Retinoblastoma Patient with a Unique Population of Ultrashort Single Stranded DNA Fragments in Blood Plasma

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Editorial

A 5 year old male retinoblastoma (2A) patient and four same age/sex healthy donors were taken for blood plasma cfDNA extraction [1]. To purify ssDNA, a consequent treatment of DNA extract with exonucleases λ and III, S1 nuclease, and proteinase K was followed then by a cascade ultrafiltration on K75/K25 SPM TechSep membranes (Mirabel, France) [2]. λ/III-nuclease resistant 25K to 75K compounds ssDNA fragments were analyzed by size exclusion/anion exchange HPLC (Figure). For this purpose, its key parameters were estimated as the followings: stationary phase–polymethylamidopropylmethacrylamide; column PRP-X600 AE, 4.6 mm × 150.0 mm, 5.0 μ particles, 1.6 meq/mL (Hamilton Corp., USA); 1,800 p.s.i., 22°C to 25°C, 0.8 mL/min elution rate. Both synchronous linear elution LiCl (0 M to 2.5 M) and pH (8.0 to 4.0) gradients were formed on 100 mM Tris/acetonitrile (85:15, v/v). Waters/Hamilton compatible Breeze 200 SLE Analytical System, W2998 UV-Detector (254 nm), W600E gradient former (Waters, Inc., USA).

Sample loading: 80 μg-100 μg DNA in 50 μL 100 mM Tris-HCl (pH 8.0)/acetonitrile (85:15, v/v). All ssDNA measurements and 2.0% agarose gel electrophoresis DNA size control were performed according to [3,4].

As a result, ssDNA ultrashort fragments (70n to 120n) were found in plasma of retinoblastoma patient, 6.47 ng x mL−1. To the contrast, in control donors, a much smaller population of ssDNA (2.40 ng to 2.82 ng × mL−1) consisting of essentially larger, 350n to 400n, sequences.

Noteworthy, a separation efficiency shown by our original HPLC technique (Figure) allows to reveal the size/charge–different populations within an ssDNA pool in cancer plasma which is not always possible in both PCR-based DNA size estimations [1,3] and a routine agarose gel

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Figure: Fractionation of plasma ssDNA fragments by HPLC on Polymethylamidopropylmethacrylamide. 1. Retinoblastoma patient; 2. Healthy donors; 3. S1 nuclease negative control. Arrows show the retention times for 50n, 100n and 200n single-stranded poly(dT) markers (Takara Biomedical Corp., Rep. Korea).
electrophoretic procedures [4,5]. Particularly, a PCR measurement of ssDNA size [1,3,5] may look not a right choice assuming the DNA repair related origin of these short cfDNA fragments. The later would mean a possible release of ssDNA directly in the "cancer-booming" DNA defects replacement [5].

A long-lasting record on the cancer related cell-free DNA (cfDNA) counts nearly 20 years. Nonetheless, all publications available have no clear indication to eukaryotic, DNA repair related, origin of ssDNA [1,6] whereas the letters may contribute namely to the ultrashort ssDNA plasma pool reflecting a known acceleration-breakdown of DNA repair in malignancies [1,3,6].

This work is the first report ever on the cancer related appearance of initially formed single stranded ultrashort DNA fragments in blood plasma of oncology patient. Considering this study a preliminary but attention catching description of presumably unique case, we might expect further research on this and related subjects.

References


