Enhanced Expression of Calreticulin in the Nucleus of Radioresistant Squamous Carcinoma Cells in Response to Ionizing Radiation

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ABSTRACT

Ionizing radiation has been shown to modulate gene and protein expression as well as cellular signal transduction pathways. However, the biochemical and molecular mechanisms that underlie the cellular response to radiation are not fully understood. The effects of ionizing radiation on the expression of nuclear proteins have now been investigated in radioresistant human head and neck squamous carcinoma cells (SQ-20B cells) using the techniques of two-dimensional PAGE, silver staining, and computer-assisted quantitative analysis. Radiation (600 cGy) induced the expression of 10 proteins and suppressed the expression of 5 proteins in the nuclei of SQ-20B cells as detected 4 h after treatment. Electroelution and NH2-terminal amino acid sequence analysis revealed that one of the radiation-induced proteins was the Ca2+-binding protein calreticulin. The expression of calreticulin was increased approximately 6-fold in the nuclei of irradiated SQ-20B cells. Calreticulin and the other proteins whose expression was affected by radiation may contribute to the radioresistance phenotype of SQ-20B cells.

INTRODUCTION

Radiation therapy plays a significant role in the treatment of cancer patients. Determination of the effects of radiation on tumor cells at the biochemical and molecular levels is vital to our understanding the cellular radiation response. Several studies have shown that radiation modulates the expression of specific genes. Thus, radiation increases the expression of various cytokine and growth factor genes including those for tumor necrosis factor (1, 2), interleukin-1 (3), platelet-derived growth factor, and fibroblast growth factor (4). Radiation also increases the expression of nuclear factor κ B (5), Gadd45 (6), and EF-1β (7). Sherman et al. (8) have shown that ionizing radiation increases the expression of c-jun, c-fos and jun-B at the transcriptional level, and Hallahan et al. (9–11) have demonstrated that ionizing radiation increases the expression of the transcription factor EGR1 and that the induction of both EGR1 and c-jun expression is mediated by protein kinase C. Finally, Wilson et al. (12) have shown that ionizing radiation increases the expression of c-myc in primary human B cells, whereas Boothman and colleagues (13, 14) have characterized several X-ray-induced transcripts as well as transcription factors and DNA binding sites that may mediate the induction of these transcripts. It is thought that expression of these various genes is related to the cellular capacity for surviving radiation injury. The effects of ionizing radiation on cellular signal transduction pathways have also been investigated. Thus, radiation activates phospholipase D (15), stimulates tyrosine kinases in human B lymphocytes (16), and specifically induces the tyrosine phosphorylation of p34cdc2 (17).

Other investigations have focused on changes in protein expression levels induced by exposure of cells to radiation. Lambert and Borek (18) compared the effects of X-rays on normal and oncogene-transformed rat cells and detected 14 proteins that were differentially expressed between the normal and oncogene-transformed cells.

Boothman et al. (19) showed that exposure of human melanoma cells to X-rays induced the expression of several specific polypeptides. In addition, a DNA-binding protein that is activated as a result of exposure of lymphoblastoid cells to γ-radiation has been characterized (20) and purified (21). Although these studies have detected several proteins whose expression is modulated by radiation, the roles of these proteins in the cellular response have not been elucidated.

We have applied the powerful combination of two-dimensional PAGE, silver staining, and quantitative computer analysis to characterize changes in the expression of specific nuclear proteins in response to ionizing radiation. We have studied human head and neck squamous carcinoma cell line SQ-20B, which has been extensively characterized as relatively radioresistant (22–24), to focus on those proteins that may contribute to the phenotypic expression of radioresistance.

MATERIALS AND METHODS

Cell Culture, Radiation Treatment, and Nuclei Preparation. The head and neck squamous carcinoma cell line SQ-20B was established and characterized as described previously (22–24). Radiation dose-response analysis (radiation survival curves) showed a D0 value of 2.4 Gy for SQ-20B cells, and clinical correlations were made on the basis of the response of the tumors from which SQ-20B cells were derived to radiation therapy (recurrence or radio-curability; Refs. 23 and 24). Both criteria indicated a relatively radioresistant phenotype of SQ-20B cells.

Cells were maintained in culture medium consisting of DMEM, 10% fetal bovine serum, 2 mm glutamine, 2 mm penicillin-streptomycin, and 0.4 μg/ml hydrocortisone and were routinely passaged at 70–80% confluency (approximately 3–4 days after subculturing). Cells were grown to confluence (as judged by light microscopy, approximately 5–7 days after subculturing), and the following morning irradiated (600 cGy) with a 60Co irradiator (Theratron-80). Control cells were not exposed to radiation. Cells were harvested 4 h after irradiation by washing with PBS, trypsinization (0.05% trypsin, 0.53 mM EDTA; GIBCO-BRL), and centrifugation at 2500 x g. Nuclei were then prepared from cell pellets as previously described (25) and frozen. Protein concentration was determined with the Pierce BCA protein assay system.

FACS. Control and irradiated SQ-20B cells were harvested in duplicate 0–25 h after irradiation, and 1 × 106 cells were resuspended in FACS buffer [250 mM sucrose, 40 mM trisodium citrate (pH 7.6), 5% DMSO]. Staining of cells with propidium iodide (26) and DNA histogram analyses were performed in the FACS Core Facility of the Lombardi Cancer Research Center. FACS analysis revealed the percentage of cells in various stages of the cell cycle.

2D-PAGE. Nuclei from control and irradiated SQ-20B cells were analyzed using 2D-PAGE (27) with the ISO-DALT 2D-PAGE System (Large Scale Biologicals, Inc.). The procedure represented a slightly modified version of that of Anderson and Anderson (28) as described previously (22). Frozen nuclei were thawed and solubilized in a solution containing 9 mM urea, 4% NP40, 2% amilorines (pH 8–10), and 1% DTT. Approximately 50 μg protein were applied to 1D tube gels (25.5 cm) containing 3.5% polyacrylamide, 9% urea, 2% amilorines (one part pH 3.5–10, three parts pH 4–8), and 2% NP40. The gels were prefocused at 200 V for 2 h with 0.085% phosphoric acid in the lower chamber and 0.02 N NaOH in the upper chamber. Molecular mass and pI standards (Sigma) were included in at least one of the tube gels in each batch. Isoelectro focusing was performed at 1200 V for approximately 20 h. The ISO-DALT 2D-PAGE System allows the simultaneous running of samples of 20 tube gels (and subsequently 20 second-dimension slab gels), thus...
allowing the advantage of reproducibility. After isoelectric focusing, the tube gels were cut (1.85 cm from the acidic end, 5.2 cm from the basic end) and layered on slab gels (17.8 x 17.8 cm, 10% polyacrylamide). Electrophoresis was performed at 80 V for 20 to 22 h with constant cooling (RCB 300 cooling water bath; Hoefer Scientific). The gels were then placed in fixative (40% methanol, 10% acetic acid) for at least 2 h.

Silver Staining. Fixed 2D-PAGE gels were subjected to silver staining as previously described (22, 29). Briefly, gels were equilibrated for 30 min in 10% ethanol and 5% acetic acid (two changes of solution), washed with Milli-Q water (Millipore filtration unit, Model CDOFO 1205), incubated with DTT (15 μg/ml) for 30 min, washed three times in Milli-Q water, and then exposed to 0.1% silver nitrate for 30 min. The gels were rinsed with Milli-Q water and developed with 3% sodium carbonate and 0.02% formaldehyde for 5 to 15 min. Development was terminated by placing the gels in 5% acetic acid.

Computerized Quantitative Analysis. Analysis of the protein profiles obtained using 2D-PAGE was performed by densitometric scanning of the silver-stained gels, digitization of the image, computer-assisted comparison of protein profiles, quantitation of protein differences, and determination of the molecular masses and pI values of the proteins of interest. These procedures were implemented with a Sun Microsystems 3/110 computer interfaced to a Photometrics scanner and digitizer (PS200 power supply, 1035 x 1320 assay). The gels were scanned with a Nikon f2.8 macro lens, and the output was digitized in such a manner that the background noise (attributable to variations in the light box used to illuminate the image) was subtracted from the protein spot signal, which was then assigned a value of 0 (white) to 250 (black). The data were stored on the main computer, and comparative analysis of the protein profiles was performed with the ELSIE-5 software program, an updated version of a program previously described by Olson and Miller (30). The nuclear protein patterns of control and irradiated SQ-20B cells were compared, and the quantitative differences were estimated by normalization of the spot values to that of the internal standard actin (43 kDa, pI 5.3). The molecular mass and pI values were determined with the ELSIE-5 program on the basis of internal protein standards in combination with the additional molecular mass and pI standards included in each 2D-PAGE experiment.

Microsequencing. Two proteins shown by 2D-PAGE analysis to be induced by radiation treatment were purified for NH2-terminal sequencing analysis. Nuclear proteins prepared from irradiated SQ-20B cells were separated using 2D-PAGE, and the resultant gels stained by colloidal Coomassie blue G-250 which, unlike silver staining, does not interfere with the subsequent microsequencing (31, 32). The two proteins of interest were excised from the gels and electroeluted with an Amicon Centrilutor. The electroeluted proteins were subjected to SDS-PAGE (33) and then transferred overnight to a polyvinylidene difluoride membrane (Millipore) in Tris-glycine-methanol buffer with a Bio-Rad Transblot Cell at 30 V and 4°C (34). The membrane was stained with 0.1% Coomassie blue R-250 in 50% methanol and 0.1% acetic acid, and the bands of interest were excised and subjected to microsequencing by Dr. Jane Walent at the University of Wisconsin Biotechnology Center.

RNA Isolation and Northern Blot Analysis. Total RNA from control and irradiated (600 cGy) SQ-20B cells was isolated with the RNAzol B method (Tel-Test, Inc.). Briefly, cells were lysed with RNAzol B reagent, and the RNA was extracted by addition of chloroform and centrifugation (12,000 x g, 4°C). The RNA was isolated by precipitation (isopropanol for 15 min at 4°C), centrifugation (12,000 x g, 4°C), and ethanol washing. Twenty μg RNA from each sample were electrophoresed on 1.4% agarose gels under alkaline conditions (18% formaldehyde) and transferred to nitrocellulose. After baking at 80°C under vacuum for 2 h, the blot was hybridized at 42°C in 50% formamide, 5X SSC, 1X Denhardt’s solution, 50 mM Na,PO, (pH 6.5), 1% glycine, 250 μg/ml tRNA, and 32P-labeled cDNA probe. The calreticulin cDNA probe (1.8 kb) was isolated from a plasmid plasmid containing the calreticulin insert, which was kindly provided by Dr. Marek Michalak. The glyceraldehyde-3-phosphate dehydrogenase cDNA probe (1.4 kb; Ambion, Inc.) was used as a loading control. Both cDNA probes were labeled with [32P]dCTP (New England Nuclear) using a megaprime end-labeling kit (Amersham). Hybridized blots were washed sequentially with 2X SSC and 0.1% SDS, 0.1X SSC, 0.1% SDS, and 0.1X SSC and then autoradiographed (10 days for the calreticulin cDNA probe, 2 days for the glyceraldehyde-3-phosphate dehydrogenase cDNA probe).

RESULTS

FACS Analysis of Confluent Cells. To investigate possible differences in cell cycle distribution in confluent cultures of control versus irradiated SQ-20B cells, we performed FACS analysis 0–25 h after irradiation. The percentage of cells in G0-G1, S-phase, and G2-M did not differ significantly between control (Fig. 1A) and irradiated (Fig. 1B) SQ-20B cells. Thus, the use of confluent cells in this study avoided complications due to cell cycle redistribution (for example, G2 arrest) in the interpretation of results.

Radiation-induced Changes in Protein Expression. We have compared the 2D-PAGE nuclear protein profiles of control (Fig. 2A)
and irradiated (Fig. 2B) SQ-20B cells in order to focus on those proteins that may play a role in the regulation of gene expression or in DNA damage and repair. The 2D-PAGE system used has the advantage of allowing the simultaneous analysis of up to 20 samples, with minimal variation in the protein profiles as detected by silver staining. To ensure that any observed differences in protein expression were due to radiation treatment, we analyzed control and irradiated samples on triplicate 2D-PAGE gels. The degree of induction or suppression (fold change) of protein expression by irradiation was calculated by normalization of the computer-derived spot value (obtained by computer digitization of the silver-stained gel) for each protein to that of the internal standard actin (43 kDa, pl 5.3) from the same gel. The mean value for the actin spot was 131 ± 28 arbitrary units for each 2D-PAGE gel in the analysis. The resulting normalized spot/actin value was then used to determine the fold change between control and irradiated samples. This 2D-PAGE analysis system reliably assesses proteins within the molecular mass range from 15 to 150 kDa and the pl range between 4 and 7.

We detected 10 proteins (molecular mass, 24–108 kDa; pl, 4.3–6.5) whose expression was increased 1.4–10-fold by ionizing radiation (proteins 1–10) and five proteins (molecular mass, 23–29 kDa; pl, 4.8–5.7) whose expression was decreased 2.3–55-fold (proteins a–e, Table 1).

Purification and Microsequencing of Radiation-induced Proteins. To identify the radiation-induced proteins detected by 2D-PAGE, we applied the techniques of electroelution and NH2-terminal amino acid sequence analysis. We initially chose proteins 1 and 2 (Fig. 2 and Table 1) for further analysis for two reasons: (a) both were stainable with Coomassie blue and present in sufficient quantities for the subsequent manipulations, and (b) both migrated to positions in

<table>
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* Values are means of triplicate samples, each normalized against actin. The differences between control and irradiated samples were statistically significant (P ≤ 0.05, Student’s t test; Ref. 35).
RADIATION AND CALRETICULIN EXPRESSION

Fig. 3. SDS-PAGE gels of purified proteins 1 and 2. Proteins 1 and 2 were excised and electroeluted from 2D-PAGE gels, subjected to SDS-PAGE, transblotted, and stained with Coomassie blue R-250. Molecular weight markers, kDa. Arrows, bands corresponding to protein 1 (apparent molecular mass, 100 kDa, A) and protein 2 (apparent molecular mass, 63 kDa, B).

Fig. 4. Comparison of the amino acid sequence of protein 2 with that of calreticulin. The upper 13 amino acids were detected by HPLC analysis of the residues released from protein 2 by automated Edman degradation (uppercase letters, amino acids that were readily assigned; lowercase letters, amino acids that were more difficult to assign). A search of the Swiss Protein Sequence Data Bank with the nine most reliable amino acids (uppercase letters) resulted in a 100% match with amino acids 21-29 of the Ca2+-binding protein calreticulin (lower sequence; Ref. 36).

The NH2 terminus of protein 1 was blocked, but we were able to obtain amino acid sequence data for protein 2. We used the nine most readily identified amino acids (Fig. 4) to probe the Swiss Protein Sequence Data Bank. This search resulted in a 100% match of all nine amino acids with the protein calreticulin. The 13 amino acids detected by automated Edman degradation of protein 2 correspond to amino acids 19-31 of calreticulin predicted from the cDNA sequence data (36). Because calreticulin is an endoplasmic reticulum membrane protein, the NH2-terminal 18-amino acids, which constitute the signal peptide, would have been cleaved to yield the mature protein and, therefore, undetectable by Edman degradation. Calreticulin has an apparent molecular mass of 60–63 kDa in SDS-PAGE gels (Laemmli system) and is highly acidic (pl 4.14–4.67; Ref. 36). These values are in good agreement with the corresponding values estimated from our 2D-PAGE gels (Table 1).

Northern Blot Analysis. To determine whether the radiation-induced increase in calreticulin protein expression was due to a corresponding increase in calreticulin mRNA expression, we performed Northern blot analysis on control and irradiated samples of SQ-20B RNA (Fig. 5). The calreticulin probe detected a band of 1.8 kb in SQ-20B cells (Fig. 5, top panel). The level of calreticulin mRNA expression in SQ-20B cells was not increased up to 8 h after exposure of 600 cGy ionizing radiation. Rehybridization of the blot with glyceraldehyde-3-phosphate dehydrogenase was used as a loading control (Fig. 5, bottom panel). These results confirmed that the observed increase in calreticulin in the nucleus of SQ-20B cells was not the result of increased levels of RNA transcripts.

DISCUSSION

The purpose of this study was to examine the changes in expression of nuclear proteins induced by exposure of radioresistant cells to ionizing radiation. Our goal was to characterize and identify such proteins through 2D-PAGE analysis, computer-assisted quantitation of silver-stained gels, protein purification, and NH2-terminal sequencing. We chose silver staining (29) rather than isotopic radiolabeling and autoradiography to detect proteins of interest on the basis of our previous studies showing that 2D-PAGE analysis of isotopically labeled cellular extracts results in many artifacts that do not reflect changes in protein expression (22). Moreover, the proteins of interest had to be stainable with Coomassie blue to ensure their expression in sufficient (μg) quantities for purification and automated Edman degradation (32).

Fig. 5. Time course of calreticulin (CRT) mRNA expression in control and irradiated SQ-20B cells. A Northern blot containing 20 μg/lane total RNA was hybridized to a 32P-labeled calreticulin cDNA probe (top panel). The level of expression of calreticulin mRNA (1.8 kb) did not increase with radiation of 600 cGy as judged by comparison of the control (left lanes) and irradiated (right lanes) samples at 0, 4 and 8 h after radiation exposure. As a loading control, the same blot was hybridized to a 32P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.4 kb) cDNA probe (bottom panel).
We examined confluent cells to avoid the effect of radiation-induced cell cycle redistribution which may have impaired our ability to determine which proteins are differentially expressed in control versus irradiated cells as a direct consequence of radiation. FACS analysis confirmed that the percentage of cells in G1, G2, or M stage was not significantly altered by radiation treatment. Furthermore, immunoblot experiments with antibodies specific for cell cycle-related proteins (cyclin A, cyclin B1, and proliferating cell nuclear antigen) revealed no differences in protein expression between control and irradiated SQ-20B cells (data not shown). Therefore, it is unlikely that the radiation-induced changes in protein expression observed by 2D-PAGE analysis are simply a consequence of cell cycle redistribution.

We have shown that irradiation of SQ-20B cells increased the expression of 10 proteins and decreased the expression of 5 proteins. The expression of these 15 proteins did not appear to be affected by changes in protein expression observed by 2D-PAGE analysis are specific for cell cycle-related proteins (cyclin A, cyclin Bl, and ability to determine which proteins are differentially expressed in sision is impractical, we can only speculate that these radiation-induced proteins play a role in expression of the radiosensitivity phenotype.

We definitively identified one of the 10 radiation-induced proteins in SQ-20B cells as calreticulin. The molecular mass (63 kDa) and pI (4.3) estimates obtained from our 2D-PAGE gels are consistent with those published for calreticulin (reviewed in ref. 36). Calreticulin is a Ca2+-binding protein that was first described in the sarcoplasmic reticulum of skeletal muscle cells. More recently, it has been identified in several nonmuscle cells and referred to by various names, including calregulin, reticuloplasmin, CRP55, CaBP3, and Erp60. Calreticulin is thought to be localized to the rough endoplasmic reticulum (36). However, Krause et al. (37) and Van Delden et al. (38) have provided evidence for the presence of calreticulin in specialized Ca2+ storage and release vesicles, termed “calciosomes” (39), in HL-60 cells. In addition, Opas et al. (40) have shown that calreticulin may also be present in the nucleus and nuclear envelope, and, in fact, contains a putative nuclear targeting signal in the primary amino acid sequence (PPIKIDPD, residues 188–195; Ref. 36). Its primary amino acid sequence also reveals that calreticulin is a member of the KDEL (endoplasmic reticulum retention sequence) family of proteins, which includes glucose-regulated protein 94, BiP (glucose-regulated protein 78), and protein disulfide isomerase (41). We suggest that the approximately 6-fold increase in calreticulin expression observed in the nucleus of SQ-20B cells after exposure to ionizing radiation may be attributable to translocation of this protein from the endoplasmic reticulum (or calciosomes) to the nucleus. Proteins of the KDEL family have a high degree of sequence homology to the stress-induced proteins heat shock protein 70 and heat shock protein 90/83. Furthermore, under stress conditions, both BiP and glucose-regulated protein 94 have been detected in the nucleus in addition to the endoplasmic reticulum (41). Relocalization of calreticulin to the nucleus could therefore represent a stress response of SQ-20B cells to ionizing radiation.

Calreticulin expression is increased in tumor cells and proliferating cells although its function is unknown (36). Perhaps the observed increase in calreticulin expression after radiation treatment was a consequence of increased proliferation. Although we cannot rule out this possibility, it seems unlikely that the increase in calreticulin in the nucleus was solely a consequence of increased cellular proliferation, because the cells were confluent before radiation treatment and the cell cycle distribution was essentially unchanged after radiation treatment (Fig. 1). In addition, cycloheximide treatment did not alter the observed radiation-induced increase in calreticulin in the nucleus of SQ-20B cells (data not shown). This suggests that calreticulin increase in the nucleus of SQ-20B cells after radiation treatment is not the result of increased protein expression.

Radiation-induced changes in the expression of a Ca2+-binding protein have not been previously described. Given that (a) calreticulin copurifies with an inositol 1,4,5-triphosphate-sensitive intracellular Ca2+ storage compartment in HL-60 cells (37, 38), (b) diacylglycerol resulting from inositol 1,4,5-triphosphate mobilization activates protein kinase C (15), (c) protein kinase C is activated in response to radiation (11), and (d) radiation or Ca2+ mobilization stimulates phospholipase D (15), we speculate that calreticulin plays a role in the signal transduction pathway that mediates the cellular response to radiation. Thus, the radiation-induced generation of free radicals and consequent oxidation of membrane phospholipids may promote phoshoinositol turnover, resulting in calreticulin-mediated Ca2+ mobilization, translocation of this Ca2+-binding protein to the nucleus, and protein kinase C activation. Since the cellular function of calreticulin is unknown, further studies are necessary to investigate this hypothesis.

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REFERENCES


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