Human T Cell Leukemia Cell Death by Apoptosis-inducing Nucleosides from CD57+ HLA-DRbright Natural Suppressor Cell Line

Tsuneatsu Mori,1 Xiang Li, Etsuko Mori and Maowu Guo

Department of Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-0071

Apoptosis-inducing nucleosides (AINs) released from CD57+ HLA-DRbright natural suppressor (57.DR-NS) cell line, derived from human decidual tissue, were isolated from 57.DR-NS cell culture supernatant by the combination of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Apoptotic cell death was strongly induced in human T cell leukemia Molt4 cells treated with AINs, absolutely depending on DNA strand breaks, with activation of the caspase cascade, especially caspase-3. The administration of AINs to Molt4 tumor-bearing severe combined immunodeficiency (SCID) mice resulted in drastic suppression of tumor growth, with a decrease of tumor size and the appearance of apoptotic signals in tumor tissue. Thus, AINs are candidates for development as anticancer agents.

Key words: Decidual cell — Natural suppressor cell — Apoptosis-inducing nucleosides — DNA strand breaks — Caspase-3

In our previous studies on humoral tumor immunity,1–3) we demonstrated that human natural antibodies against Forssman antigen lysed several human malignant cells and that the specific active immunization of tumor-bearing rats with Forssman antigen resulted in tumor regression. On the other hand, cellular antitumor activity is mainly dependent on natural killer (NK) cell-mediated functions.4) Natural suppressor (NS) cells carrying CD57 surface markers have been shown to regulate various lymphoid responses, sharing some features with NK cells.5, 6) Furthermore, NS cells not only act as strong inhibitors of lymphoproliferative responses,7, 8) but also inhibit the proliferation of tumor cells.9, 10) Decidua cells positive for NK cell markers, including the majority of CD56 and a minority of CD57, defined morphologically as large granular lymphocytes (LGL), have been found in abundance in human and murine decidua, where they play an immunoregulatory role in implantation and invasion into the endometrium of the blastocyst.11–16)

In our previous reports,17–21) we described the CD57+ HLA-DRbright natural suppressor (57.DR-NS) cell line, cloned from human decidual tissue.22) The 57.DR-NS cell line could induce apoptosis not only in human leukemia cells,23) but also in human gastric carcinoma cells,24) mediated by factors of low molecular weight released into the culture fluid. Six components were isolated by means of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The six active components were determined to be nucleosides and modified nucleosides in nature by the combination of nuclear magnetic resonance and mass spectrometric analysis.23) We collectively termed them “apoptosis-inducing nucleosides (AINs).” However, the molecular mechanisms of AINs-induced apoptosis in target cells remained unknown. In the present study, we examined the initial molecular mechanisms, such as DNA strand breaks and activation of caspase-3, during AINs-induced apoptosis of human leukemia Molt4 cells. Furthermore, the antitumor effects of AINs on human leukemia-bearing severe combined immunodeficiency (SCID) mice were examined by monitoring tumor cell growth and cell death.

MATERIALS AND METHODS

Cells and cultures 57.DR-NS cells have been maintained in RPMI 1640 (Nissui, Tokyo) supplemented with 10% fetal calf serum (FCS), 0.25 mM Na pyruvate, 2 mM L-glutamine, 0.75% Na bicarbonate and 100 µg/ml of kanamycin at 37°C in a humidified incubator containing 5% CO2. Human T cell leukemia cell line Molt4 cells were also maintained in the same medium. To collect the culture supernatant, 107 57.DR-NS/Molt4 cells were inoculated into a semi-bulk culture petri dish (diameter 15 cm, Nalge Nunc International, IL) in the medium described above. The culture supernatant (500 ml/10 dishes/experiment) was removed 72 h later, centrifuged and filtered through a 0.45 µm Millipore disposable filter unit (Millipore, Tokyo).

Isolation of AINs The 57.DR-NS cell culture supernatant collected (500 ml) was applied to an octadecyl column (Bond Elut C18, size 60 ml, Varian, CA). The active components retained in the column were eluted with acetonitrile and the eluate was evaporated under a stream of N2. Then, the sample was spotted on a TLC plate (F254, Merck, Darmstadt, Germany) and developed with chlo-
roform/methanol/water (C/M/W, 60:40:8, v/v/v). The components that migrated between the solvent front and the band of phenol red, previously added to the culture medium, were extracted with C/M 1:1 (v/v) followed by evaporation under a stream of N2. Finally, the residue was subjected to C18-reverse phase HPLC (Beckman, CA) with a TSK gel ODS-80TM column (Tosoh, Tokyo), eluted with a linear gradient from 0 to 5% acetonitrile in 0.1% trifluoroacetic acid during 360 min at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected. The elution profile was monitored in terms of the absorbance at 214 nm. Fractions in each major peak (P) were pooled, freeze-dried and examined for activity. Control samples were prepared similarly, using Molt4 cell culture supernatant, and designated Pw.

Measurement of DNA strand breaks The strand unwinding rate correlated well with the number of breaks, or alkali-sensitive sites induced in DNA of target cells treated with DNA-damaging stress.5–7 Total fluorescence was derived from cultures in which DNA unwinding was prevented and background fluorescence, from cell suspensions after sonication to permit complete DNA unwinding. The percent residual double-stranded DNA after alkali treatment was calculated as follows; Percent of double-stranded DNA=(sample fluorescence−background fluorescence)/(total fluorescence−background fluorescence)×100. Therefore, triplicate assay tubes were prepared for measurements of DNA fluorescence (total, background and test conditions).

Molt4 cells (10⁶) were incubated with the AINs components, P₁, P₂ and P₄ at various doses (8, 40, 200 and 1000 µM), then resuspended in 0.2 ml of 10 mM sodium phosphate buffer (pH 7.2) containing 0.25 M MgCl₂ and lysed by adding 0.2 ml of a solution containing 9 M urea, 10 mM NaN₃ and 0.1% sodium dodecyl sulfate to each tube for 10 min at 0°C. At this time, 0.4 ml of 1 M glucose and 14 mM 2-mercaptoethanol were added to the total fluorescence tubes to prevent DNA unwinding. All tubes then received 0.2 ml of 0.2 N NaOH, which raised the pH of the test and background samples to pH 12.8 and that of the total fluorescence tubes to pH 11.2. Background aliquots were then briefly sonicated. Individual samples were incubated at 0°C for 30 min and then at 18°C for intervals from 0 to 90 min. DNA unwinding was stopped by adding 0.4 ml of 1 M glucose and 14 mM 2-mercaptoethanol to test and background tubes. Ethidium bromide, 6.7 µg/ml in 13.3 mM NaOH, was added to each tube. Finally, the relative fluorescence intensity was measured in a filter fluorometer (excitation 520 nm, analyzer 590 nm).

Flow cytometric assessment of sub-G1 DNA content After the treatment of 5×10⁵ Molt4 cells with P₁, P₂ or P₄ at the dose of 200 µM for 24, 48 and 72 h, the cells were harvested by trypsinization and fixed in 70% ethanol. The cells were stained in propidium iodide solution containing 50 µg/ml of propidium iodide, 40 mM sodium citrate, 0.1 µg/ml RNaseA and 1% Triton X-100 in phosphate-buffered saline (PBS). The cells were incubated for 60 min in the dark, and then analyzed by flow cytometry (Becton Dickinson FACScan, CA).

Assessment of DNA fragmentation After the treatment of Molt4 cells in the same manner as above, the cells were homogenized in homogenization buffer (0.3 M Tris pH 8.0, 10 mM EDTA, 0.1 M NaCl and 0.2 M sucrose) at 65°C for 30 min and 1/10 vol. of 3 M Na acetate (pH 5.0) was added. The mixture was kept at 0°C for 1 h, then total DNA was extracted with phenol/chloroform, precipitated with ethanol and incubated for 1 h at −70°C. The DNA pellets were resuspended in 20 µl of distilled water with DNase-free RNase and incubated at 37°C for 1 h. An aliquot of DNA sample was electrophoresed on 2% agarose gel containing ethidium bromide.

Morphological observation of apoptosis The Molt4 cells or tumor tissue sections treated with various doses of P₁, P₂ and P₄ were fixed in 4% paraformaldehyde-PBS at 4°C overnight. The fixed target cells or sections on slides were subjected to terminal deoxynucleotide transferase-mediated dUTP-nick end labeling (TUNEL) using an ApopTag kit according to the manufacturer’s instructions (Oncor, Gaithersburg, MD). The samples on slides were incubated with TdT enzyme to catalyze digoxigenin-dUTP and dATP binding to 3’ ends of fragmented DNA to form a heteropolymer of digoxigenin-11-dUTP and dATP at 37°C for 2 h, followed by anti-digoxigenin-peroxidase antibody for 1 h at room temperature. The samples on slides were visualized by addition of 3,3’-diaminobenzidine tetrahydrochloride (DAB) and photographed under a light microscope.

Assay of caspase-3 activity The examination of caspase-3 activity was performed by using a Caspase-3 assay kit according to the manufacturer’s instructions (PharMingen, CA). In brief, 5×10⁵ Molt4 cells were treated with 200 µM of P₁, P₂ or P₄ and harvested 24, 48 or 72 h later. The cell suspension (2×10⁵ cells/100 µl) was mixed with 400 µl of PBS and 10 µg of Ac-DEVD-AMC (N-acetyl-DEVD-7-amino-4-methylcomuiran) was added. The fluorogenic AMC liberated from Ac-DEVD-AMC was measured by flow cytometry using UV excitation at 380 nm.

Inhibition of caspase activity Molt4 cells (5×10⁵) were incubated with 200 µM P₁, P₂ or P₄ alone or in the presence of 100 µg/ml of the caspase inhibitor Z-Asp-CH₂-DCB (carbobenzoxy- L-aspart-1-yl-[2,6-dichlorobenzoyl]-oxy]methane) or the caspase-3 inhibitor, Ac-DEVDCCHO (N-acetyl-DEVD-aldehyde), purchased from Peptide Inst. Inc. (Osaka), for 72 h at 37°C in a 5% CO₂ incubator. Then, the cells were harvested and subjected to flow-cytometric analysis and DNA fragmentation assay.
Examination of anti-tumor activity of AINs on Molt4 tumor-bearing SCID mice

Three-week-old SCID mice were purchased from CLEA Japan (Tokyo). Molt4 cells (10^8) were subcutaneously inoculated into the neck in ten SCID mice per experiment. Two weeks later, when the tumor was sufficiently grown, similar-sized tumor-bearing mice were randomly assigned into two groups of five each. In one group, P1, P2 and P4 were dissolved at 400 µg each in 0.2 ml of PBS (total 1.2 mg/mouse/injection) and administered eighteen times to the Molt4 tumor-bearing mice alternately into the tumor and the tail vein. In the other group (control), Pm at the same dose was administered similarly. The anti-tumor activity with AINs was monitored by measuring the tumor size (longitude×latitude, cm^2) once a week for 3 weeks. Finally, the tumor tissues were removed and examined by TUNEL and DNA fragmentation methods.

Statistical analysis
Differences from control values were analyzed by using Student’s t test. Probability values <0.05 were considered to be significant.

RESULTS

Preparation of AIN samples by TLC and HPLC

Culture supernatant of 57.DR-NS cells was partially purified on a preparative octadecyl column followed by TLC. The bioactive fraction that migrated to the area above phenol red on TLC was further subjected to reverse-phase HPLC. Six major peaks (P1–P6) were obtained by HPLC (Fig. 1A). The structures of the six active components were determined in our previous study as follows: P1, 2′-deoxyuridine; P2, ribothymidine; P3, 2′-O-methyluridine; P4, thymidine; P5, 2′-O-methylinosine; P6, 2′-O-methylguanosine.

On the other hand, the elution pattern of Molt4 cell culture supernatant in HPLC gave a quite different profile from that of 57.DR-NS cell culture supernatant (Fig. 1B), affording only an inactive peak (Pm). No active peaks corresponding to P1–P6 from 57.DR-NS cell culture supernatant were obtained.

Fig. 1. Elution patterns in HPLC. In chart A, six active peaks (P1–P6) were obtained from 57.DR-NS cell culture supernatant. In chart B, one nonactive peak (Pm) was obtained from Molt4 cell culture supernatant.

Fig. 2. Dose-response relationship and time course of DNA strand breaks. DNA unwinding in Molt4 cells began after incubation with P1, P2 or P4 and progressed in a dose- and time-dependent manner. □ P1, □ P2, □ P4.

Molt4 Cell Death by AINs
DNA strand breaks in target cells by AINs  The assay was based on the observation that the rate of DNA unwinding in alkaline solution is inversely related to the length of intact double-stranded DNA segments. Incubation with 8, 40, 200 and 1000 µM P1, P2 and P4 for 24 h caused DNA strand breaks in the target Molt4 cells in a dose-dependent manner (Fig. 2A). Furthermore, alkali-resistant double-stranded DNA decreased to less than 50% of control values at 24 h on incubation with 200 µM P1, P2 and P4 (Fig. 2B). The accumulation of DNA strand breaks in the target Molt4 cells progressed in a time-dependent manner up to 72 h (Fig. 2B).

Induction of apoptosis in target cells treated with AINs Flow cytometric analysis showed that the ratio of sub-G1 DNA content (M1) of Molt4 cells treated with P1, P2 and P4 at the dose of 200 µM for 24, 48 or 72 h increased to 5.1, 30.3 or 90.1% (P1), 5.2, 40.2 or 70.2% (P2) and 4.6, 70.2 or 81.1% (P4) respectively in a time-dependent manner (Fig. 3A). The control values were 5.3, 6.0 and 10.2% (C) at the dose of 0 µM, respectively. The DNA fragmentation assay of Molt4 cells was performed over 24 to 72 h, following the treatment of target cells with 200 µM P1, P2 or P4 (Fig. 3B-a). DNA ladders were observed at 72 h (Fig. 3B-a, lanes 9, 10 and 11). No ladders were found at

![Fig. 3](image-url)
24 and 48 h (lanes 1, 2, 3 and 5, 6, 7), or in the control (lanes 4, 8 and 12).

The morphological changes in Molt4 cells treated with 200 µM P1, P2 or P4 for 72 h were also examined. Molt4 cells treated with P1 were stained by the TUNEL method, showing DNA fragments in the nucleus and a part of the cytoplasm, in addition to blebbing of membranes and shrinkage of cells (Fig. 3B-b). No such changes or staining were observed in the control cells (Fig. 3B-c). The results demonstrated that the P1, P2 or P4 component of the AINs isolated by HPLC could induce apoptosis in Molt4 cells in a time-dependent manner, corresponding to the cytometric analysis of the ratio of sub-G1 DNA content of Molt4 cells treated with these AINs (Fig. 3A).

Activation of caspase-3 in target cells treated with AINs To identify the main protease species in the apoptotic pathway, cleavage of a fluorogenic substrate (Ac-DEVD-AMC) of caspase-3 was examined by flow cytometry (Fig. 4). The number of cells positive for caspase-3 cleavage activity (M2) at 24 h was 7.2% (P1), 4.3% (P2) and 6.2% (P4) in Molt4 cells treated with 200 µM P1, P2 or P4, but increased to 37.1 or 84.3% (P1), 36.2 or 70.2% (P2) and 38.1 or 87.1% (P4) at 48 and 72 h, respectively. The control values were 5.1, 10.2 or 12.1% (C), respectively. Thus, it was concluded that the apoptotic pathway in Molt4 cells treated with P1, P2 and P4 is mediated, at least in part, by the activation of caspase-3.

Blocking of apoptosis in target cells by inhibitors of caspases and caspase-3 To characterize further the caspase-associated apoptosis, we tested the effect of inhibitors of caspases (Z-Asp-CH2-DCB) and of caspase-3 (Ac-DEVD-CHO) on the development of apoptosis. DNA fragmentation was induced in Molt4 cells treated with P1, P2 or P4 at the dose of 200 µM for 72 h (Fig. 5A, lanes 1, 2 and 3). But the addition of 100 µg/ml of inhibitor (Z-Asp-CH2-DCB or Ac-DEVD-CHO) prevented DNA fragmentation in Molt4 cells (lanes 4, 5 and 6 or lanes 7, 8 and 9), and no DNA fragmentation was seen in the control (lane 10). In addition, flow cytometric analysis showed that the accumulation of sub-G1 DNA content (M1) in Molt4 cells treated simultaneously with 200 µM P1, P2 or P4 and 100 µg/ml of Ac-DEVD-CHO in 72 h was inhibited to marginal levels (Fig. 5B-b) compared with that in Molt4 cells treated with 200 µM P1, P2 or P4 (Fig. 5B-a). Thus, it was confirmed that caspase-3 plays a critical role in P1, P2 and P4-induced apoptosis of Molt4 cells.

Curative effects of AINs in Molt4 tumor-bearing SCID mice The average tumor growth curves in a group treated with a mixture of P1, P2 and P4 and a Pα-treated control group are shown in Fig. 6A. The average tumor sizes were much smaller in the former group (P<0.001), indicating that the growth of Molt4 tumor cells was strongly inhibited by the administration of AINs. All five tumor-bearing mice survived and three out of five were completely cured, in contrast with the rapid growth of tumor tissues in all five control mice. Even in the two mice that were not cured, the apoptotic signals detected by DNA fragmentation and TUNEL methods were spread in all areas of tumor tissues. On completion of the experiment after 3 weeks, the tumor tissue was dissected from an AINs-
treated mouse and separated into three parts, according to the TUNEL staining grade: 1, positive staining region; 2, strongly positive staining region; 3, weakly positive staining region (Fig. 6B-a). DNA fragmentation was found in all parts (Fig. 6B-b).

DISCUSSION

Some investigators have postulated that macrophages produce and release thymidine and other nucleosides in order to regulate the proliferation of adjacent cells. Further studies showed that deoxyribonucleosides and thymidine are toxic to human malignant T lymphoid cells, since malignant T cell lines have high levels of deoxyribonuclease phosphorylating activity and low levels of deoxyribonucleotide dephosphorylating activity, resulting in the accumulation of triphosphate forms of deoxyribonucleotides. 8-Chloroadenosine can arrest Molt4 cell growth in vitro and inhibit human lung tumor growth in nude mice in vivo probably via apoptotic mechanisms. Furthermore, 1-β-arabinofuranosylcytosine has been used for the treatment of acute myelogenous leukemia, inducing DNA fragmentation in leukemia cells. Deoxyadenosine derivatives, such as 2-chloro-2'-deoxyadenosine, also induce apoptosis in quiescent lymphocytes, and are available for the treatment of indolent lymphoproliferative diseases. Thus, the modulation of apoptotic cell death may be an effective antitumor strategy.

It has been clarified that murine metrial LGLs can lyse Yac target cells and that NK cell populations in human
early pregnancy decidua respond to IL-2 and acquire killer activity against K562 leukemia cells and trophoblast cells including choriocarcinoma cells.38, 39) We have demonstrated that 57.DR-NS cells originated in human decidual tissue induce apoptosis not only in human leukemia K562 cells, but also in human gastric carcinoma GCIY cells through AINs, though they have no effect on human lung normal WI-38 cells.23, 24) In the present study, we examined the apoptotic mechanisms in Molt4 cells exposed to AINs released from 57.DR-NS cells and further investigated the therapeutic effects of AINs in Molt4 tumor-bearing SCID mice. By means of flow cytometry, DNA fragmentation and TUNEL methods, we demonstrated that P1 (2′-deoxy-ribose polymerase40) and p53-dependent pathways.41) The DNA strand breakage (Fig. 2), and activating the poly-ADP ribose polymerase40 and p53-dependent pathways.41) The activation of the caspase cascade is ubiquitously involved in various apoptotic mechanisms,42-44) so we examined the involvement of caspase-3 in the induction of apoptosis in Molt4 cells by AINs as shown in Fig. 4, using Ac-DEVD-CHO as a specific caspase-3 inhibitor. The generation of DNA fragments and accumulation of sub-G1 DNA content was completely inhibited in Molt4 cells treated with P1, P2 or P4 in combination with the inhibitor (Fig. 5). This result confirmed the involvement of caspase-3, which is activated by sequential proteolytic events that cleave the 32-kDa precursor at aspartic acid residues to generate an active heterodimer of 20- and 12-kDa subunits,45) corresponding to our recent findings.46) The active caspase-3, in turn, cleaves the inhibitor of caspase-activated DNase (ICAD) and then activates the caspase-activated DNase (CAD), which migrates to the nucleus and degrades DNA.47) We consider that AINs are good candidates for anticancer agents, and we are planning further studies on AINs-induced apoptosis in various malignant target cells.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan.

(Received January 21, 2000/Revised March 21, 2000/Accepted March 28, 2000)

REFERENCES

1) Mori, T., Sudo, T. and Kano, K. Expression of heterophil Forssman antigen on cultured malignant cell lines. J. Natl. Cancer Inst., 70, 811–818 (1983).

2) Mori, T., Mori, E., Sudo, T. and Kano, K. Expression of heterophil Forssman antigen as glycoprotein on transformed rat cell lines: shedding of the antigen from the cells. J. Natl. Cancer Inst., 73, 1179–1186 (1984).

3) Mori, T., Mori, E., Shigyo, Y., Sudo, T. and Kano, K. Heterophil Forssman glycoprotein: adenovirus 12 transformed rat cell lines. J. Natl. Cancer Inst., 77, 115–119 (1986).

4) Lewis, C. E. and McGee, J. O’D. Natural killer cells in tumor biology. In “The Natural Killer Cell,” ed. C. E. Lewis and J. O’D. McGee, pp. 175–193 (1992). IRL Press, Oxford.

5) Abo, T., Cooper, M. D. and Balch, C. M. Characterization of HNK-1+ (Leu 7) human lymphocytes I. Two distinct phenotypes of human NK cells with different cytotoxic capability. J. Immunol., 129, 1752–1757 (1982).

6) Tilden, A. B., Abo, T. and Balch, C. M. Suppressor cell function of human granular lymphocytes identified by the HNK-1 (Leu 7) monoclonal antibody. J. Immunol., 130, 1171–1175 (1983).

7) Strober, S. Natural suppressor (NS) cells, neonatal tolerance, and total lymphoid irradiation: exploring obscure relationships. Annu. Rev. Immunol., 2, 219–237 (1984).

8) Mortari, F., Bains, M. A. and Singhal, S. K. Immunoregulatory activity of human bone marrow. Identification of suppressor cells possessing OKM1, SSEA-1, and HNK-1 antigens. J. Immunol., 137, 1133–1137 (1986).

9) Sugiura, K., Inaba, M., Ogata, H., Yasumizu, R., Inaba, K., Good, R. A. and Ikehara, S. Wheat germ agglutinin-positive cells in a stem cell-enriched fraction of mouse bone marrow have potent natural suppressor activity. Proc. Natl. Acad. Sci. USA, 85, 4824–4826 (1988).

10) Sugiura, K., Inaba, M., Ogata, H., Yasumizu, R., Saradina, E. E., Inaba, K., Kuma, S., Good, R. A. and Ikehara, S. Inhibition of tumor cell proliferation by natural suppressor cells present in murine bone marrow. Cancer Res., 50, 2582–2586 (1990).

11) Daya, S., Clark, D. A., Devlin, C., Jarrell, J. and Chapat, A. Suppressor cells in human decidua. Am. J. Obstet. Gynecol., 151, 267–270 (1985).

12) Croy, B. A., Reed, N., Malashenko, B. A., Kim, K. and Kwon, B. S. Demonstration of YAC target cell lysis by murine granulated metrial gland cells. Cell. Immunol., 133, 116–126 (1991).

13) Linnemeyer, P. A. and Pollack, S. B. Murine granulated metrial gland cells at uterine implantation sites are natural killer lineage cells. J. Immunol., 147, 2530–2535 (1991).

14) Hayakawa, S., Saito, S., Nemoto, N., Chishima, F., Akiyama, K., Shiraishi, H., Hayakawa, J., Karasaki-Suzuki, M., Fujii, K. T., Ichijo, M., Sakurai, I. and Satoh, K.
Expression of recombinase-activating genes (RAG-1 and 2) in human dendritic mononuclear cells. J. Immunol., 153, 4934–4969 (1994).

15) Golander, A., Zakuth, V., Shechter, Y. and Spirer, Z. Suppression of lymphocyte reactivity in vitro by a soluble factor secreted by explants of human decidua. Eur. J. Immunol., 11, 849–851 (1981).

16) Clark, D. A., Flanders, K. C., Banwait, D., Millar-Book, W., Manuel, J., Stedronska-Clark, J. and Rowley, B. Murine pregnancy decidua produces a unique immunosuppressive molecule related to transforming growth factor β-2. J. Immunol., 144, 3008–3014 (1990).

17) Mori, T., Noda, Y., Kanzaki, H., Irahara, M., Kamada, M., Mori, T. and Kano, K. Antibodies in pregnancy and fertilization. In “Antibodies: Protective, Destructive and Regulatory Role,” 9th International Convocation on Immunology, ed. F. Milgrom, C. J. Abeyounis and B. Albini, pp. 382–388 (1984). Karger, New York.

18) Tatsumi, K., Mori, T., Mori, E., Kanzaki, H. and Mori, T. Immunoregulatory factor released from a cell line derived from human decidua tissue. Am. J. Reprod. Immunol. Microbiol., 13, 87–92 (1987).

19) Fukuda, A., Mori, T., Mori, E., Tatsumi, K., Kanzaki, H. and Mori, T. Effects of the supernatant of mixed lymphocyte cultures and decidua cell line cultures on mouse embryo development in vitro. J. In Vitro Fertil. Embryotransf., 6, 59–64 (1989).

20) Mori, T., Takada, M., Guo, M. W., Mori, E. and Isemura, M. Studies on mammalian implantation in vitro (I): production of laminin from human decidua cell line and its effects on murine trophoblastic out growth. J. Reprod. Dev., 38, 258–292 (1992).

21) Mori, T., Guo, M. W., Shen, W. Y., Mori, T. and Sudo, T. Immunomolecular mechanisms in mammalian implantation. Endocr. J., 41, S17–S31 (1994).

22) Tatematsu, K., Noda, Y., Kanzaki, H. and Mori, T. Natural killer cells in pregnancy and fertility. Acta Obstet. Gynaecol. Jpn., 36, 2115–2120 (1984) (in Japanese).

23) Mori, T., Guo, M. W., Li, X., Xu, J. P. and Mori, T. Isolation and identification of apoptosis inducing nucleosides from CD57+ HLA-DRbright natural suppressor cell line. Biochem. Biophys. Res. Commun., 251, 416–422 (1998).

24) Li, X., Xu, J. P., Guo, M. W., Mori, E. and Mori, T. Apoptotic cell death of human gastric tumor induced by nucleosides from CD57+ HLA-DRbright natural suppressor cell line. Int. J. Oncol., 14, 687–694 (1999).

25) Kohn, K. W., Erickson, L. C., Ewig, R. A. G. and Friedman, C. A. Fractionation of DNA from mammalian cells by alkaline elution. Biochemistry, 5, 4629–4637 (1976).

26) Birmboim, H. C. and Jevcak, J. J. Fluorometric method for rapid detection of DNA strand breaks in human white blood cells produced by low doses of radiation. Cancer Res., 41, 1889–1892 (1981).

27) Seto, S., Carrera, J. C., Kubota, M., Wasson, D. B. and Carson, D. A. Mechanism of deoxyadenosine and 2-chlorodeoxyadenosine toxicity to nondividing human lymphocyte. J. Clin. Invest., 75, 377–383 (1985).

28) Nelson, D. S. Production by stimulated macrophages of factors depressing lymphocyte transformation. Nature, 246, 306–307 (1973).

29) Stadecker, M. J., Calderson, J., Karnovsky, M. L. and Unuë, E. R. Synthesis and release of thymidine by macrophages. J. Immunol., 119, 1738–1743 (1973).

30) Carson, D. A., Kaye, J., Matsumoto, S., Seegmiller, J. E. and Thompson, L. Biochemical basis for the enhanced toxicity of deoxyribonucleosides toward malignant human T cell lines. Proc. Natl. Acad. Sci. USA, 76, 2430–2433 (1979).

31) Van-Lookeren-Campagne, M. M., Villalba-Diaz, F., Jastorf, B. and Kessin, R. H. 8-Chloroadenosine 3',5'-monophosphate inhibits the growth of Chinese hamster ovary and MolT4 cells through its adenosine metabolite. Cancer Res., 51, 1600–1605 (1991).

32) Ally, S., Clair, T., Katsaros, D., Tortora, G., Yokozaki, H., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

33) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

34) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

35) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

36) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

37) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

38) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

39) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).

40) Vintermyr, O. K., Boe, R., Brustugun, O. T., Maronde, E., Finch, R. A., Avery, T. L. and Cho-Chung, Y. S. Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. Cancer Res., 49, 5650–5655 (1989).
40) Jeggo, P. A. DNA repair: PARP—another guardian angel? *Curr. Biol.*, **8**, R49–R51 (1998).

41) Miller, J. DNA damage, p53 and anticancer therapies. *Nat. Med.*, **1**, 879–880 (1995).

42) Guo, M. W., Xu, J. P., Mori, E. and Mori, T. Identification of Fas antigen associated with apoptotic cell death in murine ovary. *Biochem. Biophys. Res. Commun.*, **203**, 1438–1446 (1994).

43) Mori, T., Xu, J. P., Mori, E., Sato, E., Saito, S. and Guo, M. W. Expression of Fas-Fas ligand system associated with atresia through apoptosis in murine ovary. *Horm. Res.*, **48**(S3), 11–19 (1997).

44) Xu, J. P., Li, X., Mori, E., Sato, E., Saito, S., Guo, M. W. and Mori, T. Expression of Fas-Fas ligand system associated with atresia in murine ovary. *Zygote*, **5**, 321–327 (1997).

45) Rotonda, J., Nicholson, D. W., Fazil, K. M., Gallant, M., Gareau, Y., Labelle, M., Peterson, E. P., Rasper, D. M., Ruel, R., Vaillancourt, J. P., Thornberry, N. A. and Becker, J. W. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nat. Struct. Biol.*, **3**, 619–625 (1996).

46) Li, X., Guo, M. W., Mori, E. and Mori, T. Activation of caspase-3 in Molt4 cells by apoptosis inducing nucleosides from CD57+HLA-DRbright natural suppressor cell line. *Biochem. Biophys. Res. Commun.*, **268**, 514–520 (2000).

47) Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A. and Nagata, S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, **391**, 43–50 (1998).