Activation of Meiosis-Specific Genes Is Associated with Depolyploidization of Human Tumor Cells following Radiation-Induced Mitotic Catastrophe

Fiorenza Ianzini, 1,2,3 Elizabeth A. Kosmacek, 1,3 Elke S. Nelson, 1 Eleonora Napoli, 1 Jekaterina Erenpreisa, 4 Martins Kalejs, 4 and Michael A. Mackey 1,3

Departments of Pathology and Radiation Oncology, University of Iowa, Department of Biomedical Engineering, Seamans Center, University of Iowa, Iowa City, Iowa, and Latvia Biomedical Research and Study Centre, Riga, Latvia

Abstract

Cancer is frequently characterized histologically by the appearance of large cells that are either aneuploid or polyploid. Aneuploidy and polyploidy are hallmarks of radiation-induced mitotic catastrophe (MC), a common phenomenon occurring in tumor cells with impaired p53 function following exposure to various cytotoxic and genotoxic agents. MC is characterized by altered expression of mitotic regulators, untimely and abnormal cell division, delayed DNA damage, and changes in morphology. We report here that cells undergoing radiation-induced MC are more plastic with regards to ploidy and that this plasticity allows them to reorganize their genetic material through reduction division to produce smaller cells which are morphologically indistinguishable from control cells. Experiments conducted with the large-scale digital cell analysis system are discussed and show that a small fraction of polyploid cancer cells formed via radiation-induced MC can survive and start a process of depolyploidization that yields various outcomes. Although most multipolar divisions failed and cell fusion occurred, some of these divisions were successful and originated a variety of cell progeny characterized by different ploidy. Among these ploidy phenotypes, a progeny of small mononucleated cells, indistinguishable from the untreated control cells, is often seen. We report here evidence that meiosis-specific genes are expressed in the polyploid cells during depolyploidization. Tumor cells might take advantage of the temporary change from a promitotic to a promeiotic division regimen to facilitate depolyploidization and restore the proliferative state of the tumor cell population. These events might be mechanisms by which tumor progression and resistance to treatment occur in vivo. [Cancer Res 2009;69(6):2296–304]

Introduction

The fact that drug and/or ionizing radiation exposures trigger cell cycle checkpoints that inhibit entry into or progression through mitosis has been exploited in cancer treatment because these effects are considered major factors in these agents' toxicity (1, 2). However, cells lacking p53 function also lack G2 checkpoint function and will escape these constraints. This is an important fact for cancer treatment outcome because >50% of human tumors (especially solid tumors) are p53 nonfunctional (3, 4), and thus, lack G2 checkpoint surveillance and are often resistant to cytotoxic and genotoxic treatments. Thus, the ultimate goal of eradicating cancer by subjecting patients to surgical intervention and rounds of radiation and/or chemotherapeutic agent treatments might be compromised by the tumor response to these later treatments. Cancer cells are genetically very heterogeneous, containing aneuploid, tetraploid, and polyploid populations, and the presence of polyploid cells in malignant tumors has been established in the literature (5, 6), but their origin and biological significance are still elusive. The question of whether selection of polyploid cells during radiation treatment might contribute to the development of resistance to such treatment was posed by Revesz and Norman in an article published in 1960 (7), and polyploid tumor cells have been found to be more resistant to radiation treatment than their diploid counterparts (8), whereas various clinical trials carried out on patients treated with radiation therapy and/or chemotherapeutic agents for prostate cancer have pointed out that a high degree of aneuploidy in these patients is associated with reduced overall survival (9, 10). Reversion of extremely hyperploid cancer cells (metaphases with more than 1,000 chromosomes) to smaller pseudodiploid cells over a period of 12 days was reported by Sandberg and collaborators (6) on peritoneal effusion cells from a patient with aggressive cancer of the colon. These earlier studies have raised questions as to the fate of polyploid tumor cells produced during anticancer treatment, the ability of these cells to undergo depolyploidization, and the role of these processes in tumor progression. In 2005, Zybina and Zybina reported that normal mammalian cells are able to undergo depolyploidization (11), and that whole genome segregation occurs in the giant nuclei (more than 100N) of the trophoblasts (the cells that form the outer layer of the blastocyst and mediate the implantation of the embryo into the endometrium) originating cells containing 2N, 4N, and 8N chromosome complements. These results suggest that depolyploidization might be a common phenomenon and that cells may possess an internal control for segregation of entire genomes. Reduction division in somatic cells of higher organisms is considered a rare event (12); nevertheless, this might not be the case in tumor cells in which a highly unstable genome is present (13). In fact, high-frequency genetic exchange events occurring during reduction division (meiosis) may contribute to a genetic diversity that could render tumor cells more apt to survive after anticancer treatments.

Depolyploidization in solid and circulating tumors following radiation-induced mitotic catastrophe (MC; reviewed in refs. 14–18)
has been described by us in cytologic studies (19), and although MC is generally lethal (20), we and others have reported that a small fraction of cells which undergo radiation-induced MC and become polyploid can survive long enough to establish a growing population of cells (21–23). In the present article, by using the large-scale digital cell analysis system (LSDCAS), which allows for nonperturbing live cell imaging measurements, we show that polyploid tumor cells formed via radiation-induced MC are able to survive for many days postirradiation and undergo multipolar divisions. Most of these divisions fail and cell fusion occurs, however, a fraction of these divisions marks the beginning of the process of depolyplodization, giving rise to smaller daughter cells that possess only one nucleus and whose size is indistinguishable from that of the untreated control cells. Some of these mononucleated smaller cells originating through reduction division events give rise to viable descendants. These cells will de facto have a disarranged genomic composition; nevertheless, abnormal chromosome arrangements attributable to MC may endow the tumor cells with properties that not only differentiate them from normal somatic cells, but may also impart growth advantages over other cells and may make them more resistant to subsequent treatment. Molecular analysis of the polyploid cells formed via radiation-induced MC shows that meiosis-specific genes are activated during the depolyplodization process. Polyploid cells undergoing depolyplodization also present morphologic features similar to those characteristic of meiotic prophase I. Thus, a small percentage of irradiated cells seems to acquire pseudomeiotic properties enabling them to escape radiation-induced cell death. This fact has implications in the clinical setting: we speculate that tumors in vivo might take advantage of this switch from a promitotic to a promeiotic division regime to balance their disrupted genetic make-up and thus might acquire a more stable growth state and stabilize genetic alterations, eventually becoming resistant to subsequent cytotoxic treatment.

Materials and Methods

Cell culture. HeLa S3 human cervical cancer cells were grown in Joklik's MEM (Life Technologies), HeLa clone 3 human cervical cancer cells were grown in Ham's F-10 Nutrient Mixture medium (Life Technologies), HCT116 human colon cancer cells were grown in McCoy’s 5A modified medium (Life Technologies), and MDA-MB435 human breast cancer/melanoma cells were grown in high-glucose DMEM (Life Technologies). The origin of the latter cell line (MDA-MB435) has been under debate for some time (24, 25), as the actual tissue from which it is derived is not clear. This cell line is still a very good model for solid tumor response to radiation exposure regardless of its origin. We will mention this cell line only by its nomenclature from this point on without specifying the tissue of origin. All media were supplemented with 10% fetal bovine serum (Hyclone) and antibiotics from this point on without specifying the tissue of origin. We will mention this cell line only by its nomenclature.

Cell lines were supplemented with 10% fetal bovine serum (Hyclone) and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin; Life Technologies). All cell lines were grown in 5% CO2 in air incubators at 37°C. Media were changed every 2 days.

γ-Irradiation. γ-Irradiation was delivered at room temperature using an 8,148 Ci 137Cs source at the dose rate of 0.92 Gy/min.

Immunofluorescence. Harvested cells were suspended in 100% FCS, cytospun onto poly-l-lysine–coated microscope slides, and fixed in either 4% paraformaldehyde or in PBS (pH 7.4; 15 min). Slides were then washed twice in PBS at room temperature, and permeabilized for 10 min in PBS containing 0.25% Triton X-100 and then washed in PBS thrice for 5 min each. Slides were subsequently blocked for 30 min in 1% bovine serum albumin in PBS containing 0.05% Tween 20 (PBST). Fifty microliters of the appropriate dilution of antibody was applied to each sample and slides were incubated overnight at 4°C. Samples were then washed thrice in PBST for 5 min each, at which point, 50 μL of the appropriate secondary antibody was applied at the appropriate dilutions and slides were incubated at room temperature in the dark for 1 h, and then washed thrice in PBS for 5 min each in the dark. Antibodies were diluted in 1% bovine serum albumin in PBST. Samples were counterstained and mounted with DAPI Vectashield Mounting Media (Vector Laboratories) and evaluated under an Olympus B51 epifluorescence microscope.

Cytologic preparations. Harvested cells were suspended in 100% FCS, cytospun onto poly-l-lysine–coated microscope slides, and let dry overnight and fixed in acetone/100% ethanol, 1:1 (v/v), at 4°C for 30 min. Slides were then allowed to dry for 15 min at room temperature. Sample hydrolysis was performed by incubating the slides in 5N HCl at room temperature for 1 min, followed by five rinses in distilled water for 1 min each. Sample staining was performed on blot-dry slides by incubating the slides in 0.05% toluidine blue in McIlvan’s solution (pH 5) for 10 min at room temperature followed by a series of rinses in distilled water until removal of excess staining, at which point, slides were blotted dry and dehydrated by incubating slides twice in butanol at 37°C for 3 min. Sample clearing was then performed by incubating slides twice in xylene for 3 min each at room temperature. Finally, coverslips were mounted with Cytosel 60 Mounting Media and slides evaluated under an Olympus B51 epifluorescence microscope.

LSDCAS. LSDCAS is an automated live cell imaging microscope and image analysis system capable of analyzing thousands of living cells for a period of a few days up to weeks in a single experiment (21, 26). LSDCAS is designed to allow quantitative study of cell populations grown under conditions identical to those used in routine biochemical/molecular investigations of a variety of phenomena and is useful for determining the kinetics of various cellular mechanisms on a cell-by-cell basis. For live cell imaging, 24 h prior to irradiation, cells were plated at 1 × 105 cells in a T25 flask containing 3 mL of complete medium. After irradiation, cells were fed 3 mL of warm CO2 equilibrated medium and transported to the LSDCAS facility where 150 random fields were manually selected and acquisition initiated to run for 12 days. Image analysis was performed by using Cas/Analyze, an in-house developed software package, which allows for the quantitation of division-related events (26).

Quantitative real-time reverse transcriptase PCR assay. Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. cDNA was synthesized using SuperScript II RT (Invitrogen) according to the manufacturer’s protocol. Reverse transcription was performed using 1 μg of total RNA and a mixture of oligo(dT) and random hexamer primers. One-twentieth of this reaction, or cDNA corresponding to 50 ng RNA was then used for PCR. PCR was performed using Immolase DNA Polymerase (Bioline) in 25 μL reactions containing 1× Immolase buffer, 1.5 mmol/L MgCl2, and 200 μmol/L each of deoxynucleotide triphosphate, 200 nmol/L of primer, 200 nmol/L of probe, and 0.75 units of Immolase. Primers and FAM-labeled probes were obtained from Integrated DNA Technologies. Reactions were performed in triplicate with the DNA Engine Opticon 2 Thermalcycler (MJ Research, Bio-Rad) using the following cycling parameters: 94°C for 1 min, followed by 40 cycles of 94°C for 10 min followed by 60°C for 1 min. The 60s acidic ribosomal protein P0 was used as a control to normalize for pipetting error. Data were analyzed using Opticon Monitor Analysis Software Version 2.02 (MJ Research). Samples were quantitated using a standard curve constructed of known copy numbers of p-GEM-T PCR cloning vector (Promega) containing a PCR amplicon in the multiple cloning region. This standard curve generated from total RNA is used to calculate the relative mRNA in the starting material. The average amount of mRNA for the gene of interest, calculated as a control to normalize for pipetting error. Data were analyzed using Opticon Monitor Analysis Software Version 2.02 (MJ Research). Samples were quantitated using a standard curve constructed of known copy numbers of p-GEM-T PCR cloning vector (Promega) containing a PCR amplicon in the multiple cloning region. This standard curve generated from total RNA is used to calculate the relative mRNA in the starting material. The average amount of mRNA for the gene of interest, calculated using the standard curve, is then normalized to the values for β-actin. Fold increases of mRNA levels in the treated cells are then calculated relative to control samples.

 Autoradiography assay. Total RNA extraction was performed as described above using an RNeasy mini kit (Qiagen). The RT2 First-Strand kit (SuperArray) was used to eliminate genomic DNA and reverse transcribe 1 μg of total RNA to cDNA. This template DNA was then added to the RT2 SYBR Green qPCR Master Mix (SuperArray) and 25 μL were loaded into each well of the RT2 Profiler PCR Array (SuperArray). The SuperArray was custom-designed to contain primers for the following genes: STC2P, STC3P.
Results

The polyploid cells formed via radiation-induced MC begin depolyploidization by forming metaphase plates and segregating nuclei. Following 10 Gy of γ-irradiation, HeLa S3 cells undergo MC and form polyploid cells (Fig. 1A). By the end of the first week postirradiation, the majority of the cells that underwent MC die. At 7 to 9 days postirradiation and later, the polyploid cells that have escaped death form metaphase plates (Fig. 1B), and initiate a depolyploidization process through which nuclei are segregated in what seems to be viable descendants (Fig. 1C).

Previous work (19, 27) using DNA image cytometry revealed that when division is resumed, the cells’ DNA content (which had increased six to eight times over the control values) is reduced to that of the untreated populations. Note that the segregated cells contain only one nucleus and are morphologically indistinguishable from control cells (Fig. 1D). Noteworthy to mention is the fact that, although informative, cytology data can be difficult to interpret when describing phenomena that are per se dynamic.

The advantages of using a live cell imaging methodology, such as LSDCAS, are evident in the studies discussed here, as by employing this technology, we are able to directly observe cellular behavior and phenomena that can only be inferred when analyzing a fixed cell population on a microscope slide. The LSDCAS image results (Fig. 2; video clip 1 in the supplement) are discussed below, and show that a fraction of the polyploid cells formed via radiation-induced MC escape death and gives rise to a progeny of smaller cells (indistinguishable from the control cells) through a depolyploidization process.

Polyploid cells formed via radiation-induced MC start a program of depolyploidization reverting to a morphology identical to that of control cells and have the potential for long-term survival. LSDCAS imaging data (Fig. 2; video clip 1 in supplement, needs QuickTime Player) show that, as a consequence of a 5 Gy γ-irradiation exposure, HeLa clone 3 cells undergo MC, become polyploid, and initiate rounds of multipolar divisions. Some of these multipolar divisions are only attempted divisions, as cytokinesis fails to complete and cell fusion occurs (Fig. 2B; 72 and 82 hours postirradiation). Other multipolar divisions are successful (Fig. 2C; 101 and 173 hours postirradiation). The end result is the production of a heterogeneous population of daughter cells containing different numbers of nuclear fragments. As shown in the Supplemental Data, 305 cells were imaged in all flasks over a period of 266 hours; in this time frame, there were 210 multipolar divisions; of these divisions, 118 produced at least one small mononucleated cell, whereas the total number of the small mononucleated cells produced by multipolar divisions was 198 (note that this is different from the previous number —118— because in a single multipolar division, often more than one mononucleated cell is produced); four mononucleated cells were still alive at the end of the movie. In this query, only the

Figure 1. Nuclei segregation in polyploid cell formed via radiation-induced MC. Photomicrographs of irradiated HeLa S3 cells (10 Gy, γ-rays), cytospin preparations. A, a typical multinucleated MC cell (day 9 postirradiation); B, multiple metaphase plates at days 7 and 10 postirradiation; C, nuclei segregation (10 d postirradiation); D, sham-irradiated control.

SPO11, REC8, DMC1, MOS, STAG3, and CCNB1 (cyclin B1). The PCR reactions were run in a DNA Engine Opticon 2 Thermalcycler (MJ Research, Bio-Rad) using the following cycling parameters: 40 cycles of 95°C for 15 s, 55°C for 30 s, and 70°C for 30 s, cycles were followed by a melting curve analysis to verify product purity. The data were analyzed using the 2^−ΔΔCt method with two separate housekeeping genes, ACTB (β-actin) and RPLP0, and reported as fold increase over control.

Immunohistochemistry. Paraffin-embedded tissue sections from patients with cervical cancers were deparaffinized in xylene and rehydrated through a graded alcohol series, applied to slides, and washed in distilled water. Slides were incubated for 8 min with 3% hydrogen peroxide in distilled water to block endogenous peroxidase. At which point, slides were rinsed in Dako buffer, pretreated with proteinase K (Dako) for 10 min at room temperature, and rinsed twice in Dako buffer for 5 min each. Mouse anti-DMC1 monoclonal antibody (Abcam) was diluted 1:200 in Dako diluent, applied to slides, and incubated for 6 h at room temperature. Slides were then rinsed twice in Dako buffer for 5 min each and incubated in Dako Envision+ System–labeled Polymer-HRP for 1 h at room temperature. Slides were rinsed twice in Dako buffer for 5 min each and developed with Dako Dab Plus for 5 min, and again rinsed twice in Dako buffer for 5 min each. At which time, Dako DAB Enhancer was applied and slides incubated for 3 min, rinsed in distilled water, and counterstained with hematoxylin (Surgipate) for 3 min. Slides were finally washed in tap water, and tipped twice in ammonia water to enhance hematoxylin staining. Slides were dehydrated in distilled water, 70%, 95%, 100% ethanol, and xylene and mounted with Mount-Quick sealant.
mononucleated cells (generated by a multipolar division) that have divided at least twice were counted as survivors; these cells are considered to have potential for further normal divisions. In the still images in Fig. 2B, the generation of the smaller mononucleated cell is shown 82 hours postirradiation (cell 4c); this cell is able to normally divide, giving rise to two mononucleated daughter cells (Fig. 2C; 101 hours postirradiation, cells 4c1 and 4c2). Note that these cells contain only one nucleus and if they were to be observed in standard microscopic cell preparations, they might be mistaken for unaffected cells that escaped MC cell killing whereas, in fact, they originate from a polyploid MC cell. Both these daughter cells are able to divide successfully as well (Fig. 2C; 137 hours postirradiation, cells 4c1a, 4c1b, 4c2a, and 4c2b). At 266 hours (11 days and 2 hours) postirradiation, cells 4c1a, 4c1b, 4c2a, and 4c2b have stayed within the field of view and are still alive. Multipolar divisions of irradiated cells that have undergone MC is not a sole characteristic of HeLa cells, but a quite common phenomenon in irradiated tumor cells, as shown by image data obtained by us observing the human head and neck squamous cell carcinoma, FaDu, the human ovary adenocarcinoma cell lines SKOV3, OVCAR, and EG (data not shown), and the MDA-MB435 cells (video clip 2 in supplement, needs QuickTime Player). Video clip 2 shows images of multipolar divisions occurring in the MDA-MB435 cells irradiated with a dose of 5 Gy of γ-irradiation. The MDA-MB435 cells are also able to produce smaller mononucleated cells through a reduction division process. These results indicate that a small fraction of cells with morphology identical to that of control cells originate from polyploid cells formed via radiation-induced MC.

Meiotic genes are expressed during depolyploidization of polyploid cells formed via radiation-induced MC. At days 7 and 9 postirradiation, morphologic features reminiscent of the meiotic synaptonemal complexes were present in the HeLa S3 cells’ nuclei (Fig. 3). Synaptonemal complexes are meiosis-specific supramolecular tripartite proteinaceous structures that develop during pairing of homologous chromosomes in the late zygotene and pachytene stages of meiosis I. This structure forms between two homologous chromosomes and mediates chromosome pairing, synapsis, and crossover (28, 29), and consists of two parallel lateral regions and a central element and it comprises three specific components, the SC protein-1 (SYCP1), the SC protein-2 (SYCP2), and the SC protein-3.
(SYCP3). A series of experiments were designed to determine if components of the synaptonemal complex are expressed in cells undergoing depolyploidization. To this end, we performed quantitative real-time reverse transcriptase PCR (RT-PCR) experiments. Quantitative RT-PCR data of irradiated HeLa S3 cells reveals a 1-fold to 2.5-fold increase in SYCP3 mRNA expression at days 4 to 11 postirradiation, with a recurring peak increase (~1.5-fold) at days 18 to 20 postirradiation (Fig. 4A). A second experiment performed for longer times confirmed the fold increases reported and revealed a 1.5-fold increase of SYCP3 mRNA at 24 days postirradiation. The same experiment was performed using the human colon cancer cells HCT116-379.2, with mutated p53 gene—thus lacking p53 function (a kind gift from Dr. Bert Vogelstein, Johns Hopkins University, Baltimore, MD). Expression of SYCP3 mRNA was also present in this cell line (Fig. 4B), with a 1.5-fold to 1.8-fold increase at 2 to 16 hours postirradiation and recurring peak increases at 32 and 48 hours postirradiation.

Using these same cell lines, we have also measured the expression of two other meiosis-specific genes, Rec8 and DMC1. Rec8 is a family member of meiotic cohesins that are a set of meiosis-specific proteins involved in sister chromatid cohesion and homology pairing during meiosis (30); DMC1 is essential for meiotic homologous recombination and for cell cycle progression (31, 32). Figure 4B, reports the Rec8 mRNA expression in irradiated HeLa S3 cells and the DMC1 mRNA expression in the irradiated syngenic HCT116-379.2 (mutated p53) and HCT116-40,16 (wild-type p53, also a kind gift from Dr. Bert Vogelstein) cells. For Rec8, mRNA expression increases between days 2 and 10, with an increase as high as 5-fold by day 5 postirradiation, a second peak is also visible at day 25 postirradiation. For DMC1, in both cell lines, mRNA expression increases in the range of 1.2-fold to 1.6-fold at 16, 40, and 48 hours postirradiation. It is noteworthy to mention that radiation exposure leads to increases in the expression of DMC1 mRNA in both the wild-type and the mutated p53 HCT116 cells. Thus, allowing for the speculation that certain types of solid tumors, albeit with functional p53, might attempt to activate the same mechanisms of survival as for the nonfunctional p53 tumors, thus escaping p53-mediated programmed cell death and gaining a survival advantage.

DMC1 mRNA expression was also measured in the MD-MBA435 cells (Fig. 4C) irradiated with 5 Gy of γ-rays. Increases in DMC1 mRNA were seen between 0 and 5 days postirradiation, with a 5-fold peak increase at 4 days postirradiation. Immunofluorescence studies, carried out with HeLa S3 cells, confirm the translation of the DMC1 mRNA in the functional protein (Fig. 4D). DMC1 focal staining is noticeable at different time points in the polyploid γ-irradiated HeLa S3 cells; representative time points of 4, 23, and 25 days postirradiation are presented in Fig. 4D.

Note that the meiosis-specific genes were found activated in all the cell lines reported, at times when polyploid MC cells had already formed in irradiated cells (LSDCAS data not shown; LSDCAS data in ref. 27). These observations support our speculation that the activation of pseudomeiotic pathways in irradiated tumors plays a role in the depolyploidization processes.

SuperArray custom-designed assay reveals the activation of other meiosis-specific genes in irradiated MDA-MB435 cells. Intrigued by the results described above, we decided to analyze the possible expression of other meiosis-specific genes involved in homologous pairing, meiotic recombination, and cohesion in the MDA-MB435 cells. An RT-PCR SuperArray was designed using primers for the following genes: SYCP2, SYCP3, SPO11, STAG3, DMC1, RECS, and MOS; CCMB1 (cyclin B1) was also enclosed in the array. SPO11 is a meiotic recombination protein that mediates DNA cleavage in double-strand breaks that initiate meiotic recombination (33) and is also involved in axial element and synaptonemal complex formation and in the maintenance of meiotic chromosome condensation and proper spindle formation (34). STAG3 is expressed specifically in testis and is involved in chromosome pairing and maintenance of the synaptonemal complex structure during the pachytene phase of meiosis in a cohesin-like manner. During anaphase I, STAG3 dissociates from the centromeres allowing chromosome segregation (35). MOS regulates oocyte maturation by arresting the unfertilized cells in M phase and is destroyed before fertilization and after exit from meiosis II (36). Cyclin B1 is the regulatory subunit of the maturation-promoting factor and, when phosphorylated by the maturation-promoting factor catalytic subunit cdc2, it plays a role both during meiosis and mitosis. In meiosis, cyclin B1 is responsible for meiotic maturation of oocytes; whereas in mitosis, it is responsible for the cell cycle G2-M phase transition. The function of SYCP, RECS and DMC1 genes have been described above. The results of the SuperArray assay are reported in Fig. 4C (right). All the meiotic genes tested are expressed in MDA-MB435 cells after exposure to 5 Gy of γ-irradiation. Moreover, as previously reported by us (15), cyclin B1 mRNA is also increased at days 4 and 5 postirradiation. The increases in mRNA expression seen in all the RT-PCR data presented in this article are comparable to the increases shown by other investigators for mRNA expression of a variety of meiotic genes (37, 38). These data show that meiosis-specific genes involved in homologous pairing and recombination are activated in polyploid tumor cells formed via radiation-induced MC that have escaped death. This activation occurs in concert with the onset of a depolyploidization process as shown by the LSDCAS data discussed in Fig. 2.

Tissue specimens from human cervical cancer are positive for DMC1 staining. Immunohistochemistry performed with the meiosis-specific protein DMC1 on human cervical cancer tissues shows that this protein is preferentially expressed in these specimens (Fig. 5C) as well as in the testis tissue (Fig. 5A), serving as a positive control, whereas the normal tissue presents little

![Image](cancerres.aacrjournals.org)
staining (Fig. 5B). To our knowledge, this is the first time that the expression of a meiosis-specific gene has been reported in human tumor tissue specimens.

**Discussion**

During the last 15 years, our interest has focused on the effects of radiation exposure on the cell cycle and on phenomena associated with cell cycle dysregulation. Under conditions that can compromise genome integrity, cells activate checkpoint control pathways that delay them from entering and exiting mitosis. The first set of checkpoints controls entry into mitosis and, in response to DNA damage or other detrimental effects, delays the activation of the positive regulator of mitosis, cyclin B1/cdc2. Exit from mitosis is controlled by the spindle assembly checkpoint that prevents the activation of the anaphase-promoting complex that is required to target securin and cyclin B1 for proteolysis. Destruction of these proteins allows for the separation of chromatids, thus marking anaphase, and exit of the cell from mitosis, marking telophase, respectively (ref. 39; and reviewed in ref. 40). A broad class of agents (20, 41–45) induces a loss of regulation of the cell cycle with the end result that if the cell population does not immediately die, it often undergoes MC. We have shown that radiation-induced MC occurs in a variety of mammalian cell lines with impaired p53 function and it is characterized by aberrant nuclear morphology observed following premature mitotic entry (14–18, 41, 46). Using live cell imaging, we have also determined that irradiated HeLa cells undergo MC and are able to survive for many generations post-exposure (21); 50% of the surviving colonies exhibited MC that persisted throughout colony formation (21). Moreover, other live cell imaging experiments conducted with irradiated human-hamster hybrid GM10115 cells showed the presence of a high frequency of surviving clones containing an elevated incidence of polyploid cells formed via MC. Specifically, 0.07% of the cell

![Figure 4. mRNA and protein expression of meiosis-specific genes in various human tumor cell lines exposed to different doses of γ-irradiation. A, SYCP3 mRNA expression in HeLa S3 and HCT-116 379.2 cells. B, Rec8 and DMC1 mRNA expression in HeLa S3 and HCT-116 40.16 and HCT-116 379.2 cells. C, DMC1 mRNA expression in MDA-MB435 cells and custom-designed RT-PCR SuperArray showing mRNA expression of various meiosis-specific genes in MDA-MB435 cells. mRNA levels were assayed using qRT-PCR techniques as described in Materials and Methods. Each sample (and the reference gene, β-actin) was run in duplicate. Measurements were repeated at least twice, representative experiments are reported in A and B. For the MDA-MB435 cells (C, left) three repeats were performed. SuperArray data (C, right) are semiquantitative. D, immunofluorescence for DMC1 protein in HeLa S3 cells. Focal staining of DMC1 (green) is visible at various times postirradiation. Condensed chromosomes are also visible at 25 h postirradiation. Positive controls: untreated mouse testis cells. Primary antibody: DMC1 (1:100 dilution; Abcam); secondary antibody goat anti-mouse FITC IgG (whole molecule, 1:100 dilution; Sigma); counterstaining 4,6-diamidino-2-phenylindole (red or blue).](https://www.aacrjournals.org)
population underwent MC and continued to divide for up to 12 days following irradiation (6,000 individual cells were studied for 10 days postirradiation), making MC a possible contributor to radiation-induced genomic instability. Captivated by these results and by cytologic studies recently published by us (19) in which we have reported the formation of large polyploid cells in irradiated HeLa cells, we initiated the series of experiments described in this article to determine the mechanisms underlying the process of depolyploidization.

Using LSDCAS imaging data, we report here that as result of γ-irradiation, HeLa clone 3 and MDA-MB435 cells undergo MC and become polyploid; within the first 5 days postirradiation, the polyploid cells initiate a depolyploidization process. Mononucleated smaller cells are produced that are morphologically indistinguishable from the untreated cells; these cells are able to produce a cell progeny and are still alive 12 days postirradiation. These results indicate that a small fraction of cells can survive MC. Both the morphologic and the molecular results discussed in this article delve into the possible mechanisms used by a tumor cell to escape death following radiation-induced MC. We find that irradiated tumor cells (colon, breast/melanoma, cervix) impaired in p53 function present morphologic traits resembling features characteristic of meiotic prophase, and that at the time of the appearance of these morphologic features, an induction of genes involved in meiotic pathways are observed. These data suggest that polyploid tumor cells conserve their original individual genomic integrity and can reinitiate cell division via reduction division. In an article published in 1982, Dawkins (47) pointed out that meiosis can be envisioned as a mechanism that elaborate chemostats, such as diploid organisms, have evolved to maximize the fitness of the haploid organisms they carry, that is, the gametes. Likewise, some tumor cells might attempt to switch to meiotic divisions to maximize their fitness and their chance at survival.

The results discussed here are in line with the data reported by Illidge and collaborators (48), Erenpreisa and collaborators (23), and Ivanov and collaborators (49) that found polyploid giant cells formation after irradiation of Burkitt’s lymphoma cells. Our results are also in line with the data reported by Prieur-Carrillo and collaborators (22) that, using computerized video time-lapse techniques, found survivors of irradiated giant human bladder carcinoma cells up to a week postirradiation. Taking into account the results reported above and our data discussed in this article, it seems that polyploid cells, originating from MC, present a survival advantage over the remaining portion of the cell population. In the same article referred to above (23), Erenpreisa and collaborators also showed the presence of chromosome double-loops in the giant cells by the second week postirradiation. These formations are referred to as polyploid bouquets. The process of bouquet formation involves the clustering of telomeres (which are randomly distributed in premeiotic interphase and early leptotene) in a small region of the nuclear envelope during the zygotene stage. The bouquet coincides with homologous chromosome pairing and synopsis and is suggestive of a role for telomeres in the homology search process (50). This telomere-mediated reorganization of the early meiotic prophase nucleus has the effect of reducing the search space required for chromosomes to find their partners. The fact that irradiated tumor cells present features resembling the bouquet formation suggests that this process might contribute to rendering the polyploid cells able to undergo a successful reduction division.

Using the same cell line, Ivanov and collaborators (49) have also reported that a portion of lymphoblastoid Namalwa cells exposed to severe genotoxic treatment undergo MC, giving rise to polyploid cells, some of which resemble cells in meiotic prophase. Homologous DNA recombinational repair occurs in these cells and they replicate their DNA for more than a few rounds of mitotic cycles producing descendants. This is an important aspect, as the signal for initiation of recombination is the production of DNA double-strand breaks mediated by the meiosis-specific endonuclease enzyme SPO11 (51), and the SPO11 gene is found activated in
and/or genotoxic treatments, to escape death, and to promote progression. Thus, understanding the effects of anticancer treatment on polyploid tumor cell populations and the process of depolyplodization might shed light into mechanisms of tumor resistance and progression, and might furnish new insight into treatment methodologies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 9/8/2008; revised 12/4/2008; accepted 12/10/2008; published OnlineFirst 3/3/09.

Grant support: Partially supported by the NIH (CA/GM94801 and CA86862), NASA (NAG8-1851), the Latvian-USA Governmental Exchange grant enabling visits between Riga and Iowa City and vice versa, and the Whitaker Foundation Special Opportunity Award.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The authors are also indebted to Jean L. Ross from the Central Microscopy Research Facility at the University of Iowa, for her skillful assistance in collecting the cytology photographs; Janis R. Rodgers from the Department of Pathology's Histology Research Laboratory at the University of Iowa, for her expert assistance in tissue preparation and staining; and Paul J. Davis, who contributed some of the analysis algorithms employed in the LSDCAS data imaging analysis.

References
33. Kennedy S, Giroux CN, Kleckner N. Meiosis-specific DNA double-stranded breaks are catalyzed by Spol1, a member of a widely conserved protein family. Cell 1997; 88:375–84.
Activation of Meiosis-Specific Genes Is Associated with Depolyploidization of Human Tumor Cells following Radiation-Induced Mitotic Catastrophe
