Prognostic role of Ape/Ref-1 subcellular expression in stage I-III breast carcinomas

FABIO PUGLISI¹, FABIO BARBONE², GIANLUCA TELL⁵, GIUSEPPE APRILE¹, BARBARA PERTOLDI³, CONCETTA RAITI³, MARK R. KELLEY⁶, GIUSEPPE DAMANTE⁴, ALBERTO SOBRERO¹, CARLO ALBERTO BELTRAMI³ and CARLA DI LORETO³

¹Chair of Clinical Oncology, ²Chair of Hygiene, ³Chair of Anatomic Pathology and ⁴Chair of Genetics, University of Udine, Udine, Italy; ⁵Department of Biochemistry, Biophysics and Macromolecular Chemistry University of Trieste, Italy; ⁶Herman B Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA

Received September 20, 2001; Accepted October 24, 2001

Abstract. The purpose of the study was to evaluate the prognostic role of the DNA repair protein APE/Ref-1 in breast carcinomas. Immunohistochemical analysis for APE/Ref-1 was performed in a series of 133 consecutive stage I-III breast carcinomas. The relationship between APE/Ref-1 and other prognostic and predictive factors such as tumor size, nodal status, histologic grade, p53 expression, hormonal receptor status, vascular invasion and necrosis was investigated. The prognostic value of APE/Ref-1 was studied by univariate and multivariate analysis. The predominant pattern of APE/Ref-1 immunohistochemical expression was nuclear, although cytoplasmic and mixed nuclear/cytoplasmic localization was also observed. The percentage of cells with APE/Ref-1 cytoplasmic stain directly correlated with the percentage of p53 positive cases ($\rho=0.28$, $p=0.013$). The small group of women whose tumors showed mixed nuclear/cytoplasmic APE/Ref-1 localization ($n=5$) experienced a significantly poorer survival ($p=0.014$) and Cox proportional hazard model analysis identified APE/Ref-1 as an independent prognostic factor. The results suggest that subcellular localisation of APE/Ref-1 may influence the aggressiveness of breast carcinomas.

Introduction

Genomic stability is achieved by avoiding errors during DNA duplication and repairing damage that spontaneously may occur. Base loss from human cellular genome has been estimated to nearly 10^4 per cell per day under physiological conditions, representing the most important spontaneous mammalian DNA damage (1-5). Moreover, this high rate of depurination can be increased by a variety of factors, such as ionizing radiations or cytotoxic agents. To counteract this intrinsic threat to genome integrity, cells express endonucleases, which specifically recognize AP (apurinic/apyrimidinic) sites of base loss (6).

The human AP endonuclease 1 (APE/Ref-1) protein belongs to a homologous family of DNA repair proteins, which are highly conserved from bacteria (e.g., *E. coli*) to insects, plants and rodents (7-9). Structure and function studies of the 37 kDa APE/Ref-1 protein have described at least three different activities pertaining to distinct protein domains which function independently (10,11): i) APE/Ref-1 rapidly initiates a DNA repair process by introducing DNA strand breaks on the 5' side of baseless sites. The deoxyribose phosphate residue is subsequently removed by a phosphodiesterase, followed by abolishing the nucleotide gap by DNA polymerase and DNA ligase, which seals the nick, thus restoring the correct DNA sequence (12). ii) APE/Ref-1 may also regulate the DNA-binding activity of several transcription factors involved in cell proliferation and differentiation (c-Fos, c-Jun, c-Myb, Pax proteins) (13-15). The N-terminal domain and the Cys65 residue of APE/Ref-1 are necessary for this function, while the same residue is not required for endonuclease activity (16). iii) Moreover, recent data indicate APE/Ref-1 as the first non-covalent modifier of p53 function in cells. In fact, APE/Ref-1 plays a crucial role in the activation of p53, both in redox-dependent and independent ways (17).

Since APE/Ref-1 is a main actor in the maintenance of DNA integrity and in the control of cell proliferation, its expression has been studied in a variety of tumors, including lung (18), colon (19) and breast cancer (20). In this latter tumor, APE/Ref-1 can be detected both in the nucleus and in the cytoplasm and nuclear expression was inversely associated

Correspondence to: Dr Fabio Puglisi, Clinica Oncologica, Policlinico Universitario, Piazzale S.M. Misericordia I-33100 Udine, Italy
E-mail: fabio.puglisi@med.uniud.it

Abbreviations: AP, apurinic/apyrimidinic or baseless; APE, AP endonuclease; Ref-1, redox effector factor 1; BER, base excision repair; ABC, avidin-biotin-peroxidase complex; ER, estrogen receptor; PR, progesterone receptor; PBS, phosphate buffered saline; OS, overall survival; HR, hazard ratio

Key words: DNA repair, breast carcinoma, immunohistochemistry, prognosis, multivariate analysis

with lymph node status and angiogenesis, although survival did not correlate with APE/Ref-1 status (20).

In the present work, we examined the prognostic value of APE/Ref-1 in a series of 133 consecutive breast carcinomas. We also studied the relationship between APE/Ref-1 and other well-known prognostic and predictive factors, including tumor size, nodal status, histologic grade, p53 expression, estrogen receptor status (ER), vascular invasion and necrosis.

Patients and methods

Breast cancer specimen were obtained from 133 female patients diagnosed at the University and the General Hospitals of Udine, Italy, between 1992 and 1997. The median patient age at diagnosis was 60.5 years (range 33-88, 25th-75th percentiles 51-70). All primary cancer patients had no distant metastases. Sections of the resected tumors were examined for the histological type (according to the guidelines of the World Health Organization), histological grade (according to Elston and Ellis criteria), the presence or absence of vascular invasion and the presence or absence of necrosis. Tumors were histologic variants of invasive breast carcinomas, comprising 120 ductal, 6 lobular, 1 tubular and 6 other types. In all cases, tumor size was measured in the fresh state and confirmed by re-assessment in the microscopic sections. Median tumor size was 1.7 cm (range 0.2-7.5, 25th-75th percentiles 1.2-2.4). Lymph node metastases were present in 41.35% of patients. Histological grade was evaluable in 126 cases, comprising 37.3% G1, 34.9% G2 and 27.8% G3.

The pathologic stage (pStage) was I in 58 patients, II in 58 and III in 17 patients. Other tumor characteristics are shown in Table I.

Complete follow-up information was available on 133 patients, with a median follow-up duration of 66.03 months (range 10.6-98.70). At the time of analysis, 106 (79.7%) patients were alive.

Various regimes of adjuvant therapy were administered to the patients and information about treatment was not available for all cases. For these reasons adjustment for endocrine therapy and/or chemotherapy was intentionally avoided.

Immunohistochemistry. The immunohistochemical study was performed on formalin fixed, paraffin-embedded sections of resected specimens by the avidin-biotin-peroxidase complex (ABC Elite Vectastain, Vector, Burlingame, CA, USA) using specific antibodies [p53(D07) YLEM, Avezzano, Italy; ER(1D5) Dako, Glastup, Denmark]. The sections were freshly cut immediately before immunostaining.

After blocking of endogenous peroxidase with hydrogen peroxide, the sections for ER and p53 were pretreated in a 750 W microwave oven set at maximum power for 2 min and at 200 W for 6 min with 0.01 M citrate buffer, pH 7.3; then sections were cooled and washed twice in phosphate buffered saline (PBS). Following overnight incubation with primary antibodies diluted in PBS (D07 1:100; 1D5 1:400), the avidin-biotin-peroxidase complex was applied for 30 min at room temperature. Diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) was used as chromogen, and hematoxylin was used as counterstaining. Appropriate positive and negative controls were included. For negative controls,

the primary antiserum was omitted and replaced with mouse immunoglobulin (Dako).

The percentage of nuclei positive for ER and p53 was scored. Approximately 1000 tumor cells were counted for each case.

For statistical purposes a cut-off point of $\geq 10\%$ of immunoreactive cells was chosen to define tumors positive for ER and p53.

Immunohistochemistry for APE/Ref-1. For each case, a minimum of four serial sections of representative formalin-fixed paraffin embedded blocks of breast cancer tissues were cut onto silane-coated slides and dewaxed. After blocking of endogenous peroxidase, sections were subjected to two microwave irradiations for 5 min at 800 W and for 10 min at 200 W in 10 mM citrate buffer, pH 6.0 for antigen retrieval. The sections were incubated with rabbit antiserum to APE/Ref-1 diluted 1:50 in PBS overnight at 4°C. After washing, the biotinylated goat anti-rabbit antibody at a dilution of 1:200 and the avidin-peroxidase detection system (ABC Elite Vectastain Vector Laboratories, Burlingame, CA, USA) were applied both for 30 min at room temperature. Peroxidase activity was detected with 3,3'-diaminobenzidine tetrachloride (Sigma) and a light haematoxylin counterstain was applied. Negative controls were carried out replacing the primary antiserum with pre-immune rabbit serum. The specificity of antiserum to APE/Ref-1 was previously described (21).

A brown staining in the nucleus and/or in the cytoplasm indicated the cellular presence of APE/Ref-1 (Fig. 1). The APE/Ref-1 expression was evaluated calculating separately nuclear and cytoplasmic reactivity in 1000 neoplastic cells and was expressed as a percentage of positive cells.

Immunoreactivity for APE/Ref-1 was independently scored by two observers and reported as percentage of neoplastic cells with evidence of 'nuclear' and/or 'cytoplasmic' stain. Therefore, with respect to subcellular localization of immunostaining, each tumor was classified as nuclear, cytoplasmic or nuclear/cytoplasmic. The cut-off points to separate various categories were optimised at $\geq 56\%$ and 15.95% for nuclear and cytoplasmic localization respectively, on the basis of the median value of the immunoreactive cases. The different groups were defined as follows: i) the 'nuclear' group with $\geq 56\%$ of cells with APE/Ref-1 immunoreactive nuclei and $< 15.95\%$ of cells with cytoplasmic expression; ii) the 'cytoplasmic' group, with $\geq 15.95\%$ of cells APE/Ref-1 positive in the cytoplasm and $< 56\%$ of cells with Ref-1 nuclear reactivity; iii) the 'nuclear/cytoplasmic' group, with both $\geq 56\%$ of cells showing APE/Ref-1 positive nuclei and $\geq 15.95\%$ of cells showing a Ref-1 cytoplasmic reaction; iv) the 'negative' group in which the percentage of cells with nuclear and cytoplasmic APE/Ref-1 expression was below the defined cut-off points.

No different scores for intensity of immunostaining were adopted because of the negligible differences observed among the positive cases.

Statistical analysis. The Mann-Whitney or the Kruskal-Wallis analyses were performed, as appropriated, to test the hypothesis of a difference in APE/Ref-1 expression among

Figure 1. Ductal breast carcinomas showing nuclear (A), mixed nuclear/cytoplasmic (B), cytoplasmic (C) and negative (D) immunohistochemical staining for APE/Ref-1.

Figure 2. Kaplan-Meier survival curves are shown for tumors with different localization of APE/Ref-1 immunostaining (log-rank test, $p=0.01$).

the two or three groups of each tumor characteristics. Association between categorical variables was evaluated by means of χ^2 analysis. To evaluate the correlation between continuous variables Spearman's test was used.

Survival data of the patients were obtained from the Laboratory Information System of the Institute of Anatomic Pathology. Overall survival (OS) was calculated as the time from the date of surgery to the date of death or last follow-up. Post-operative survival curves were constructed using the Kaplan-Meier method and then compared using the log-rank test.

The influence of APE/Ref-1 expression, adjusted for other covariates, was assessed in a multivariate analysis by the Cox proportional hazards model. Cox models were performed on

stepwise (backward) procedures that always forced APE/Ref-1 into the model along with any variables which satisfied an entry level of $p<0.05$ and, as the model added terms, did not exceed an exit level of $p>0.05$.

Results

The predominant pattern of APE/Ref-1 expression observed in this series of breast carcinomas was nuclear. In particular, the median percentage of cells with nuclear stain was 46.9% (25th-75th percentiles, 19.7-72.75) among all cases and 56% (25th-75th percentiles, 26.07-74.87) among nuclear immunoreactive cases. The median percentages of cells with cyto-

Table I. Mortality among 133 women with breast cancer by clinical and pathology characteristics.

	Total	Deaths	Person-months	HR ^a	95% CI	p-value
APE/Ref-status						
Nuclear	90	18	5793	1	-	
Cytoplasmic	27	5	1689	0.96	0.36-2.59	0.9352
Nucleocytoplasmic	5	3	194	4.99	1.45-17.17	0.0107
Negative	11	1	832	0.40	0.05-2.97	0.3679
Age (years)						
<50	35	8	2279	1	-	
50-69	66	9	4280	0.61	0.23-1.58	0.3048
≥70	32	10	1949	1.50	0.58-3.70	0.4259
Estrogen receptors						
Pos	93	15	6180	1	-	
Neg	31	10	1789	2.27	1.02-5.05	0.0447
Unknown	9	2	539			
Grade						
1	47	6	3110	1	-	
2	44	8	2917	1.41	0.49-4.07	0.5230
3	35	12	2045	3.05	1.14-8.14	0.0259
Unknown	7	1	436			
pT						
1	84	14	5676	1	-	
2-3	49	15	2832	2.46	1.15-5.27	0.0203
Nodal metastases						
0	78	13	5146	1	-	
1-3	35	7	2309	1.20	0.48-3.02	0.6937
≥4	20	7	1053	2.67	1.06-6.70	0.0368
pStage						
I	58	8	3825	1	-	
II	58	13	3789	1.62	0.67-3.90	0.2853
III	17	6	894	3.18	1.10-9.19	0.0328
Vascular invasion						
Absent	87	14	5694	1	-	
Present	34	11	2017	2.15	0.99-4.68	0.0542
Unknown	12	2	797			
Necrosis						
Absent	92	15	6053	1	-	
Present	36	11	2126	2.51	1.16-5.43	0.0195
Unknown	5	1	329			
p53 status						
Negative	38	3	2380	1	-	
Positive	38	9	2375	3.03	0.82-11.19	0.0967
Unknown	57	15	3753			

^aUnadjusted hazard ratio; 95% CI, confidence interval.

plasmic reactivity was 1.6% (25th-75th percentiles, 0-20.8) among all cases and 15.95% (25th-75th percentiles, 3.10-45.5) among cases with cytoplasmic positivity only.

No significant difference in APE/Ref-1 nuclear expression was observed depending on the pStage (p=0.96), nodal status (p=0.57), tumor size (p=0.52), histologic grade (p=0.14),

Table II. Multivariate analysis of APE/Ref-1 pattern, histologic grade, number of metastatic lymph nodes, and tumor size among 133 women with breast cancer.

	HR ^a 95% CI p-value	HR ^b 95% CI p-value	HR ^c 95% CI p-value	HR ^d 95% CI p-value	HR ^e 95% CI p-value	HR ^f 95% CI p-value	HR ^g 95% CI p-value
APE/Ref-1							
Nuclear-cytoplasmic	4.77 1.34-17.00 0.0161	4.47 1.28-15.59 0.0189	5.22 1.54-17.69 0.0080	5.13 1.46-17.96 0.0106	5.22 1.55-17.59 0.0076	4.64 1.36-15.81 0.0060	5.89 1.74-19.89 0.0043
Histologic grade							
3	1.87 0.81-4.33 0.1425	2.19 0.97-4.94 0.0585	1.96 0.87-4.43 0.1044		2.53 1.18-5.40 0.0170		
No. of lymph node metastases							
≥4	1.29 0.47-3.55 0.6165	1.69 0.66-4.36 0.2780		1.54 0.58-4.05 0.3864		2.30 0.96-5.49 0.0620	
pT							
>1	1.93 0.82-4.56 0.1339		2.06 0.92-4.65 0.0800	2.24 0.97-5.15 0.0579			2.57 1.20-5.50 0.0155

^aFrom a proportional hazard model that simultaneously included ref, grade, number of ln meta and T; ^bFrom a proportional hazard model that simultaneously included APE/Ref-1 status, histologic grade and number of lymph node metastases; ^cFrom a proportional hazard model that simultaneously included APE/Ref-1 status, histologic grade and pT; ^dFrom a proportional hazard model that simultaneously included APE/Ref-1 status, number of lymph node metastases and pT; ^eFrom a proportional hazard model that simultaneously included APE/Ref-1 status and histologic grade; ^fFrom a proportional hazard model that simultaneously included APE/Ref-1 status and number of lymph node metastases; ^gFrom a proportional hazard model that simultaneously included APE/Ref-1 status and pT; 95% CI, confidence interval.

necrosis (p=0.36), vascular invasion (p=0.86), oestrogen receptor status (p=0.44) and p53 status (p=0.83).

There appeared to be no differences in level of cytoplasmic APE/Ref-1 expression according to the pStage (p=0.49), nodal status (p=0.67), tumor size (p=0.19), histologic grade (p=0.77), necrosis (p=0.18), vascular invasion (p=0.94) and oestrogen receptor status (p=0.11). Percentage of cells with APE/Ref-1 cytoplasmic stain directly correlated with percentage of p53 positive cells (p=0.28, p=0.013).

Table I summarises the unadjusted hazard ratios (HR) describing the risk of death associated with tumor characteristics. The univariate analysis showed that tumor size, histologic grade, nodal status, hormonal receptor status, APE/Ref-1 expression and necrosis all had prognostic value. A marginal effect on survival was also noted for vascular invasion and p53 status. Kaplan Meier survival curves for tumors with different patterns of APE/Ref-1 expression are shown in Fig. 2. Although APE/Ref-1 nuclear/cytoplasmic immunostaining was observed only in five cases, all the women whose tumors showed mixed localisation experienced a significantly poorer survival rate (log-rank test, p=0.014).

When APE/Ref-1 expression was included in the Cox multivariate analysis, it retained significance as an independent prognostic factor in the models tested (Table II).

Discussion

Base losses [apurinic/aprimidinic (AP) sites] from human DNA represent the most important spontaneous DNA damage in cells (2,4-6). The rate at which these lesions occur is high *per se* and can be increased by the insult of DNA damaging agents such as drugs or radiation (20). In the absence of cellular defence mechanisms to repair and prevent a high rate of these DNA damages, cell death and/or mutagenesis are inevitable. All cells have developed various strategies to maintain the integrity of genome.

APE/Ref-1, also known as Hap-1/APEX is an enzyme that plays a key role in the process of base excision repair (BER). It belongs to the family of the so-called AP endonucleases whose main function is to initiate a repair cascade culminating in replacement of the AP site with the correct bases (6,10). Besides the activity in DNA repair, APE/Ref-1 is involved in the redox regulation of transcription factors by stimulating the DNA binding activity of various proto-oncogene products such as c-Jun, c-Fos, c-Myb and Pax proteins (13-15).

Derangement of DNA repair mechanisms is well recognized as a pathogenetic event in cancer progression (23).

Accordingly, there is accumulating interest about the biologic role of APE/Ref-1 in tumors. In particular it has

been hypothesised that different subcellular localisation of the protein could correspond to specific functions related to the process of DNA repair. Therefore, alterations in normal activity of APE/Ref-1 have been suggested as possible determinants of abnormal phenotypes.

A prognostic role of APE/Ref-1 was described by Kakolyris *et al* in a consecutive series of early operable non-small cell lung carcinomas (18). The authors reported a survival benefit for patient whose tumors expressed nuclear APE/Ref-1 over the defined cut-off value. No significant difference in survival was noted according to cytoplasmic, mixed nuclear and cytoplasmic, or negative APE/Ref-1 expression. Interestingly, the best survival advantage was seen when tumors expressed nuclear APE/Ref-1 positivity together with p53 negativity (likely expression of a wild-type p53).

Other studies addressing the prognostic value of APE/Ref-1 found interesting associations between APE/Ref-1 pattern and tumoral characteristics. Recently, different expression of APE/Ref-1 between ovarian adenocarcinomas of low malignant potential and other ovarian carcinomas has been reported (24). A cytoplasmic immunostaining was predominantly encountered in ovarian carcinomas whereas only two carcinomas of low malignant potential presented some areas with this pattern. On the basis of these observations the authors speculate that, in ovarian cancers, the presence of a strong cytoplasmic staining could represent a marker of malignant behaviour. Qualitative (subcellular localization) rather than quantitative differences in APE/Ref-1 expression were also reported during the various steps of the sequence normal colonic mucosa-adenoma-carcinoma (19).

The pattern of APE/Ref-1 immunostaining and its prognostic role in breast cancer has been previously investigated (20). No relationship between APE/Ref-1 status and survival was observed although APE/Ref-1 nuclear localisation was associated with negative nodal status and with low angiogenesis.

In the present study we have demonstrated a survival disadvantage for patients whose tumors showed APE/Ref-1 stain in both nuclear and cytoplasmic compartments (HR=4.99, confidence limits=1.45-17.17, p-value=0.01). In addition, on multivariate analysis, the prognostic significance of APE/Ref-1 was retained independently from the other main predictors of survival such as histological grade, nodal status and tumor size (Table II). However, our results merit to be interpreted with caution because of the paucity of cases presenting the combination of nucleus and cytoplasmic APE/Ref-1 expression (only 4% of the study population). The biological significance depends entirely on whether or not this small group of 5 patients represents a discrete entity. Nevertheless, the validity of our findings is strengthened by the observation that consistent results are obtained after small perturbation of the cut-off points (data not shown).

No adequate information on adjuvant treatment of patients was available in our retrospective unselected series. So, no conclusions can be drawn about a potential role of APE/Ref-1 expression as predictor of response to therapy. This hypothesis is however very tempting on the basis of pre-clinical data indicating the APE/Ref-1 activity in mechanisms of repair after the insult of DNA damaging agents.

The discordance between the present report and the work by Kakolyris *et al* regarding the prognostic role of APE/Ref-1 in breast cancer could be explained on the basis the different method of APE/Ref-1 evaluation, respectively mixed qualitative/quantitative and qualitative alone. In fact, the same authors found a relationship between APE/Ref-1 pattern and survival in their lung cancer series when they considered the percentage of APE/Ref-1 stained cells according to its localization inside the cell (18).

The implications for the poor survival rate (more aggressiveness) observed for women in which breast tumors showed nuclear/cytoplasmic localization instead of those showing only cytoplasmic localization of APE/Ref-1, are difficult to understand. In a very recent report, Kelley *et al* suggested that a cytoplasmic localization of APE/Ref-1 in prostate cancers could be due to its reverse movement from the nucleus to cytoplasm (25). Another possibility could be envisaged and is related to the fact that, due to its relatively high molecular weight, APE/Ref-1 requests an active mechanism for its localization into the nucleus, such as importins (26), and a subsequently inducible mechanism of regulation (i.e., phosphorylation, de-phosphorylation) as is the case of the NF-AT transcription factor (27). In this view, the altered subcellular localization of APE/Ref-1 could be an epiphenomenon of the alteration of the mechanism of nuclear shuttling, due to the neoplastic phenotype of the cells. Moreover, it should be kept in mind that several lines of evidence suggest that the APE/Ref-1 protein is highly heterogeneous in the cell, having possible phosphorylation-dependent redox and repair activities. Of note is the recent observation that another post-translational checkpoint for APE/Ref-1 activity is represented by the redox control of its activity (unpublished data). Therefore, the only presence of APE/Ref-1 in a cellular compartment is not sufficient, *per se*, to carry out the potential activity as a repair or redox enzyme. The actual form in which the APE/Ref-1 protein exists (e.g., reduced, oxidized, reduced and phosphorylated, oxidized and phosphorylated, reduced in the redox domain and oxidized in the repair domain, etc.) determined in these previous studies is unknown. The determination of the status of APE/Ref-1 in these cancers may provide clues regarding the role it is playing in their initiation, progression and aggressiveness.

Acknowledgments

The authors wish to thank Mrs. Maura Pandolfi for her significant technical help. Supported in part by grants of CNR (target project on biotechnology) and Regione Friuli Venezia Giulia to GD.

References

1. Lindahl T: Instability and decay of the primary structure of DNA. *Nature* 362: 709-715, 1993.
2. Lindahl T: Repair of intrinsic DNA mutations. *Mutat Res* 238: 305-311, 1990.
3. Loeb LA and Preston BD: Mutagenesis by apurinic/aprimidinic sites. *Ann Rev Genet* 20: 201-230, 1986.
4. Mol CD, Hosfield DJ and Tainer JA: Abasic site recognition by two apurinic/aprimidinic endonuclease families in DNA base excision repair: the 3' ends justify the means. *Mutat Res* 460: 211-229, 2000.

5. Wilson DM, Takeshita M and Demple B: Abasic site binding by the human apurinic endonuclease, Ape, and determination of the DNA contact sites. *Nucleic Acids Res* 25: 933-939, 1997.
6. Barzilay G and Hickson ID: Structure and function of apurinic/apyrimidinic endonucleases. *Bioessays* 17: 713-719, 1995.
7. Robson CN and Hickson ID: Isolation of cDNA clones encoding a human apurinic/apyrimidinic endonuclease that corrects DNA repair and mutagenesis defects in *E. coli* xth⁻ (exonuclease III) mutants. *Nucleic Acids Res* 19: 5519-5523, 1991.
8. Sander M, Lowenhaupt K and Rich A: *Drosophila* Rpr1 protein: an apurinic endonuclease with homologous recombination activity. *Proc Natl Acad Sci USA* 88: 6780-6784, 1991.
9. Babiychuk E, Kushnir S, van Montagu M and Inze D: The *Arabidopsis thaliana* apurinic endonuclease Arp reduces human transcription factors Fos and Jun. *Proc Natl Acad Sci USA* 91: 3299-3303, 1994.
10. Rothwell DG, Barzilay G, Gorman M, Morera S, Freemont P and Hickson ID: The structure and functions of the HAP1/Ref-1 protein. *Oncol Res* 8: 275-280, 1997.
11. Xanthoudakis S, Miao CG and Curran T: The redox and DNA-repair activities of Ref-1 are encoded by non-overlapping domains. *Proc Natl Acad Sci USA* 91: 23-27, 1994.
12. Craig N, Johnson S, Komachi K and Lindhal T: DNA repair. In: *Molecular Biology of the Cell*. Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson D (eds). Garland, New York, pp242-251, 1994.
13. Xanthoudakis S, Miao CG, Wang F, Pan YCE and Curran T: Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. *EMBO J* 11: 3323-3335, 1992.
14. Xanthoudakis S and Curran T: Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. *EMBO J* 11: 653-665, 1992.
15. Tell G, Scaloni A, Pellizzari L, Formisano S, Pucillo C and Damante G: Redox potential controls the structure and DNA binding activity of the paired domain. *J Biol Chem* 273: 25062-25072, 1998.
16. Walker LJ, Robson CN, Black E, Gillespie D and Hickson ID: Identification of residues in the human DNA repair enzyme HAP-1 (ref-1) that are essential for redox regulation of Jun DNA binding. *Mol Cell Biol* 13: 5370-5376, 1993.
17. Jayaraman L, Murthy KGK, Zhu C, Curran T, Xantoudakis S and Prives C: Identification of a redox/repair protein Ref-1 as a potent activator of p53. *Genes Dev* 11: 558-570, 1997.
18. Kakolyris S, Giatromanolaki A, Koukourakis M, Kaklamanis L, Kanavaros P, Hickson ID, *et al*: Nuclear localization of human AP endonuclease 1 (HAP1/Ref-1) associated with prognosis in early operable non-small cell lung cancer (NSCLC). *J Pathol* 89: 351-357, 1999.
19. Kakolyris S, Kaklamanis L, Engels K, Turley H, Hickson ID, Gatter KC, *et al*: Human AP endonuclease 1 (HAP 1) expression in a colorectal adenoma-carcinoma sequence. *Cancer Res* 57: 1794-1797, 1997.
20. Kakolyris S, Kaklamanis L, Engels K, *et al*: Human AP endonuclease 1 (HAP 1) protein expression in breast cancer correlates with lymph nodes status and angiogenesis. *Br J Cancer* 77: 1169-1173, 1998.
21. Xu Y, Moore DH, Broshears J, Liu L, Wilson TM and Kelley MR: The apurinic/apyrimidinic endonuclease (APE/ref-1) DNA repair enzyme is elevated in premalignant and malignant cervical cancer. *Cancer Res* 17: 3713-3719, 1997.
22. Walker LJ, Craig RB, Harris AL and Hickson ID: A role for the human DNA repair enzyme HAP1 in cellular protection against DNA damaging agents and hypoxic stress. *Nucleic Acids Res* 22: 4884-4889, 1994.
23. Loeb KR and Loeb RA: Significance of multiple mutations in cancer. *Carcinogenesis* 21: 379-385, 2000.
24. Moore DH, Michael H, Tritt R, Parson SH and Kelley MR: Alterations in the expression of the DNA repair/redox enzyme APE/ref-1 in the epithelial ovarian cancers. *Clin Cancer Res* 6: 602-609, 2000.
25. Kelley MR, Cheng L, Foster R, Tritt R, Broshears J and Koch M: Elevated and altered expression of the multifunctional DNA base excision repair and redox enzyme Ape/ref-1 in prostate cancer. *Clin Cancer Res* 7: 824-830, 2001.
26. Kaffman A and O'Shea E: Regulation of nuclear localization: a key to a door. *Annu Rev Cell Dev Biol* 15: 291-339, 1999.
27. Shibasaki F, Price ER, Milan D and McKeon F: Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. *Nature* 382: 370-373, 1996.