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SINGLE STRANDED DNA FRAGMENTS IN RETINOBLASTOMA PATIENT BLOOD PLASMA: LINK TO ONCOGENESIS AND DIAGNOSTIC VALIDITY

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A significant population of ultrashort (50n – 150n) single-stranded DNA fragments were found in exosome-free blood plasma of retinoblastoma patient (6.84 ng x mL-1), but not in plasma of healthy donors. An original HPLC technique has been employed. 5.0 year old male retinoblastoma (2A) patient and four same age/sex healthy donors were taken for blood plasma cfDNA extraction. A consequent treatment of DNA extract with exonucleases and III, S1 nuclease, and proteinase K was followed then by a cascade ultrafiltration on K75/K25 SPM TechSep membranes (Mirabel, France). /III-nuclease resistant 25K – 75K compounds were analysed by size exclusion/anion exchange HPLC. For this purpose, its key parameters were estimated as the followings: stationary phase – polymethylamidopropylmethacrylamide; column PRP-X600 AE, 4.6 x 150.0mm, 5.0 particles, 1.6 meq/mL (Hamilton Corp., USA); 1,800 p.s.i., 22° – 25°C, 0.8 mL/min elution rate. Both synchronous linear elution LiCl2 (0 – 2.5M) and pH (8.0 – 4.0) gradients were formed on 100mM Tris/acetonitrile (85:15, v/v). Waters/Hamilton compatible Breeze 200SLE Analytical System, W2998 UV-Detector (254nm), W600E gradient former (Waters, Inc., USA). Sample loading: 80 – 100 g DNA in 50 L 100mM Tris-HCl (pH 8.0)/acetonitrile (85:15. v/v). As mentioned above, ssDNA short fragments were found in plasma of retinoblastoma patient. To the contrast, in control donors, a smaller population of ssDNA (2.40 – 2.82 ng x mL-1) was found consisting of essentially larger, 350n – 400n, sequences. A separation efficiency shown by our HPLC technique 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 and a routine agarose gel electrophoretic procedures. The later would mean a possible release of ssDNA directly in the "cancer-booming" DNA defects replacement. HPLC proposed is a simple and reliable tool for further epigenic and diagnostic studies on patients with retinoblastoma.

Biography

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