

Studies of Apurinic/Apyrimidinic Endonuclease/ref-1 Expression in Epithelial Ovarian Cancer: Correlations with Tumor Progression and Platinum Resistance

Sarah Freitas, David H. Moore,¹ Helen Michael, and Mark R. Kelley

Departments of Obstetrics and Gynecology [S. F., D. H. M.], Pathology [H. M.], and Pediatrics [M. R. K.], Indiana University School of Medicine, Indianapolis, Indiana 46202

ABSTRACT

Purpose: A crucial step in the DNA base excision repair pathway involves the cleavage of an apurinic/apyrimidinic site by an apurinic/apyrimidinic endonuclease (APE). The major APE in mammalian cells is APE/ref-1, a multifunctional enzyme that acts not only as a DNA repair enzyme but as a redox-modifying factor for a variety of transcription factors. The purpose of this study is to determine whether APE/ref-1 expression differs with ovarian cancer progression and metastasis and in platinum-resistant disease.

Experimental Design: Ovarian tissue sections were obtained from the Cooperative Human Tissue Network for studies of APE/ref-1 expression and the metastatic process and from our institutional Department of Pathology for studies of APE/ref-1 expression and platinum resistance. Tissue microsections from formalin-fixed, paraffin-embedded specimens of epithelial ovarian cancers were stained using a monoclonal antibody to APE/ref-1 followed by standard immunohistochemical techniques. Slides were then analyzed for the percentage of positively staining nuclei as well as staining intensity using a blinded coding system.

Results: All epithelial ovarian cancers expressed APE/ref-1. There were no significant differences in the percentage or intensity of nuclear staining in primary tumors from patients with early- versus advanced-stage disease or in primary tumors versus metastasis from patients with advanced disease. Both platinum-sensitive and platinum-refractory tumors demonstrated a range from minimal to high intensity staining nuclei with a median value of 2+ staining on a scale of 0–3+. The median value for the

percentage of nuclei involved was 70% in the platinum-sensitive group and 90% in the platinum-refractory group ($P = 0.118$).

Conclusions: APE/ref-1 expression is ubiquitous among epithelial ovarian cancers and is unaltered with the metastatic process. APE/ref-1 expression does not appear to differ between platinum-sensitive and platinum-refractory ovarian cancers and thus is not a useful biomarker for platinum resistance. Combined with evidence that APE/ref-1 expression and function may not be equivalent in all cell types and tissues, future work will investigate APE/ref-1 as a potential therapeutic target.

INTRODUCTION

Ovarian cancer is the leading cause of gynecological cancer death in the United States. The relatively asymptomatic nature of early-stage disease and the lack of adequate screening tests have resulted in the majority of cases presenting with stage III or stage IV disease. Standard treatment for advanced-stage disease involves cytoreductive surgery followed by the administration of platinum-based combination chemotherapy. Approximately 15% of patients will not respond to this primary chemotherapy regimen; furthermore, of those that initially respond to chemotherapy, the majority of patients will eventually relapse and die from chemoresistant disease.

Understanding the factors that determine the chemoresistance of cells is therefore an important area of investigation. DNA is thought to be the main target for the toxicity of platinum. The reactive complex binds to DNA to form inter- and intrastrand cross-links, resulting in inhibition of both DNA and RNA synthesis. The survival of cells after exposure to platinum is governed by complex interactions between a large number of proteins involved in DNA damage recognition, DNA repair, and the response of cells to unrepaired or misrepaired DNA damage.

Evidence of the significance of DNA repair as a mediator of platinum resistance first came from the study of DNA repair-deficient mutants of bacteria, where it was found that low ability to repair DNA damage results in hypersensitivity to DNA-reactive drugs (1). Conversely, it has also been demonstrated that cisplatin resistance to human ovarian cancer cells is associated with increased DNA repair capacity. Masuda *et al.* (2) reported increased DNA repair capabilities *in vitro*, relative to the degree of cisplatin resistance, among cisplatin-resistant cells versus cisplatin-sensitive controls. Therefore, it is reasonable to anticipate that variations in the levels of expression of such proteins may result in variations in clinical chemosensitivity.

DNA damage may occur as a result of oxidizing agents, hydrolysis, UV or ionizing radiation, or alkylating agent chemotherapy. Apurinic/apyrimidinic sites are the most common form of DNA damage, and the DNA base excision repair pathway is primarily responsible for the repair of this type of

Received 11/1/02; accepted 3/20/03.

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.

This work was supported in part by the Gynecologic Oncology Group Committee of Experimental Medicine (National Cancer Institute Grant CA27469-19) and a Department of the Army New Investigator Award (OC990085 CDMRP).

¹ To whom requests for reprints should be addressed, at Professor and Chief of Gynecologic Oncology, Indiana University School of Medicine, 535 Barnhill Drive, RT 433, Indianapolis, IN 46202. Phone: (317) 274-2422; Fax: (317) 274-4878; E-mail: dhmoore@iupui.edu.

damage (3). Excision repair involves recognition of DNA damage, incision of DNA on either side of the lesion, generation of a new DNA strand to replace the excised segment, ligation of the new segment to the existing strand of DNA, and restoration of the normal chromatin conformation. A crucial step in the base excision repair pathway involves the cleavage of an apurinic/apyrimidinic site in DNA by an APE.² The major APE in mammalian cells is APE/ref-1, a multifunctional enzyme that acts not only as an APE but as a redox regulation factor for DNA binding of transcription factor (4). *In vitro* evidence suggests that APE/ref-1 activity is the rate-limiting step in the repair of DNA oxidative damage (5).

APE/ref-1 expression has been studied in other cancers in relation to resistance to chemotherapy and radiotherapy. Koukourakis *et al.* (6) found that nuclear expression of APE/ref-1 [also referred to as human APE (HAP1/Ref-1)] in head and neck cancer was associated with resistance to cisplatin chemoradiation therapy and poor outcome. Herring *et al.* (7) reported that there was an inverse association between intrinsic radiosensitivity and the levels of HAP1 in cervical carcinoma. There is recent evidence that APE/ref-1 expression and base excision repair capabilities may differ among cell types and tissues. Based on demonstrable differences in immunohistochemical expression, we previously postulated that APE/ref-1 function may differ between epithelial ovarian cancers *versus* normal ovarian tissues and benign ovarian neoplasms (8). The purpose of the present study was to determine (a) whether APE/ref-1 expression is different in primary tumors of early *versus* advanced ovarian cancers, (b) whether APE/ref-1 expression is different in the primary tumor *versus* metastasis in advanced ovarian cancers, and (c) whether APE/ref-1 expression differs in platinum-sensitive *versus* platinum-refractory epithelial ovarian cancers.

MATERIALS AND METHODS

Tissue Specimens. Ovarian tissues prospectively collected via the Gynecologic Oncology Group were obtained from the Cooperative Human Tissue Network (Columbus, OH). Requested were tissues from primary ovarian tumors in patients with stage I disease and both primary ovarian tumors and metastasis from patients with advanced ovarian cancers. Gynecologic Oncology Group tissue acquisition via the protocol #136 mechanism did not occur without prior Institutional Review Board approval and written informed consent from participating patients. Tissue blocks from formalin-fixed, paraffin-embedded ovarian cancer specimens were cut in approximately 6- μ m sections, fixed on glass slides, and shipped to the authors' laboratory for further processing.

Archival ovarian tissues used for the correlative studies of APE/ref-1 expression and platinum resistance had been obtained during primary staging procedures by gynecologic oncology surgeons at the Indiana University School of Medicine and stored in the Department of Pathology. Patients with platinum-sensitive ($n = 20$) and platinum-refractory ($n = 17$) tumors

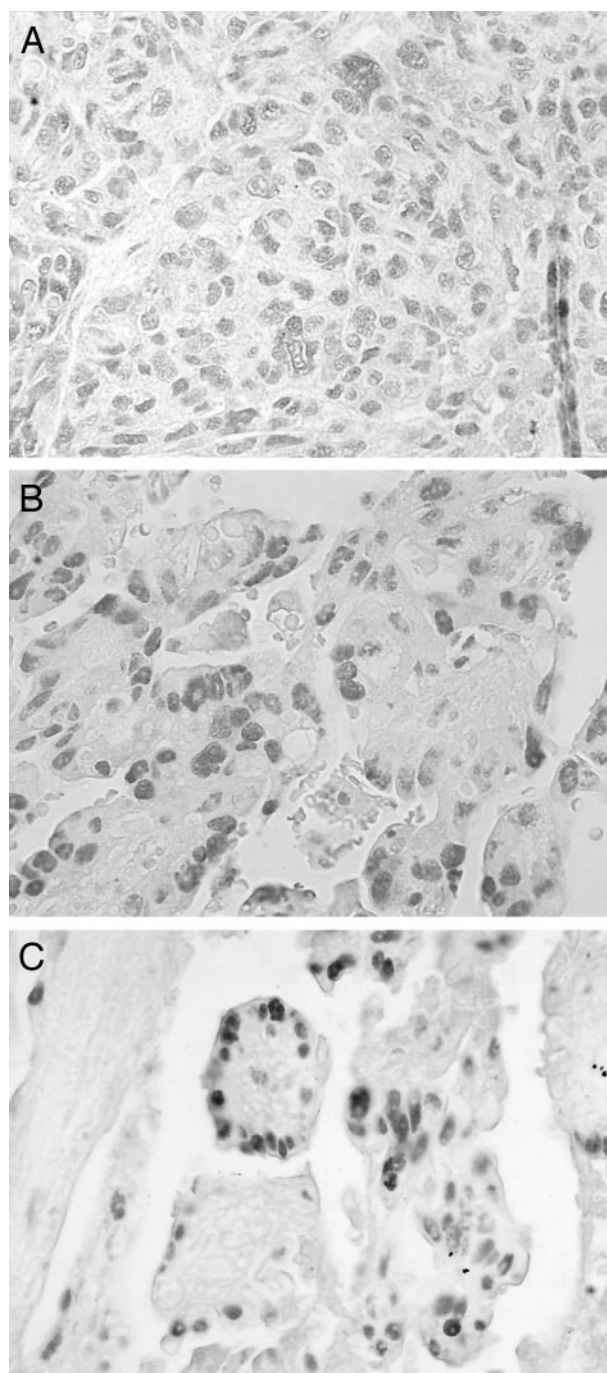


Fig. 1 A, 1+ staining of tumor cell nuclei. Very light staining of nuclei is present (H&E, $\times 400$). B, 2+ nuclear staining. Nuclei display darker staining, but chromatin pattern is still visible (H&E, $\times 400$). C, 3+ nuclear staining. Nuclei are very darkly stained, and nuclear detail is obscured by the stain (H&E, $\times 400$).

were identified from the gynecologic oncology database of patients treated for ovarian cancer between July 1996 and June 1998 at Indiana University School of Medicine. All patients had received primary platinum-based chemotherapy after surgery. Platinum sensitivity was defined by response to first-line plati-

² The abbreviation used is: APE, apurinic/apyrimidinic endonuclease.

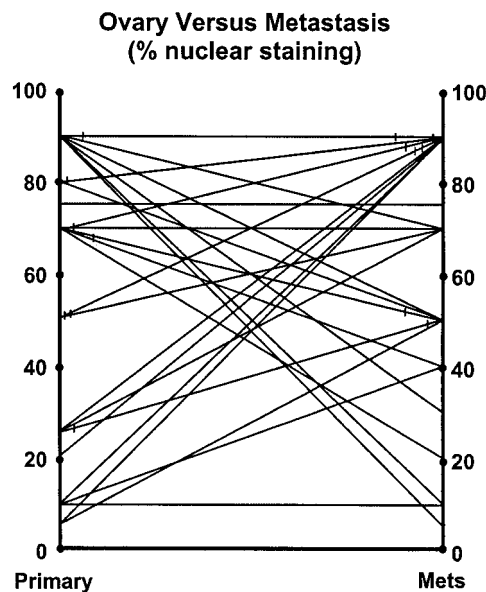


Fig. 2 Percentage of nuclei with positive APE/ref-1 immunostaining in the primary tumors of early versus advanced ovarian cancer. Mean values, early (60%) versus advanced (56%), $P =$ nonsignificant.

num-based therapy or a progression-free interval of >6 months off treatment. Patients who progressed during treatment, had stable disease in response to initial platinum-based therapy, or relapsed within 6 months were considered to have platinum-refractory disease. Criteria for persistence or recurrence included clinical evidence of disease (e.g., physical examination findings or measurable disease on computed tomography or magnetic resonance imaging) or a sustained rise in serum CA-125.

All surgical specimens were fixed in 4% buffered formaldehyde and embedded in paraffin. Tissue blocks were cut in 6- μ m sections and fixed on glass slides. Slides were immunostained and analyzed using a blinded coding system such that staining procedures and microscopic assessments were performed without knowledge of the histopathological diagnosis and response to primary chemotherapy.

Antibody Preparation. The initial anti-APE/ref-1 polyclonal antibody was produced using overexpression of the human APE/ref-1 protein in a pGEX-glutathione *S*-transferase *Escherichia coli* system (Pharmacia, Uppsala, Sweden), as described previously (9, 10), and was obtained from Novus Biologicals (Littleton, CO). However, we have more recently produced a monoclonal antibody (Affinity Bioreagents Inc., Golden, CO) that was used in the studies herein presented. Both polyclonal and monoclonal antibodies yielded identical results. Antibody purity was confirmed with Western blot analysis before each use (9, 10), and each batch of slides was simultaneously processed using the same antibody concentration. Tissues determined previously to express APE/ref-1 were used as positive controls.

Immunohistochemistry. Tissue sections were coated with anti-APE/ref-1 antibody (mouse antihuman APE/ref-1 monoclonal antibody) and incubated overnight at 4°C at a 1:200

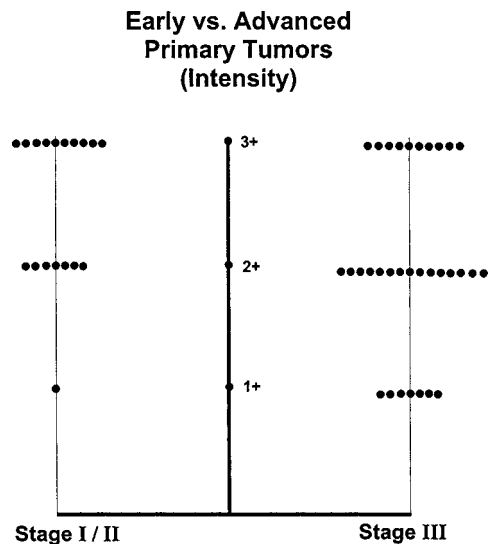


Fig. 3 Intensity of APE/ref-1 immunostaining in the primary tumors of early versus advanced ovarian cancer. Mean values, early (2.5+) versus advanced (2.1%), $P =$ nonsignificant.

dilution in 10% goat serum in PBS. The next day, sections were washed three times for 5 min in PBS and incubated with biotinylated goat antimouse IgG (Vector Laboratories, Burlingame, CA) at 15 μ /ml in 10% goat serum for 1 h. After two PBS washes for 5 min each, sections were incubated with avidin and biotinylated horseradish peroxidase complex (ABC elite kit; Vector Laboratories) for 45 min. Slides were then incubated with diaminobenzidine (Vector Laboratories). After the development of color signal, the sections were washed briefly in distilled H₂O, counterstained with eosin, dehydrated through a graded alcohol to xylene sequence, coverslipped, analyzed, and selectively photographed. As a negative control, preimmune IgG (50 μ /ml) was used as the primary antibody in place of anti-APE.

Slides were analyzed using light microscopy by the same gynecologic pathologist for the percentage of nuclei staining positive for APE/ref-1 and the intensity of staining on a 0–3+ scale (Fig. 1). The pathologist was blinded to clinical data. Any appreciable brown staining was considered positive and graded as follows: 1+, barely detectable staining; 2+, easily seen fine granules were present diffusely throughout the nucleus or cytoplasm; or 3+, staining was so strong that nuclear detail was obscured. For scoring purposes, the nuclear intensity was graded according to the strongest staining areas within that sample. The percentage of cells exhibiting positive staining was estimated. A corresponding H&E stain was reviewed to determine diagnosis and map the location of the various histological patterns with the staining patterns observed in the immunohistochemistry preparations.

RESULTS

From the Cooperative Human Tissue Network, we obtained slides from 20 patients with early-stage and 40 patients with advanced-stage epithelial ovarian cancer. For patients with advanced-stage disease, slides were available from both primary

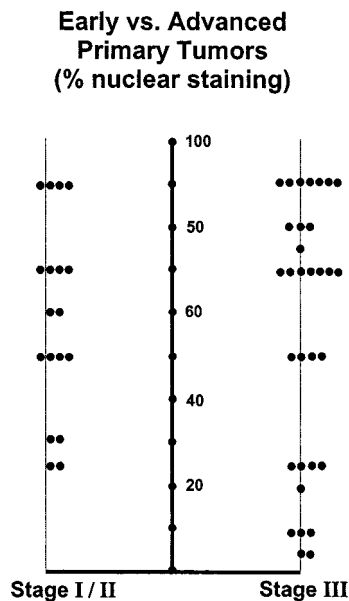


Fig. 4 Percentage of nuclei with positive APE/ref-1 immunostaining in the primary tumor *versus* metastasis in patients with advanced ovarian cancer.

and metastatic tumor. Slides from seven patients were deemed invaluable and excluded [no tumor seen (three patients), only low malignant potential tumor seen (one patient), no viable tumor or extensive necrosis (two patients), or inadequate sample (one patient)]. All epithelial ovarian cancers expressed APE/ref-1. Overall, there were no differences in the percentage or intensity of nuclear staining in primary tumors from patients with early- *versus* advanced-stage disease (Figs. 2 and 3) or in the primary tumors *versus* metastasis from patients with advanced disease (Figs. 4 and 5). The staining pattern in the primary tumor usually differed from that of the metastasis, but there was no consistent trend. In some cases, APE/ref-1 immunorexpression was increased in the metastasis, in some cases it was decreased, and in a few cases it was the same. Cytoplasmic staining was predominant in four patients with early-stage ovarian cancer and seven patients with advanced-stage ovarian cancer. Among patients with advanced-stage disease, cytoplasmic staining was present in the primary tumor only (four patients), the metastasis only (two patients), and in both (one patient).

A total of 37 tissue blocks from Indiana University School of Medicine were processed and analyzed for APE/ref-1 expression with respect to platinum resistance. One specimen was eliminated when no tumor could be found in the processed slide. The groups were well matched for age and stage. All patients had FIGO stage III or IV disease, except for two patients in the platinum-sensitive group who had stage IIA disease and one patient in the platinum-refractory group who had stage IC disease.

The median value for nuclear intensity for both groups was 2+, with both groups showing a range of low- to high-grade staining. The median value for percentage of nuclei involved was 70% in the platinum-sensitive group and 90% in the platinum-refractory group. Using a Mann-Whitney rank-sum test,

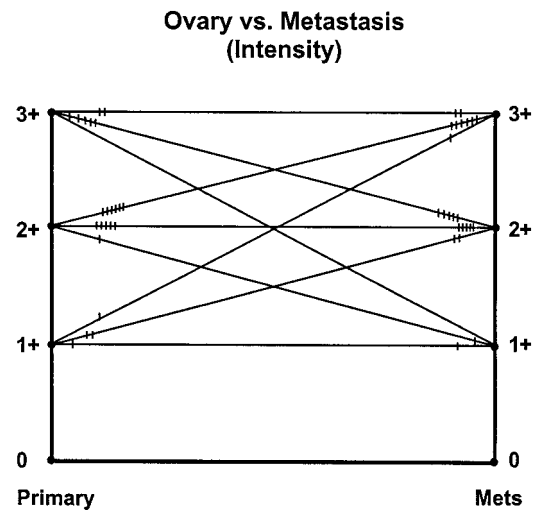


Fig. 5 Intensity of APE/ref-1 immunostaining in the primary tumor *versus* metastasis in patients with advanced ovarian cancer.

the two groups were compared (Signa Stat Program, Version 2.0; SPSS Inc.), and this difference was not significant ($P = 0.118$). Cytoplasmic staining was uniformly absent in all samples. The majority of the tumor specimens demonstrated variations in the staining intensity, with some samples showing 1+ staining in one portion of the slide and 2+ staining in another portion. In nearly all cases, the percentage of positive nuclear staining was high, with only 5 of the 36 evaluable specimens showing staining of <50% of nuclei. Only one tissue sample showed no staining at all.

If APE/ref-1 expression were to be used in the clinical setting as a biomarker for platinum resistance, a meaningful difference in platinum-resistant *versus* platinum-sensitive populations should be large (for example, a difference of intensity of at least 1+ grade on a scale of 0–3+ or a difference in percentage of nuclei involved of 50%). The results of a power analysis on these data suggest that our sample size was sufficient to detect a difference of this magnitude, had one existed.

DISCUSSION

The ability to identify cases of platinum-refractory ovarian cancer before initiating treatment would obviate unnecessary toxicities from predictably ineffective platinum-based chemotherapy regimens in favor of alternative investigational treatments. This study observed no difference in the pattern of staining of APE/ref-1 between the two study groups; therefore APE/ref-1 cannot be used as a clinical marker of platinum resistance. Furthermore, immunohistochemical expression of APE/ref-1 is not altered with more advanced stages of ovarian cancer or with the metastatic process.

There are several possible explanations for these observations. One factor that could influence results is that immunohistochemical methods observe quantitative, not qualitative, differences in APE/ref-1 protein. Recent *in vitro* studies have investigated whether changes in APE/ref-1 expression observed at the protein level translated into changes in APE/ref-1 DNA

repair capabilities. Moore *et al.* (8) used an apurinic/aprimidinic site oligonucleotide cleavage assay to demonstrate that protein levels correlated well with appropriate changes in DNA repair activities. The DNA repair domain of APE/ref-1 resides near the COOH terminus (11). The DNA repair activity of APE/ref-1 may be inactivated by phosphorylation (12). Various investigators have linked single-nucleotide polymorphisms in DNA repair genes with cancer susceptibility (13, 14). Hu *et al.* (15) demonstrated that a single amino acid substitution in APE/ref-1 resulted in increased susceptibility to ionizing radiation. It is presently unknown whether the antibody used in these experiments can distinguish between these subtle physical and functional states of APE/ref-1 protein. Consequently, observed expression differences via immunohistochemistry may not reflect alterations in DNA repair capabilities.

A second factor that must be considered is that APE/ref-1 functions extend beyond DNA repair. Xanthoudakis and Curran (16) found that APE/ref-1 is involved in the redox-activated binding of transcription factors Fos, Jun, and activator protein-1. Subsequently, APE/ref-1 has been shown to activate a host of other transcription factors including nuclear factor κ B, cAMP response element-binding protein, p53, hypoxia-inducible factor 1 α , and HIF-like factor and facilitate their DNA binding via redox regulation (17–21). The redox activities of APE/ref-1 reside in a separate domain near the NH₂ terminus (11). It is possible that the effect of APE/ref-1 expression on platinum sensitivity may be related to its other functions, specifically the redox control of transcription factor binding to DNA. At present, there are no data demonstrating that DNA repair functions and redox regulatory functions of APE/ref-1 are regulated in a coordinate fashion. In fact, Prieto-Alamo and Laval (22) constructed a mutated protein endowed with normal APE activity but devoid of redox function. When this mutated protein was expressed in a cell line, elevated APE activity was measured, however, sensitization to the lethal effects of compounds requiring bioreduction (mitomycin C, porfiromycin, and daunorubicin) was no longer observed (22). In addition to functioning as a modulator of transcriptional activity through redox regulation, there is evidence that APE/ref-1 is itself modulated via post-transcriptional redox modifications. Hirota *et al.* (23) discovered an association between APE/ref-1 redox activity and thioredoxin, a small ubiquitous protein with multiple biological functions including cellular defense mechanisms against oxidative stress. Translocation of thioredoxin from the cytoplasm to the nucleus was essential for the association between APE/ref-1 and thioredoxin and for the subsequent enhancement of activator protein-1 binding (23). There is evidence that platinum induces thioredoxin and that cells which overexpress thioredoxin display increased resistance to platinum (24). It is quite apparent that APE/ref-1 redox regulation involves a complex and still misunderstood cascade of events.

Previously, it was mentioned that nuclear expression of human APE/ref-1 (HAP1) is associated with resistance to chemotherapy and poor outcome in head and neck cancer (6). Conversely, cytoplasmic (but not nuclear) expression of HAP1 is associated with poor predictors of outcome (angiogenesis, lymph node positivity, and poor tumor differentiation) in breast carcinoma (25). Uncertainties regarding *in vivo* functional status notwithstanding, it may be that APE/ref-1 is expressed by all

epithelial ovarian cancers but under usual circumstances does not otherwise function in this disease in a rate-limiting capacity to influence the process of tumor metastasis or affect the clinical emergence of platinum resistance. This possibility does not limit the relevance of base excision repair in general or APE/ref-1 function in particular to ovarian carcinoma. *In vitro* studies have shown that cell lines overexpressing specific DNA repair genes are rendered resistant to ionizing radiation and certain chemotherapeutic agents (26, 27). The subsequent identification of small molecule inhibitors, small interfering RNAs, or other emerging technologies may lead to novel pharmacological strategies designed to selectively target and inhibit specific enzymes in DNA repair pathways and thereby sensitize tumor cells to radiation therapy or chemotherapy.

This study suggests that resistance to platinum-based chemotherapy in ovarian cancer is independent of the DNA base excision repair pathway. APE/ref-1 expression does not appear to differ between platinum-sensitive and platinum-refractory ovarian cancers and thus is not a useful biomarker for platinum resistance. Additional studies are needed to confirm these findings and in particular to clarify the functional role of APE/ref-1 in ovarian cancer. APE/ref-1 expression is ubiquitous among epithelial ovarian cancers and is unaltered with the process of metastasis. Future work should investigate APE/ref-1 as a potential therapeutic target.

REFERENCES

- Chaney, S. G., and Sancar, A. DNA repair: enzymatic mechanisms and relevance to drug response. *J. Natl. Cancer Inst. (Bethesda)*, *88*: 1346–1360, 1996.
- Masuda, H., Tanaka, T., Matsuda, H., and Kusaba, I. Increased removal of DNA-bound platinum in a human ovarian cancer cell line resistant to *cis*-diamminedichloroplatinum(II). *Cancer Res.*, *50*: 1863–1866, 1990.
- Chou, K. M., and Cheng, Y. C. An exonucleolytic activity of human apurinic/aprimidinic endonuclease on 3' mispaired DNA. *Nature (Lond.)*, *415*: 655–659, 2002.
- Hansen, W. K., and Kelley, M. R. Review of mammalian DNA repair and translational implications. *J. Pharmacol. Exp. Ther.*, *295*: 1–9, 2000.
- Ramana, C. V., Boldogh, I., Izumi, T., and Mitra, S. Activation of apurinic/aprimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. *Proc. Natl. Acad. Sci. USA*, *95*: 5061–5066, 1998.
- Koukourakis, M. I., Giatromanolaki, A., Kakolyris, S., Sivridis, E., Georgoulas, V., Funtzilias, G., Hickson, I. D., Gatter, K. C., and Harris, A. L. Nuclear expression of human apurinic/aprimidinic endonuclease (HAP1/Ref-1) in head-and-neck cancer is associated with resistance to chemoradiotherapy and poor outcome. *Int. J. Radiat. Oncol. Biol. Phys.*, *50*: 27–36, 2001.
- Herring, C. J., West, C. M. L., Wilks, D. P., Davidson, S. E., Hunter, R. D., Berry, P., Forster, G., MacKinnon, J., Rafferty, J. A., Elder, R. H., Hendry, J. H., and Margison, G. P. Levels of the DNA repair enzyme human apurinic/aprimidinic endonuclease (APE1, APEX, Ref-1) are associated with the intrinsic radiosensitivity of cervical cancers. *Br. J. Cancer*, *78*: 1128–1133, 1998.
- Moore, D. H., Michael, H., Tritt, R., Parsons, S. H., and Kelley, M. R. Alterations in the expression of the DNA repair/redox enzyme APE/ref-1 in epithelial ovarian cancers. *Clin. Cancer Res.*, *6*: 602–609, 2000.
- Duguid, J. R., Eble, J. N., Wilson, T. M., and Kelley, M. R. Differential cellular and subcellular expression of the human multifunctional apurinic/aprimidinic endonuclease (APE/ref-1) DNA repair enzyme. *Cancer Res.*, *55*: 6097–6102, 1995.

10. Xu, Y., Moore, D. H., Broshears, J., Liu, L., Wilson, T. M., and Kelley, M. R. The apurinic/aprimidinic endonuclease (APE/ref-1) DNA repair enzyme is elevated in premalignant and malignant cervical cancer. *Anticancer Res.*, *37*: 13–20, 1997.
11. Xanthoudakis, S., Miao, G. G., and Curran, T. The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. *Proc. Natl. Acad. Sci. USA*, *91*: 23–27, 1994.
12. Yacoub, A., Kelley, M. R., and Deutsch, W. A. The DNA repair activity of human redox/repair protein APE/ref-1 is inactivated by phosphorylation. *Cancer Res.*, *57*: 5457–5459, 1997.
13. Shen, M. R., Jones, I. M., and Mohrenweiser, H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res.*, *58*: 604–608, 1998.
14. Ford, B. N., Ruttan, C. C., Kyle, V. L., Brackley, M. E., and Glickman, B. W. Identification of single nucleotide polymorphisms in human DNA repair genes. *Carcinogenesis (Lond.)*, *21*: 1977–1981, 2000.
15. Hu, J. J., Smith, T. R., Miller, M. S., Mohrenweiser, H. W., Golden, A., and Case, L. D. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. *Carcinogenesis (Lond.)*, *22*: 917–922, 2001.
16. Xanthoudakis, S., and Curran, T. Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. *EMBO J.*, *11*: 653–655, 1992.
17. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y. C., and Curran, T. Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. *EMBO J.*, *11*: 3323–3325, 1992.
18. Mitomo, K., Nakayama, K., Fujimoto, K., Sun, X., Seki, S., and Yamamoto, K. Two different cellular redox systems regulate the DNA-binding activity of the p50 subunit of NF- κ B *in vitro*. *Gene (Amst.)*, *145*: 197–203, 1994.
19. Ema, M., Hirota, K., Mimura, J., Abe, H., Yodoi, J., Sogawa, K., Poellinger, L., and Fujii-Kuriyama, Y. Molecular mechanisms of transcriptional activation by HLF and HIF-1 α in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO J.*, *18*: 1905–1914, 1999.
20. Jayaraman, L., Murthy, K. G., Zhu, C., Curran, T., Xanthoudakis, S., and Prives, C. Identification of redox/repair protein Ref-1 as a potent activator of p53. *Genes Dev.*, *11*: 558–570, 1997.
21. Lando, D., Pongratz, I., Poellinger, L., and Whitelaw, M. L. A redox mechanism controls differential DNA binding activities of hypoxia-inducible factor (HIF) 1 α and the HIF-like factor. *J. Biol. Chem.*, *275*: 4618–4627, 2000.
22. Prieto-Alamo, M. J., and Laval, F. Overexpression of the human HAP1 protein sensitizes cells to the lethal effect of bioreductive drugs. *Carcinogenesis (Lond.)*, *20*: 415–419, 1999.
23. Hirota, K., Matsui, M., Iwata, S., Mishiyama, A., Mori, K., and Yodoi, J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc. Natl. Acad. Sci. USA*, *94*: 3633–3638, 1997.
24. Sasada, T., Iwata, S., Sato, N., Kitaoka, Y., Hirota, K., Nakamura, K., Nishiyama, A., Taniguchi, Y., Takabayashi, A., and Yodoi, J. Redox control of resistance to *cis*-diamminedichloroplatinum (II) (CDDP): protective effect of human thioredoxin against CDDP-induced cytotoxicity. *J. Clin. Investig.*, *97*: 2268–2276, 1996.
25. Kakolyris, S., Kaklamanis, L., Engels, K., Fox, S. B., Taylor, M., Hickson, I. D., Gatter, K. C., and Harris, A. Human AP endonuclease 1 (HAP1) protein expression in breast cancer correlates with lymph node status and angiogenesis. *Br. J. Cancer*, *77*: 1169–1173, 1998.
26. Barret, J. M., and Hill, B. T. DNA repair mechanisms associated with cellular resistance to antitumor drugs: potential novel targets. *Anticancer Drugs*, *9*: 105–123, 1998.
27. Limp-Foster, M., and Kelley, M. R. DNA repair and gene therapy: implications for translational uses. *Environ. Mol. Mutagen.*, *35*: 71–81, 2000.