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Microsatellite Instability in Cancer Identified by Non-Gel Sieving Capillary Electrophoresis

To the Editor:

Microsatellite instability has been shown to be relevant to various human diseases, including cancers. An increase or decrease in the number of (CA)n repeat units between lymphocyte and tumor DNA from the same patient was found by using gel electrophoresis (*1, 2*).

Capillary electrophoresis (CE) is a promising alternative to gel electrophoresis for high-speed and reproducible separation of DNA, and its use is feasible for automated DNA analysis (*3*). Non-gel sieving CE is a specialized form of capillary zone electrophoresis performed with buffer containing polymer additives such as methylcellulose (*4*). We optimized the analytical condition of microsatellite instability testing by using non-gel sieving CE.

CE was performed with a BioFocus 3000™ CE apparatus (Bio-Rad Labs., Hercules, CA). The capillary cartridge contained a 50 μm (i.d.) × 36 cm polyacrylamide-coated capillary. Prior to sample migration, the running buffer was filtered and the samples were degassed by centrifugation. Pressurized injection at 414 kPa for 1 s was performed to introduce 10 nL (50–100 pg) of the DNA sample for analysis. Electrophoresis was performed for ~10 min at 222 V/cm at 30°C in PCR Product Analysis Buffer™ (Bio-Rad Labs.). The ultraviolet detector was set at 260 nm, with a range of 0.02 *A*. The postrun analysis of data was then performed with the BioFocus 3000 software (version 3.0).

The amplification of the (CA)n microsatellite marker, D2S123, was described previously (*5*). Prior to CE analysis, the purification of polymerase chain reaction (PCR) products has been necessary to avoid interferences by some components in the samples. Therefore, we examined the use of unpurified PCR products for CE analysis. When we applied the unpurified (CA)n repeats amplified under ordinary conditions, extra peaks were found in front of the target peaks. When we applied 200 μmol/L dNTP and 160 nmol/L primers separately to study the extra peaks, 200 μmol/L dNTP gave the same peaks as the extra ones, whereas 160 nmol/L primers did not (data not shown). Therefore, PCR was carried out with 20 μmol/L instead of 200 μmol/L dNTP. The PCR products then showed the same target peaks, but not the extra peaks (data not shown). Excessive peak broadening due to the difference in the salt concentration between the salt-containing PCR products and the running buffer was not observed. These data indicate that PCR products with 20 μmol/L dNTP can be subjected to CE without further purification. The CV of intraassay imprecision (n = 10) in the calibrated base pairs was ≤0.7%, indicating good reproducibility.

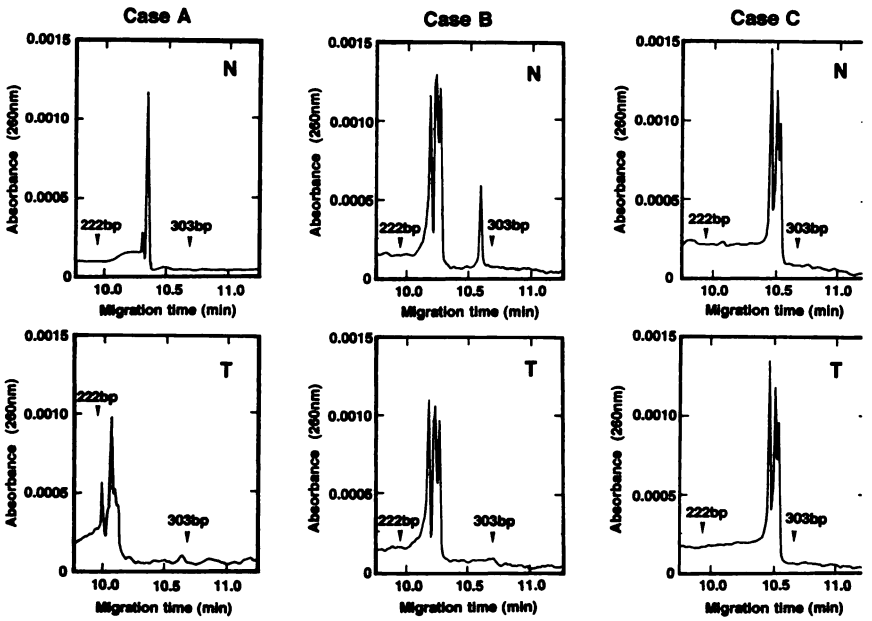

Fig. 1. Analysis of (CA)n repeats from various specimens with non-gel sieving CE.
DNA from the normal (N) and tumor (T) cells from the three representative colorectal cancer patients. The sizes of pBR 322/*AvaII*/*EcoRI* markers are indicated.

Surgical specimens were obtained from colorectal cancer patients according to the procedures of the Helsinki Declaration of 1975, as revised in 1983. The results of CE analyses of amplified D2S123 DNA derived from three of these patients are shown in Fig. 1. The electrophoretic profile of the amplified products was compared between tumor and normal DNA from the same patient. In case A, the mobility of tumor DNA was faster than that of normal DNA. In case B, the largest DNA fragment in normal cells had disappeared in the tumor. On the other hand, the mobilities of tumor and normal DNA were identical in case C. These profiles of the amplified products observed on CE were the same as or better than the data obtained on gel electrophoresis (data not shown). To confirm the differences in profiles between normal and tumor DNA, we made a double injection of both forms of DNA from case A. The electropherogram clearly showed the differences in mobility (data not shown). Thus, the genetic instability in cancer could be detected with CE as on gel electrophoresis, and the time required for CE analysis was ~10 min, i.e., much shorter than for gel electrophoresis. In case B, the disappearance of the largest DNA may indicate loss of heterozygosity. However, further studies are necessary to characterize this phenomenon as genetic instability or loss of heterozygosity.

With this technique, DNA with amplified (CA)n repeats can be used for non-gel sieving CE without further purification and analyzed in ~10 min with resolution the same as or better than that of gel electrophoresis without postseparation detection techniques such as ethidium bromide staining or silver staining.

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Blood Sample Tubes for Lactate Assay

To the Editor:

Blood preserved with fluoride/oxalate is often recommended for lactate measurements, e.g., for the Ektachem method (Eastman Kodak, Rochester, NY). Heparin is also considered to be a satisfactory anticoagulant (but not preservative). However, EDTA, citrate, and iodoacetate may interfere with color development in some assays (e.g., Ektachem).

We found that the tubes used in this hospital for glucose assays contained EDTA as anticoagulant. Although the box in which the tubes are supplied carries a statement that they contain fluoride and EDTA, this is indicated on the tube label only by the code "FE," and the supplier's catalog gives no information about the anticoagulant in these or other glucose tubes, some of which we subsequently found contained heparin.

Because blood collection tubes are often removed from their packaging and loaded onto collection trays before use, the anticoagulant labels on the boxes may not be noticed. Moreover, although the coding on the tube labels is said to comply with an international draft recommendation (ISO/DIS 6710), this is not a document that falls readily into the hands of every laboratory worker. Given the potentially significant effects of anticoagulant type on some assays, I suggest that manufacturers should clearly state the type of anticoagulant on the tube labels.

At least one major manufacturer (Becton Dickinson, Oxford, UK) already does this. Two smaller manufacturers in the UK also print preservative/anticoagulant details on the labels rather than using codes, but one of these does not indicate the type of anticoagulant that accompanies the fluoride in its glucose tubes, despite the fact that the manufacturer produces fluoride-containing tubes with three different types of anticoagulant. When approached about this, the company accepted that the omission needed to be corrected. Certainly, to avoid invalid results, anyone performing Ektachem lactate estimations should check which anticoagulant is used in the blood collection tubes.

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Toward Unraveling the Heterogeneity of High-Density Lipoproteins with a Functional Approach

To the Editor:

I have read with interest the article by Dobiášová and Frohlich (*1*). The relevance of the article is conceptual in nature because it looks at the heterogeneity of the high-density lipoproteins (HDL) from a functional rather than physical/chemical point of view. Indeed, even after further division of the HDL class into subspecies HDL_2 and HDL_3 (based on the results of ultracentrifugation); HDL_{2a}, HDL_{2b}, HDL_{3a}, HDL_{3b}, and HDL_{3c} (based on gradient-gel electrophoresis); or lipoprotein (Lp)-AI and Lp-AI/AII (i.e., containing apolipoprotein (apo)A-I only vs apoA-I plus apoA-II), the heterogeneity within each of these subspecies still remains.

Dobiášová and Frohlich elected to probe HDL heterogeneity with a functional assay they developed, the fractional esterification rate of cholesterol (FER_{HDL}) performed in plasma depleted of low- and very-low-density lipoproteins. Thus, "individuals with identical concentration of HDL free (or total) cholesterol may differ dramatically in their respective FER_{HDL}" (*1*). They concluded that "FER_{HDL} . . . reflects the composition of HDL subspecies (specifically, the relative content of HDL_{2b} and $HDL_{3b,\ c}$ or their interactions) . . ." (*1*). However, data on at least the number of the individual HDL particles identified with such assay are not provided.

In the course of studies on the findings of thyroid hormones [thyroxine (T_4), triiodothyronine (T_3)] to human plasma lipoproteins (*2–6*), my colleagues and I have collected some data that can be analyzed from the perspective of a functional approach to HDL heterogeneity (although the primary scope of those studies was not to address this issue). This useful and novel information can be summarized as follows:

1) We now know of the existence of a low-molecular-mass (68-kDa) Lp-AI particle containing 2 mol of apoA-I and 16 mol of lipids (*2*). This very low lipid content would account for the relatively high affinity of this Lp-AI for T_4, because lipids inhibit the T_4 binding to apolipoproteins (*3*).

2) This observation agrees with the findings by gel filtration chromatography of the total lipoprotein fraction (d <1.210 kg/L) equilibrated with radioactive T_4 (see panel B of Fig. 2, ref. *4*). In fact, the eluate contains about a dozen HDL-cholesterol peaks (i.e., ~12 distinct HDL subfractions) within the molecular mass range of ~440–60 kDa but only half as many [^{125}I]T_4 peaks. The radioactive peaks are evident in the range ~250–60 kDa, confirming that T_4 prefers to interact with the relatively smaller (and lipid-poorer) HDL subfractions.

3) Chromatography performed after the total lipoprotein fraction had been loaded with radioactive T_4 or T_3 or reverse T_3 (a biologically inactive metabolite) shows that the two hormones are bound to low-M_r HDL subfractions, whereas the reverse T_3 is bound to high-M_r subfractions (see panel A of Fig. 2, ref. *4*). (The physiological implications of this interesting difference are at present unclear.) More recent findings indicate that the low-M_r HDL subfractions that trans-