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COMPARISON OF PROTEIN PATTERNS OF XRS-5, A RADIOSENSITIVE CHINESE HAMSTER OVARY CELL LINE, AND CHO-K1, ITS RADIORESISTANT PARENT, USING TWO-DIMENSIONAL GEL-ELECTROPHORESIS,

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-J. Michael Kramer-

Miami University
Zoology Department
Oxford, OH 45056

Faculty Advisors:
Dr. Carol S. Giometti and Dr. Jeffrey L. Schwartz
Biological and Medical Research Division, Argonne National Laboratory
9700 S. Cass Ave., Argonne, IL 60439

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Abstract

X-ray sensitive strains of Chinese hamster ovary cell lines have been used to analyze radiation repair mechanisms. One cell line, xrs-5, first isolated by Jeggo and Kemp from CHO-K1 cells, has been shown to be very sensitive to ionizing radiation and radical forming chemical mutagens. This sensitivity is thought to be a result of a mutation in the DNA double strand break (DSB) repair mechanism, and its characterization has been a goal of several repair mechanism studies. Using two-dimensional gel electrophoresis, we have detected a protein (MW approximately 55KD) in the DNA/Nuclear Matrix (nucleoid) cell fraction of CHO-K1 cells that is absent in the nucleoid fraction of xrs-5. This protein is present, however, in both CHO-K1 and xrs-5 whole cell protein maps. To determine whether the 55KD protein is responsible for the radiosensitive and defective DSB repair phenotype of xrs-5 cells, studies are now underway to analyze revertants of xrs-5 that are proficient in DSB repair. Furthermore, an effort to sequence the protein in question is planned.

Introduction

We are constantly being exposed to ionizing radiation either through our environment (radon,cosmic radiation) or through medical procedures. Understanding the risks associated with these exposures requires an understanding of the lesions induced by radiation and the cellular processes that have developed to repair these lesions. It is well established that the major radiation induced DNA lesion of biological importance is the DNA double-stranded break (DSB). It remains unknown, however, how this lesion is repaired in mammalian cells.

Two scientists , P.A. Jeggo and L.M. Kemp (1983) have created six x-ray sensitive cell lines (xrs 1-6) from the Chinese hamster ovary cell line. These cells were heavily mutagenized by treatment with ethyl methanesulphonate and then selected for sensitivity to x-ray exposure. The six mutant cell lines have been studied extensively and their cross sensitivity to other chemicals and forms of radiation has been characterized (Jeggo and Kemp,1983). More importantly, several studies have reported that the ability of the xrs cells to repair double stranded DNA breaks is deficient in all strains. This DSB repair deficiency has been strongly correlated to the cause of their x-ray sensitivity (Kemp et al., 1984).

Hybrid studies between wild type cells and all the mutant strains have shown a genetic mutation to be recessive, and further complementation studies crossing all of the xrs varieties among themselves show the mutation to be of the same complementation group (Jeggo,1985). Interestingly, the cells exhibit different degrees of sensitivity to X-rays and to various other DNA damaging agents. Due to this phenotypic diversity the classification of the xrs cell line has been maintained as xrs-1 through xrs-6. This variety of phenotypes may result from the pleiotropic effects of a single mutant gene, or these effects may be from a number of genetic defects.

It seems a rare coincidence that all six xrs strains would be mutated in the same gene loci. However, the partially hemizygotic nature of CHO cells explains the high rate of mutation expression in the xrs complementation group. Through methylation of one of the strands of DNA, it becomes transcriptionally inactivated (Taylor and Jones, 1986). A subsequent mutation in the other stand of DNA becomes functionally dominant since it is not masked by the transcriptionally silent gene. In fact, inhibition of the methylating enzyme DNA-transmethylase by 5-azacytidine during replication restores xrs-5 cells to the wild type level of radiosensitivity (Jeggo and Holliday, 1986). While xrs-5 revertant cells are no longer radiosensitive, however, these cells are still more susceptible than wild type cells to the effects of a cross-linking drug called cisplatin (Haraf et al., unpub.). This evidence suggests that more than one repair mechanism has been altered in xrs-5.

Studies correlating cell cycle and radio-resistance also show evidence of a multiple repair mechanism in xrs-5. Biphasic survival curves demonstrate a possible multiple mechanism in xrs (Denekamp et al.,1989; Jeggo, 1990; Iliakis and Okayasu, 1990) and in a variety of other cell types (Resnic and Moore, 1979; Weibezahan and Coquerelle, 1981; Brunborg and Williamson, 1978). There are several explanations of this biphasic phenomena. One pertains to cyclic availability of the silent methylated genes. This theory suggests that recently replicated

genes are available to transcription since they are not immediately methylated. Therefore, the cell briefly gains radioresistance (Jeggo and Holliday, 1986; Denekamp, 1989). This theory is contradicted by evidence in other cell types studied for x-ray sensitivity. These cells are not revertible by azacytidine and therefore do not have methylated genes (Jeggo,1990; Jeggo and Holliday,1986). An additional possibility suggested by Jeggo, is the cyclic access of repair enzymes to the DNA. As the cell proceeds through the cycles of replication and growth, the DNA might be more or less accessible to various proteins at different periods. In this model a mutation in proteins involved in the packaging of DNA would alter such access of important proteins (Jeggo,1990).

Supporting this theory, researchers have noticed phenotypic variation in the chromosome structure of xrs-5 cells as compared to the CHO-k1 parent. Metaphase chromosomes were dramatically shorter and thicker than CHO-K1. One revertant cell line which was reverted in x-ray sensitivity also reverted to some degree back to the CHO-K1 chromosome structure demonstrating a correlation between chromosome structure and x-ray sensitivity (Schwartz et. al., 1990). Studies further correlating chromosome structure and x-ray sensitivity in other revertants are underway (Schwartz, personal communication).

This paper shows that a protein present in the nuclear protein matrix, a protein array responsible for DNA packaging and coiling (nucleoid), is missing in the xrs-5 nuclear matrix. The absence of this 55kd protein, therefore, is one possible explanation for its x-ray sensitivity and phenotypic difference in chromosome morphology.

Research analyzing the nature of DNA repair is very significant. Recent experiments with cells very similar to the xrs line have demonstrated a parallel between hamster cells and human cells. By inserting parts of human chromosome 5 into xr-1 cells (a CHO cell similar to xrs 1-6) the human DNA can rescue those cells from radiosensitivity and even restore their ability to repair DSB's. This suggests that some of the same or very similar mechanisms functioning in hamster cells are functioning in humans (Giaccia et al., 1990). Further studies on repair mechanisms, therefore, have direct applications to human cancer treatment as well as many other genetic processes. It is therefore important to further elucidate these mechanisms.

Methods and Materials

Ceil lines CHO-K1 and xrs-5 were gifts of Dr. P. Jeggo. All cell lines were grown and provided by Dr. J. Schwartz and his lab. All cell lines were maintained under exponential growth conditions in McCoys 5a medium supplemented with 10% v/v fetal calf serum, 100ug/ml streptomycin, 100units/ml penicillin and 3mM L-glutamine.

Nucleoid sample preparation

The nucleoid preparation was a modification of a preparation of Kapiszewska et al. (1989). First, I washed 1.0-2.0x10^7 cells in phosphate buffered saline three times and then spun the cells down. The packed cells were then resuspended in 1 ml 0.0005% Triton X-100, 1.5 M NaCl, 10 mM EDTA, 10 mM Tris, pH 8.0 by vortex and then allowed to lyse for 30 min at room temp. The sample was centrifuged at 150,000 x g for 30 min. and the supernatant poured off. The pellet was then solubilized in 50 uL TMNP (10 mM Tris, pH 7.4, 5 mM $MgCl_2$, 10 mM NaCl, 0.1 mM PMSF) containing 25 ug of DNAase I/30 uL sample at room temperature for 30 min. The sample was finally mixed with an equal volume of NP-40/Urea mix and centrifuged 435000 xg for 10min. Supernatant was then analyzed by 2D electrophoresis.

Whole cell sample preparation

I pelleted approx. 5.5x10^5 cells and solubilized them in 100ul of NP40-urea mix. The sample was then centrifuged at 435000g for 10 min. The supernatant was then analyzed by 2D electrophoresis.

Two-dimensional gel electrophoresis

I performed two-dimensional gel electrophoresis as described by O'Farrell (1975a) with modifications introduced by Anderson and Anderson (1978). First-dimension isoelectric focusing was performed on 10 inch rods containing 50% Biolyte pH 3-10 and 50% Biolyte pH5-7 for 30,000 volt hours. The isoelectric focussing gels were equilibrated with sodium dodecyl sulfate prior to second-dimension electrophoresis. The second-dimension sodium dodecyl sulfate gel electrophoresis was performed on 7 by 7 inch slab gels containing a linear gradient of 9-17% polyacrylamide as described by Anderson and Anderson (1978b). Proteins were detected with silver staining.

Silver staining

I fixed my gels for at least 7 hrs in 1 L of 50% ethanol containing 1% (v/v) acetic acid and 0.1% (v/v) formaldehyde. This solution was removed and the gels were rocked in 50% ethanol for 15hrs. They were rinsed in 20% ethanol for 30 mins with 5mg/l of dithiothreitol dissolved in the second 30 min rinse. The gels were stained for 1 hour in 1.41ml 10N NaOH, 10.5 ml NHOH, 5 gm $AgNO_3$ dissolved in 50 ml water (added in the order shown), to 1 L 20% ethanol immediately before use. Each box was rinsed briefly with distilled water then washed 3 times 20 minutes each with 20% ethanol. The gels were developed for approximately 30 min in 50 mg citric acid and 0.5 ml formaldehyde in 1 L 20% ethanol. The development was stopped in a solution of 0.5% acetic acid in water for 2 min. The gels were washed 3 times in distilled water at 15 min intervals, and contact prints were made on x-ray film after the washes.

I conducted these experiments over the course of three months. In that time 8 preparations of xrs-5 and CHO-k1 cell nucleoids and whole cells were made. These samples were assayed for protein concentration using a modified Bradford assay (Ramagli and Rodriguez, 1985). As they were loaded into isoeletric wells samples were then adjusted to equilibrate total protein/gel by adding NP/40 urea. Nucleoid isoelectric gels were typically loaded with 60,90, and 120ug/gel. Whole cell isoelectric gels were loaded with 55ug/gel.

Results/Discussion

Results from this experiment have shown that a 55kd protein is reproducibly absent in two-dimension gel electrophoresis patterns of the DNA/nuclear matrix (nucleoid) cell faction of a Chinese hamster cell mutant xrs-5 (figure 1). This protein is present in the nucleoid faction of the parent cell line, CHO-K1, and present in whole cell gel patterns of both cell types (figure 2). Previous studies have established that the xrs cell line, a radiosensitive derivative of the radioresistant cell line CHO-K1, was more susceptible to x-ray radiation and other DNA damaging agents due to its deficiency in the ability to repair double stranded DNA breaks (Kemp et al.,1984). This paper proposes that the 55kd protein absent in the nuclear protein matrix pattern of xrs-5 is involved in double stranded break repair. The absence of this 55kd protein in the nucleoid is one possible explanation for the x-ray sensitivity and phenotypic differences in chromosome structure of the xrs-5 cell line.

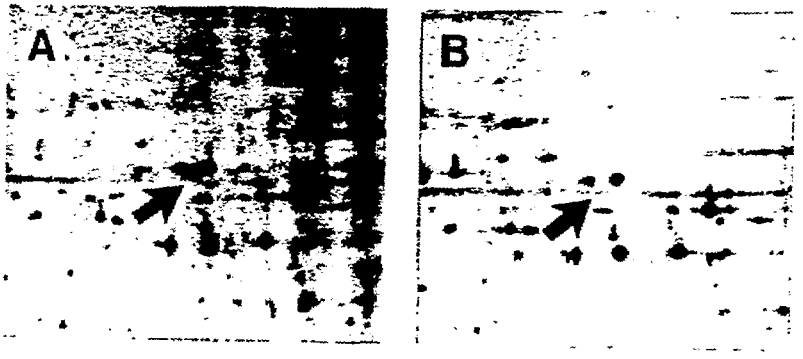

Figure 1: Two-dimensional gel electrophoresis patterns of nuclear matrix proteins showing the 55kd region of A) CHO-K1 and B) xrs-5. The arrows indicate the protein of interest.

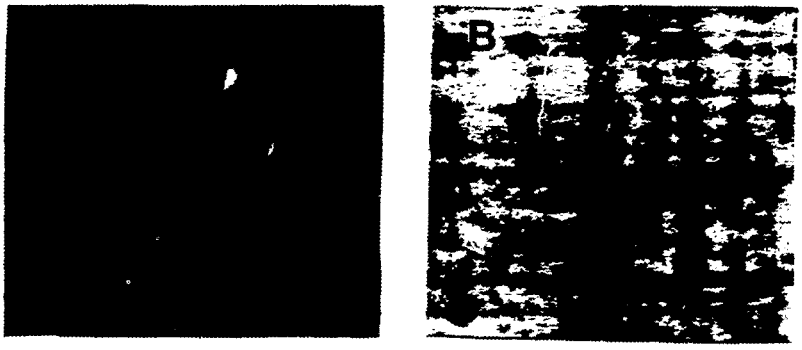

Figure 2: Two-dimensional gel electrophoresis patterns of whole cell proteins showing the 55kd region of A) CHO-K1 and B) xrs-5. The arrows indicate the protein of interest.

It is possible to suggest that proteins responsible for DNA organization may be linked to x-ray sensitivity and DSB repair (Schwartz,1990) Analysis of xrs-5 chromosome morphology after treatment with colcemid showed metaphase chromosomes were much shorter and thicker than CHO-K1 cells. This difference could also be seen in untreated cells. More interestingly, the 1.650 cell line, reverted in x-ray sensitivity from xrs-5 cells also reverted almost completely in their metaphase chromosome morphology (Schwartz,1990). It is unlikely that both characteristics x-ray sensitivity and chromosome morphology reverted at the same time. Therefore, it is believed that one genetic change has dual effects.

Chromosome conformation is very important to biological activity. Chromatin must undergo conformational changes in order to make regions more accessible to endonucleases. (Smerdon and Lieberman, 1978). Several studies have shown conformation to be important to repair mechanisms. For example, fibroblast cells taken from patients with xeroderma pigmantosum (XP), are defective in their ability to alter chromatin in preparation of excision repair of thymine dimers (Hittleman, 1986).

DNA supercoiling activity was analyzed in two murine leukemic lymphoblast cell lines of differing radiosensitivity. Results showed that they also differed in their DNA supercoiling ability. Furthermore, one dimensional SDS polyacrylamide gel electrophoresis found nucleoid protein samples missing a 55kd protein that was present in the cells proficient in supercoiling ability and radiosensitivity. Lastly, one study has attempted to separate DSB repair from the nucleoid. Exogenous plasmid vectors with no associated chromatin were inserted into xrs-5 and CHO-K1 cells with endonuclease induced breaks. No difference in the rate of rejoining was detected, indicating similar abilities of DSB repair when nuclear matrix proteins are not a factor. (Schwartz,1990)

This paper further proposes that future experiments will confirm the preliminary experiments with two radioresistant revertants, aza-2 and 1.650. These experiments revealed the presence of the same 55kd protein in 1.650 revertant nucleoid patterns. The confirmed presence of this

protein in 1.650 and AZA-2 will further strengthen the argument that the 55kd protein is involved in DSB repair. Furthermore, the 1.650 revertant line has similar chromosome structure to the parent line. However, the AZA-2 is not consistent with 1.650. While AZA-2 has reverted to partial radioresistance, its chromosome structure is still like xrs-5, short and condensed. It is hoped that studies currently underway will clarify these differences, and elucidate how and if the 55kd protein is involved in both DSB repair and chromosome packaging.

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