 60 % of newly diagnosed prostate cancers are clinically localized. Prostate cancer is curable if it is diagnosed and treated at an early stage. The treatment of locally advanced disease is not very successful. Therefore, early detection is crucial. If telomerase components can be shown to augment early detection of the disease, then it is clear that the Agilent 2100 bioanalyzer can provide a useful tool in this arena.

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The Department of Cell and Tumor Biology's mission is to obtain a detailed molecular characterization of the genetic changes, the molecules, and the irregular regulation patterns that contribute to the abnormal genotype and phenotype of the tumor cell through cutting edge efforts in both research and education.

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