DETECTION AND CHARACTERIZATION OF RAT PROTEINS RECOGNIZING AND BINDING DNA DAMAGED BY N-ACETOXY-ACETYLAMINOFLUORENE AND CIS-PLATINUM

MONIKA PIETROWSKA, JOANNA ŁANUSZEWSKA, JOANNA RZESZOWSKA-WOLNY and PIOTR WIDLAK
Department of Experimental and Clinical Radiobiology, Center of Oncology, 44-100 Gliwice, Poland

Proteins recognizing DNA damaged by the chemical carcinogen N-acetoxy-acetylaminofluorene (AAAF) were analyzed in nuclear extracts from rat tissues, using a 36 bp oligonucleotide as substrate and electrophoretic mobility shift assay. Two major complexes binding DNA damaged by AAAF were detected that possess different electrophoretic mobility. The low mobility complex contained protein loosely attached to nuclear components and extracted with 0.1 M NaCl. The amount of this protein was estimated as about $10^4$ copies per nucleus of liver cell, and its probable size was about 42 kDa as detected by Southwestern blotting assay. Its affinity for DNA damaged by AAAF was ~10-fold higher than that for undamaged DNA. Analogous AAAF-DDB complexes were also detected in extracts from brain, testis and kidney. The high mobility complex contained chromatin protein extracted with 0.4 M NaCl. The amount of this protein was estimated as about $10^3$ copies per nucleus of liver cell, and its probable size was about 25 kDa as detected by the Southwestern blotting assay. The affinity of this protein to DNA damaged by AAAF was ~70-fold higher as compared to undamaged DNA. DNA damaged by cis-platinum, benzo(a)pyrene diol epoxide and UV-radiation also bind this protein with increased affinity (the first one more strongly while the latter two less strongly than AAAF-damaged DNA). The high mobility complexes were detected also in brain, spleen and kidney, but not in testis. The levels of both damaged DNA-binding proteins detected in rat tissues were not affected in animals treated with the carcinogen 2-acetylaminofluorene.