Caspase-1α Is Down-regulated in Human Ovarian Cancer Cells and the Overexpression of Caspase-1α Induces Apoptosis

Qiang Feng,1 Peixiang Li,1 Clara Salamanca,1 David Huntsman,2 Peter C.K. Leung,3 and Nelly Auersperg1

Departments of Obstetrics and Gynecology and Pathology, University of British Columbia, Vancouver, British Columbia, Canada

Abstract

Caspase-1 plays a key role in the processing of cytokines and in the apoptosis of neurons and macrophages. Whether it also causes apoptosis of cancer cells has been unclear. In this study, we screened an array of apoptosis-related proteins in ovarian carcinoma cell lines and their tissue of origin, ovarian surface epithelium (OSE). Caspase-1α protein was abundant in OSE and in nontumorigenic OSE with extended but limited life spans (immortalized OSE), but was reduced in the cancer lines A2780 and OVCAR10. By Western blot and immunofluorescence, caspase-1α levels were greatly reduced in six of eight ovarian carcinoma lines compared with OSE. By real-time reverse transcription-PCR, steady-state transcripts of the CASP1 gene were proportional to protein levels. Caspase-1α overexpression caused significant apoptosis, but overexpression of a caspase-1α mutant without catalytic activity did not, confirming that the effect was caspase-1α-specific. Immunofluorescence of caspase-1α and terminal nucleotidyl transferase–mediated dUTP-X nick end labeling colocalization clearly established a link between apoptosis and caspase-1α expression. Caspase-9 and caspase-3 were activated in caspase-1α overexpressing A2780 cells, suggesting involvement of an intrinsic apoptotic pathway. Caspase-1α overexpression did not change the apoptotic effect of cisplatin in A2780 and OVCAR10 cells, suggesting that this agent activates a different pathway. Immunohistochemically, caspase-1α was lower in ovarian serous carcinomas than in OSE. Our study indicates, for the first time, that caspase-1α is proapoptotic in ovarian cancer cells, and raises the possibility that its down-regulation is one of the mechanisms which increase resistance to apoptosis in cancer cells. (Cancer Res 2005; 65(19): 8591-6)

Introduction

One of the hallmarks of cancer cells, including ovarian cancer (1, 2), is their increased resistance to apoptosis, which can be attained by up-regulation of antiapoptotic proteins such as Bcl-2 and survivin (3), or down-regulation of proapoptotic proteins such as caspase-8 (4). In general, caspase-1 has not been considered as proapoptotic in ovarian cancer, which implicates a different pathway. Emerging evidence suggests that during hypoxia-induced neuron apoptosis, active caspase-1 may cleave Bid and thus initiate an intrinsic apoptotic pathway (5). Thus, caspase-1 seems to be an upstream mediator of certain kinds of pathologic cell death. In relation to cancer, previous observations have been limited to indications that caspase-1 levels are reduced in neoplasms of prostate and colon (9, 10). There is no information on the role of caspase-1 in ovarian cancer. Interestingly, numerous cytogenetic studies link the loss of heterozygosity at chromosome 11q22-23 to carcinomas, including ovarian cancer (11, 12). During a preliminary screening of 25 apoptosis-related proteins in ovarian carcinoma cell lines, we discovered that in their precursor, normal ovarian surface epithelium (OSE) and in immortalized OSE (IOSE), caspase-1α protein levels are significantly higher than in ovarian cancer cells. In the present study, we provide further evidence that caspase-1α is proapoptotic in ovarian cancer, which implicates down-regulation of this caspase as a novel mechanism to enhance the resistance of cancer cells to apoptosis.

Materials and Methods

Cell culture. Human OSE cells were obtained as reported (13). OSE cells (OSE cells with an extended life span) were established by transfecting OSE with SV40 Tag/tag (14). The cisplatin-resistant cell line CP70 (15), A2780, OVCAR3, OVCAR4, OVCAR5, and OVCAR10 were kindly provided by Dr. T.C. Hamilton at Fox Chase Cancer Center, Philadelphia. SKOV3 and Caov3 were obtained from American Type Culture Collection (Manassas, VA).

Construction of expression vectors. Full-length CASP1 cDNA was obtained by reverse transcription-PCR from human OSE cDNA as described previously (7). To create a mutant at the epicenter of caspase-1 QACRG location, site-directed mutation of the caspase-1α plasmid were carried out by Bios&T (Montreal, Quebec), so that Cys285 was replaced by serine.

Apoptosis-protein profiling assay, Western blot, and immunofluorescence staining. For the profiling assay, OSE, IOSE and lines A2780 and OVCAR-10 were lysed according to protocols provided by
Kinexus. All profiling experiments as well as data processing were carried out by Kinexus (Vancouver, British Columbia, Canada). Rabbit anti-human caspase-1 polyclonal antibody (A-19), which recognizes the NH2 terminus of procaspase-1, anti-actin goat IgG and anti–glyceraldehyde-3-phosphate dehydrogenase goat IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Immunoblot analysis was done as described previously (7), using actin or glyceraldehyde-3-phosphate dehydrogenase as loading controls. Antibodies for detection of caspase-1 (both pro-form and cleaved p20 subunit of caspase-1; Fig. 4), caspase-3, and caspase-9 were from Cell Signaling Technology (Beverly, MA). For immunofluorescence microscopy, cells on glass coverslips or frozen tissue sections (5 μ thick) of normal ovaries or ovarian tumor were fixed in 4% formaldehyde/PBS for 10 minutes, washed thrice with PBS, and permeabilized for 2 minutes with ice-cold 0.01% Triton X-100/PBS. After washing with PBS, samples were incubated with blocking solution (DAKO, Mississauga, Ontario, Canada) for 1 hour, followed by 1 hour of incubation with caspase-1 antibody (A-19). Alexa 594 fluor dye conjugated goat anti-rabbit IgG was used to visualize the location of caspase-1. Cell nuclei of cultures were counterstained with Hoechst 33258 and fluorescence images were captured using a Zeiss Axiophot microscope with a Northern Eclipse digital imaging system.

Quantitative real-time PCR. To determine whether the down-regulation of caspase-1α in cancer cells was at the transcriptional level, we did quantitative reverse transcription-PCR to measure the amount of caspase-1 transcripts in OSE, IOSE, and various cancer lines. Total RNAs of OSE, IOSE, and ovarian carcinoma cell lines were prepared using Qiagen RNeasy mini kit. First-strand cDNAs were synthesized using a first-strand cDNA synthesis kit (Amersham, Baie D’urfe, Quebec, Canada). RNase-free DNase I on-column treatment was carried out during RNA extraction to eliminate possible genomic DNA contamination. First strand cDNAs were synthesized using random hexamer primers. To detect human caspase-1 message, we used the ABI TaqMan gene expression assay (Hs00236158_m1). PCR reactions were carried out and analyzed in an Applied Biosystems (Foster City, CA) 7300 real-time PCR system.

Transient transfection of A2780, OVCAR-10, and CP70. Lines A2780 and OVCAR-10 were maintained in DMEM with 5% fetal bovine serum and were transfected when 70% to 80% confluent, using a mixture of plasmids (4 μg/well) and LipofectAMINE 2000 (4-10 μL/well) prediluted in Opti-M medium. Cells were collected and analyzed for apoptosis and protein expression 24 hours after transfection. For cisplatin treatment experiments, cells were first transfected for 4 hours and then the transfection media were replaced with normal culture medium containing cisplatin (10 μg/mL). The cells were collected 24 hours after transfection. The dose of cisplatin was predetermined to achieve a moderate increase of apoptosis in CP70 cells.

Cell death ELISA and terminal nucleotidyl transferase–mediated dUTP-X nick end labeling. Cell death ELISA kit (Roche Diagnostics, Laval, Quebec, Canada) was used to measure DNA fragmentation as reported previously (6). In situ cell death detection kit, FITC (Roche Diagnostics, Laval, Quebec) was used to label apoptotic cells. For colocalization, caspase-1α protein was first visualized with goat anti-rabbit IgG conjugated with

3 http://www.kinexus.ca

Figure 1. Apoptosis-protein profiling, Western blot, and quantitative reverse transcription-PCR analysis of caspase-1 in OSE, IOSE, and ovarian cancer cells. A, immunoblot-based Kinexus KAPS apoptosis protein profiling assay showing the caspase-1α level (boxed bands) in OSE, IOSE, A2780, and OVCAR-10 cells. B, Western blot analysis of caspase-1α in OSE, IOSE, and ovarian carcinoma cell lines. Cells were cultured in medium 199/MCBI05 with 10% fetal bovine serum. Cell lysates from different cell lines containing equal amounts of protein (30 μg) were loaded in each lane. C, relative amounts of caspase-1 mRNA quantitated by real-time PCR in two OSE (gray columns), four IOSE (dotted columns), and eight ovarian cancer cell lines (solid columns). rRNA (18S) primers and probes were used as an endogenous reference for quantitative reverse transcription-PCR amplification. Comparative Ct value calculation was carried out by setting the expression level of caspase-1α in IOSE80pc cells as 1.0.
Results

Caspase-1α protein levels in ovarian surface epithelium, immortalized ovarian surface epithelium, and cancer cell lines. In order to identify changes in apoptosis during malignant progression of OSE, we profiled 25 apoptosis-related proteins using the Kinexus KAPS assay (Fig. 1A). Caspase-1α protein was most abundant in OSE, reduced somewhat in IOSE, and was very low or undetectable in the ovarian carcinoma lines A2780 and OVCAR10.

To determine whether this pattern applied to other cells, we did Western blots using the same antibody as for the profiling assay. As shown in Fig. 1B, OSE and IOSE cells retained significant amounts of caspase-1α. Out of eight ovarian cancer cell lines, six showed very low or undetectable levels of caspase-1α. A striking exception was CaOV3, which had the highest caspase-1α content of all cells. Immunofluorescence staining of ovarian cancer cells and (Fig. 2A-F, and I) and OSE (Fig. 2G and H) paralleled the Western blot results.

Immunofluorescence microscopy of ovarian serous carcinomas and normal ovaries. Staining for caspase-1 in three cases of normal ovaries and six cases of high-grade ovarian serous carcinomas showed normal OSE to be strongly caspase-1-positive (Fig. 2M). In contrast, the six tumors showed significantly reduced caspase-1 (Fig. 2N). These results indicate that caspase-1α protein diminishes with ovarian neoplastic progression.

Caspase-1α gene expression. As shown by quantitative reverse transcription-PCR (Fig. 1C), caspase-1α mRNA was abundant in OSE, IOSE, and in Caov3 cells, but was lower or undetectable in most cancer cell lines. The amount of caspase-1 gene transcripts was highest in the two OSE lines, lower in IOSE, undetectable in A2780, and barely detectable in OVCAR10. This significant down-regulation of gene expression at the transcriptional level with neoplastic progression is consistent with the changes in protein levels.

Apoptotic effects of caspase-1α overexpression. A2780 cells express no detectable caspase-1α (Fig. 2I). Transient transfection of caspase-1α significantly increased caspase-1α protein expression as shown by immunofluorescence (Fig. 2J) and Western blot (Figs. 3A and 4A). TUNEL staining of in situ apoptosis in A2780 showed colocalization of caspase-1α overexpression with TUNEL-positive cells having a typical apoptotic morphology (Fig. 2J-L). DNA fragmentation measurements showed significant increases of apoptosis in caspase-1α–overexpressing cells compared with untransfected A2780 (Fig. 3D) and OVCAR10 cells (Fig. 3B and E). These results indicate that caspase-1α is a proapoptotic factor for ovarian cancer cells. As shown in Figs. 1B and 2A, CaOV3 cells express the highest level of caspase-1α among all the ovarian carcinoma cell lines tested. Although transient transfection of caspase-1α moderately increased caspase-1α expression in CaOV3 (Fig. 3C), there was no caspase-1α–dependent increase of apoptosis compared with the C285s mutant (Fig. 3F).

Caspase-1α overexpression does not affect cisplatin-induced apoptosis. The cisplatin-resistant ovarian cancer line CP70 was transfected with caspase-1α, followed by cisplatin treatment. As shown in Figs. 3G, CP-70 cells showed a similar apoptotic response to overexpression of caspase-1α as its parental line A2780 (0 μg/mL cisplatin). In untransfected CP70 cells, cisplatin (10 μg/mL) caused a small increase in apoptosis. However, CP-70 cells transfected with caspase-1α as well as C285s mutant showed similar, much increased responses to cisplatin, suggesting that cisplatin-induced apoptosis is independent of caspase-1 activity.

Caspase-1α, caspase-3, and caspase-9 activation. Activation of caspase-1α in A2780 cells was confirmed by Western blot using antibody from Cell Signaling Technology, which recognizes both the procaspase-1 and the cleaved p20 subunit (Fig. 4A). However, that effect was erased by a single inactivating mutation of Cys285 to Ser285 in caspase-1α (Figs. 3A-B and 4A), showing that the apoptotic effect of caspase-1α is specifically dependent on its catalytic activity. In addition, as shown in Fig. 4B, procaspase-9 and procaspase-3 levels are lower in caspase-1α than in the C285s mutant transfectants, suggesting their activation downstream of

www.aacrjournals.org 8593 Cancer Res 2005; 65: (19). October 1, 2005

Cancer Res 2005; 65: (19). October 1, 2005

Downloaded from cancerres.aacrjournals.org on September 13, 2017. © 2005 American Association for Cancer Research.
caspase-1. In both untransfected and transfected CaOV3 cells, we failed to detect cleavage of caspase-1α (data not shown). There was no indication that the C285s mutant has a dominant-negative effect. This suggests that the caspase-1 pathway was not the dominant mechanism in nonspecific transfection-induced apoptosis.

**Discussion**

Caspase-1α is usually regarded as a cytokine-processing caspase. Our results are the first to report that a specific isoform of caspase-1, caspase-1α, is able to induce apoptosis in ovarian cancer cells, and that high-grade ovarian serous carcinomas and most ovarian cancer cell lines are characterized by greatly reduced constitutive expression of caspase-1α protein in comparison with their normal precursor, OSE. Furthermore, the apoptotic effect of caspase-1α in ovarian cancer is dependent on its catalytic activity as the mutational change at its catalytic center completely abolished its death effect on two different ovarian cancer cell lines. Our results also indicate that caspase-1α overexpression does not increase the cell death response of ovarian cancer cells to cisplatin. The fact that caspase-1α is equally potent to induce apoptosis in both cisplatin-sensitive A2780 and cisplatin-resistant OVCAR-10 cells could provide new therapeutic strategies to combat drug resistance.

Unlike other apoptotic caspases, caspase-1 is mostly regarded as one of the cytokine-processing caspases, which include caspase-4, caspase-5, and the murine caspases-1, -11, and -12. However, caspase-1 also plays an important role in apoptosis, particularly under certain pathologic conditions such as neurodegenerative disorders and hypoxic ischemia (5, 6). Recent data from our lab showed that overexpression of caspase-1α in HEK293 cells causes apoptosis, which is dependent on its catalytic effect (7). Not only the full-length α isoform, but a newly found NH2-terminal truncated isoform β lacks most of the CARD domain, suggesting that this domain is not required for the apoptotic function of caspase-1 (7), although it may be required for its nuclear translocation (16). In cultured neurons, overexpression of Rip2 protein causes caspase-1–dependent apoptosis. Under inflammatory conditions, Rip2 activates caspase-1 through CARD-CARD interactions (17). The Rip2/caspase-1 activation pathway converges to the intrinsic apoptotic pathway through Bid cleavage, which subsequently leads to activation of caspase-9 and caspase-3 (5). Our current report showing ovarian cancer cell death associated with the activation of caspases-1, -9 and -3 suggests involvement of the caspase-1/tBid pathway. Thus, caspase-1 is not only a cytokine-processing caspase, but also plays important roles in different forms of apoptosis. In inflammatory responses, caspase-1 becomes part of a protein...
complex, the inflammasome, which has similarities to the apoptosomes in apoptosis (8). This complex formation requires the protein CARDINAL, which is overexpressed in several types of cancer and, interestingly, in normal human ovary and ovarian cancer (8).

The OSE is the mesothelial lining of the ovary and the source of the epithelial ovarian carcinomas (13). This is the first report that OSE produces substantial quantities of caspase-1α. This fact supports our previous report that cultured OSE secretes active proinflammatory cytokines such as IL-1α, IL-6, and M-CSF (18). Another report indicated that biologically active IL-18 production supports our previous report that cultured OSE secretes active proinflammatory cytokines such as IL-1α, IL-6, and M-CSF (18). Although this study did not focus on the functions of caspase-1α in normal OSE cells, it is tempting to speculate that it could play an important apoptotic role in OSE cells in the event of tissue damage and repair during ovulation. The loss of proapoptotic caspase-1α in these cells, and the subsequent survival of damaged cells, could increase the risk of developing cancer. Importantly, our immunohistochemical observations in normal ovaries and ovarian carcinomas support the culture data and suggest that the loss of caspase-1α may also contribute to the poor outcome of late stage ovarian cancer patient survival. One of the exceptions to the general pattern was the highly malignant ovarian carcinoma line CaOV3, which expresses very high levels of caspase-1α. Further artificial overexpression of caspase-1α in the CaOV3 cells caused no increase of caspase-1α-dependent apoptosis (Fig. 3F), and we failed to detect any cleavage of caspase-1α in CaOV3. This result strongly suggests that the machinery required for caspase-1-mediated apoptosis is impaired.

The Rip2/caspase-1 apoptosis pathway is specifically activated in cell death under stress conditions (5). Endothelial cells, of similar mesothelial origin as OSE, died by caspase-1-dependent apoptosis under serum-withdrawal conditions which cause oxidative stress (20). This evidence supports the hypothesis that reduced caspase-1α may give premalignant and malignant ovarian epithelial cells an advantage to survive under unfavorable conditions.

In summary, our findings suggest that caspase-1α is a potentially important player in OSE functions and ovarian cancer. Increased resistance to apoptosis has been recognized as one of the prime mechanisms leading to neoplastic progression. Our results implicate, for the first time, caspase-1α in this process.

Acknowledgments

Received 1/24/2005; revised 7/15/2005; accepted 8/5/2005.

Grant support: Canadian Cancer Society through a grant to N. Auersperg from the National Cancer Institute of Canada.

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.

We thank Dr. C. Blake Gilks, Pathology Department, University of British Columbia, for providing normal and tumor tissue sections.

References


14. Maires-Bandiera SL, Kruk PA, Auersperg N. Simian...


Caspase-1α Is Down-regulated in Human Ovarian Cancer Cells and the Overexpression of Caspase-1 α Induces Apoptosis

Qiang Feng, Peixiang Li, Clara Salamanca, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/19/8591

Cited articles
This article cites 20 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/19/8591.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/19/8591.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.