Nitric Oxide Is an Initiator of Intercellular Signal Transduction for Stress Response after Hyperthermia in Mutant p53 Cells of Human Glioblastoma

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INTRODUCTION

Nitric oxide is known to be a multifunctional physiological substance. Recently, it was suggested that nitric oxide is involved in p53-dependent response to many kinds of stress, such as heat shock and changes in cellular metabolism. To verify this hypothesis, we examined the effect of nitric oxide produced endogenously by heat-stressed cells on nonstressed cells using a human glioblastoma cell line, A-172, and its mutant p53 (mp53) transfectant (A-172/mp53). The accumulation of inducible nitric oxide synthase was caused by heat treatment of the mp53 cells but not of the wild-type p53 (wt53) cells. The accumulation of heat shock protein 72 (hsp72) and p53 was observed in nontreated mp53 cells cocultivated with heated mp53 cells, and the accumulation of these proteins was suppressed by the addition of a specific inducible nitric oxide synthase inhibitor, aminoguanidine, to the medium. Furthermore, the accumulation of these proteins was observed in the wt53 cells after exposure to the conditioned medium by preculture of the heated mp53 cells, and the accumulation was completely blocked by the addition of a specific nitric oxide scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide, to the medium. In addition, the accumulation of hsp72 and p53 in the wt53 cells was induced by the administration of an nitric oxide-generating agent, S-nitroso-N-acetylpenicillamine, to the medium. Finally, the thermosensitivity of the wt53 cells was reduced in the conditioned medium by preculture of the heated mp53 cells as compared with conventional fresh growth medium. Our finding of the accumulation of hsp72 and p53 in nitric oxide-receptor cells cocultivated with heated nitric oxide-donor cells provides the first evidence for an intercellular signal transduction pathway via nitric oxide as intermediate without cell-to-cell interactions such as gap junctions.

ABSTRACT

Nitric oxide is known to be a multifunctional physiological substance. Recently, it was suggested that nitric oxide is involved in p53-dependent response to many kinds of stress, such as heat shock and changes in cellular metabolism. To verify this hypothesis, we examined the effect of nitric oxide produced endogenously by heat-stressed cells on nonstressed cells using a human glioblastoma cell line, A-172, and its mutant p53 (mp53) transfectant (A-172/mp53). The accumulation of inducible nitric oxide synthase was caused by heat treatment of the mp53 cells but not of the wild-type p53 (wt53) cells. The accumulation of heat shock protein 72 (hsp72) and p53 was observed in nontreated mp53 cells cocultivated with heated mp53 cells, and the accumulation of these proteins was suppressed by the addition of a specific inducible nitric oxide synthase inhibitor, aminoguanidine, to the medium. Furthermore, the accumulation of these proteins was observed in the wt53 cells after exposure to the conditioned medium by preculture of the heated mp53 cells, and the accumulation was completely blocked by the addition of a specific nitric oxide scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide, to the medium. In addition, the accumulation of hsp72 and p53 in the wt53 cells was induced by the administration of an nitric oxide-generating agent, S-nitroso-N-acetylpenicillamine, to the medium. Finally, the thermosensitivity of the wt53 cells was reduced in the conditioned medium by preculture of the heated mp53 cells as compared with conventional fresh growth medium. Our finding of the accumulation of hsp72 and p53 in nitric oxide-receptor cells cocultivated with heated nitric oxide-donor cells provides the first evidence for an intercellular signal transduction pathway via nitric oxide as intermediate without cell-to-cell interactions such as gap junctions.

INTRODUCTION

NO is an important regulatory substance for the immune response, cytoxicotropy, neurotransmission, and vasodilatation (1). NO is endogenously generated from L-arginine by NOS isoenzymes (2). The activities of the constitutive forms neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3) are dependent on elevated cellular calcium concentrations (3). It is well known that iNOS (or NOS2) is expressed in various species of mammalian cells after exposure to many kinds of inducers such as cytokines, bacterial lipopolysaccharide, heat shock, and hypoxia (4–6). It has been reported that iNOS can produce sustained high concentrations of NO after induction (3). High concentrations of NO and NO metabolites have been shown to cause DNA damage and have been shown to be mutagenic (7–9). On the other hand, it has been shown that high concentrations of NO can quench superoxide anion radicals and that the reaction product, peroxynitrite, can rearrange to nontoxic nitrate. NO can also scavenge oxidizing intermediates generated from peroxynitrite (10). Recently, it has also been reported that NO can induce hsp and wt53 protein, which play important roles in adaptation of the organism to many kinds of stress (11, 12).

The inducible hsp72 is one of the stress proteins induced transcriptionally by various environmental stressors such as heat shock, cold shock (13), UV (14, 15), ionizing radiation (14, 16), and DNA-damaging agents (17). Hsps play important roles in cellular thermotolerance and the development of thermotolerance as an adaptive response after exposure to heat shock (18, 19). It has been found that many stress proteins, including hsp72, function as a key protein acting as a cell cycle checkpoint (21, 22). Thus, it belongs to the signaling pathway by which cells regulate the G1-S transition after either genotoxic or nongenotoxic insults; in this way, wt53 functions as a transcriptional factor to control the cell cycle, especially in stressed cells. Recently, it has been considered that the major functional role of wt53 is as an integrator of stress-response signals (23). The p53-dependent cellular events, such as WAF1 induction by heat shock, cold shock, or low pH, have been demonstrated using a pair of human glioblastoma cell lines, A-172 and T98G, bearing wt53 and mp53 genes, respectively (24–26). We have established a transfected cell line, A-172/mp53, by transfecting A-172 cells with the mp53 gene (27). The genotype of A-172/mp53 cells differs from that of A-172 cells only in the p53 gene. We have confirmed that the wt53-dependent cellular events induced after hyperthermia do not occur in the A-172/mp53 cells (28).

Recently, NO is suggested to be involved in p53-dependent response to many kinds of stress such as heat shock and changes in cellular metabolism. In the present study, to verify this hypothesis we examined: (a) the kinetics of the accumulation of iNOS after heat treatment; (b) the kinetics of the accumulation of hsp72 and p53 in nontreated cells cocultivated with the heated cells; and (c) the thermosensitivities of cells in the conditioned medium by preculture of the heated cells using a human glioblastoma cell line, A-172, and its mutant p53 transfectant (A-172/mp53).

MATERIALS AND METHODS

Chemicals. SNAP was purchased from Molecular Probes, Inc. (Eugene, OR). c-PFTIO was purchased from Doujin Chemical Co. (Tokyo, Japan). Both chemicals were dissolved at 10 mM in PBS(−) and stored at −20°C until use. The protein assay kit was purchased from Nacalai Tesque (Kyoto, Japan). Anti-iNOS monoclonal antibody (Clone 6), anti-hsp72 monoclonal antibody (C92F3A-5), anti-p53 monoclonal antibody (PAb1801 or DO-1), and horse- 

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3 The abbreviations used are: NO, nitric oxide; NOS, NO synthase; iNOS, inducible NOS; hsp, heat shock protein; wt53, wild-type p53; mp53, mutant p53; SNAP, S-nitroso-N-acetylpenicillamine; c-PFTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide.
Amplification System was purchased from DuPont/NEN Research Products (Boston, MA). Giemsa solution was purchased from Merck & Co., Inc. (Rahway, NJ).

**Cells.** A human glioblastoma cell line, A-172, was purchased from JCRB Cell Bank (Setagaya, Tokyo, Japan). The two human glioblastoma cell lines, A-172 and A-172/mp53, were cultured in DMEM containing 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and 50 μg/ml kanamycin (DMEM-10). A-172 cells have the wild-type p53 gene (wt p53), whereas A-172/mp53 cells have a mutated p53 gene (mp53; Refs. 27 and 29). The doubling times of A-172 and A-172/mp53 cells were about 24 and 22 h, respectively. Plating efficiencies of both cell lines were about 50%.

**Heat Treatment.** Twenty h before heat treatment, exponentially growing cells were seeded at \(10^6\) cells/dish in 9-cm dishes or \(10^5\) cells/flask in 25-cm² flasks containing DMEM-10 without irradiated feeder cells. Cells were washed twice with DMEM-10 and then exposed to the conditioned medium containing c-PTIO for 10 h at 37°C. After the treatment, cells were harvested and used for Western blot analysis.

**SNAP Treatment.** Twenty h before treatment, exponentially growing cells were seeded at \(10^6\) cells/flask in 25-cm² flasks containing DMEM-10 without irradiated feeder cells. Cells were washed twice with DMEM-10 and then treated with SNAP (1–10 μM) in DMEM-10 for 10 h at 37°C. After the treatment, cells were harvested and used for Western blot analysis.

**Western Blot Analysis.** The cells were suspended in RIPA buffer and then treated by freezing and thawing three times. The protein contents in the supernatants obtained after centrifugation were quantified with a commercial protein assay kit. An aliquot of proteins was subjected to Western blotting analysis for iNOS, hsp72, and p53. After electrophoresis on 10% polyacrylamide gels containing 0.1% SDS and electrophoretic transfer to poly(vinylidene difluoride) membranes, proteins on the membrane were incubated with anti-iNOS, anti-hsp72, or anti-p53 monoclonal antibodies. For visualization of the bands, we used horseradish peroxidase-conjugated anti-mouse IgG antibody and the BLAST: Blotting Amplification System. The relative amounts of iNOS, hsp72, and p53 were calculated from the scanning profiles using a Macintosh (LC 475) computer with the public domain NIH Image program.

**Fig. 1.** Accumulation of iNOS in A-172 (○) and A-172/mp53 (▲) cells after heating. The cells were heated at 44°C for 15 min and then incubated at 37°C for 0–24 h. The level of iNOS in each sample was analyzed by Western blotting as described in “Materials and Methods.” The level in nontreated cells was used as a control. Bars, SE.

**Fig. 2.** Change of nitrite concentration in medium of A-172 (A) or A-172/mp53 (B) cells. Nitrite concentration in medium was measured according to the method of Saltzman (30). ○ and ▲, in the absence of aminoguanidine; ● and ▼, in the presence of aminoguanidine (100 μM).
Fig. 3. Western blot analysis of hsp72 and p53 in A-172 cells cocultivated with heated A-172/mp53 cells. A-172/mp53 cells were heated at 44°C for 15 min. After transfer of A-172 cells on slide glasses to dishes of A-172/mp53 cells, cell cultures were incubated at 37°C for 1 to 10 h. AG, aminoguanidine.

Measurement of Nitrite Concentration in Medium. Nitrite concentration in medium was measured according to the method of Saltzman (30). Forty μl of medium were mixed well with 960 μl of the reagent containing 0.5% sulfanilic acid, 0.002% N-1-naphthylethylendiamine dihydrochloride, and 14% acetic acid. After standing at room temperature for 15 min, absorbance of samples at 550 nm was measured. The solution of sodium nitrite dissolved in 14% acetic acid, after standing at room temperature for 15 min, the nitrite concentration decreased to 0.3 μM. Just after heating at 44°C for 15 min, the nitrite concentration decreased to 0.3 μM, which is similar to the level in the medium in which A-172 cells were cultured before heating. Subsequently, the nitrite concentration increased to over 1.6 μM 12 h after heating, and this level was maintained at least up to 24 h (Fig. 2B). In addition, the elevation of nitrite concentration in the medium of A-172/mp53 cells was completely suppressed by the addition of aminoguanidine (100 μM). On the other hand, in the medium of A-172 cells, the nitrite concentration decreased, and the addition of aminoguanidine did not affect the nitrite concentration after heating (Fig. 2A). These results in Figs. 1 and 2 indicate that in A-172/mp53 cells, NO generated by the accumulated iNOS after heating causes an elevation of nitrite concentration in the medium. Our results confirmed a previous report that iNOS appeared to have produced presumably up to micromolar concentrations of NO (31).

To determine whether the cellular stress response was induced by the elevation of the nitrite concentration in the medium, we examined the accumulation of hsp72 and wtp53 in the nontreated A-172 cells that were cocultivated with A-172/mp53 heated at 44°C for 15 min, in the presence or absence of 100 μM aminoguanidine as iNOS inhibitor (see “Materials and Methods”). In the absence of aminoguanidine, both hsp72 and wtp53 were accumulated in the nontreated A-172 cells cocultivated with the heated A-172/mp53 cells (Fig. 3). However, this accumulation was completely abolished by the addition of aminoguanidine to medium. In the heated A-172/mp53 cells, a remarkable accumulation of hsp72 was observed in either the presence or absence of aminoguanidine, whereas the level of mp53 remained relatively stable. These results indicate that NO produced in the heated A-172/mp53 cells induces the accumulation of hsp72 and wtp53 in the cocultivated A-172 cells, suggesting that NO may be a mediator for an intercellular signal transduction pathway through the medium, without cell-to-cell contact, in the stress response.

To confirm that the accumulation of hsp72 and wtp53 was induced by NO, we examined the accumulation of these proteins in A-172 cells using an NO-specific scavenger and an NO-generating agent. The conditioned medium of A-172/mp53 cells was prepared by culturing the cells for 10 h after heating them at 44°C for 15 min in the presence or absence of 10 or 50 μM c-PTIO as NO scavenger. The levels of hsp72 and wtp53 in A-172 cells increased markedly 10 h after exposure to the conditioned medium prepared by culturing A-172/mp53 cells for 10 h after heating at 44°C for 15 min in the absence of c-PTIO (Fig. 4, Lane 3), whereas the levels of these proteins did not change 10 h after exposure to the conditioned medium

![Image](https://cancerres.aacrjournals.org)
prepared by culturing A-172/mp53 cells for 10 h without heating (Fig. 4, Lane 2). The accumulation of these proteins was diminished by the addition of c-PTIO to the conditioned medium of the heated A-172/mp53 cells in a dose-dependent manner (Fig. 4, Lanes 4 and 5). Furthermore, we examined whether NO can induce the accumulation of hsp72 and wtp53 in A-172 cells. The accumulation of hsp72 was observed 10 h after exposure to 1 μM SNAP, and the level of hsp72 was decreased at higher SNAP concentrations (Fig. 5). The accumulation of wtp53 was observed 10 h after exposure to 1 μM SNAP, reached a peak at 5 μM, and was sustained at an elevated level up to 10 μM. The results in Figs. 4 and 5 indicate that the large amount of NO produced by the elevated level of iNOS in the heated A-172/mp53 cells is released into the medium, acts as a mediator of the intercellular signal transduction, and induces the accumulation of hsp72 and wtp53 in nonstressed A-172 cells.

In an attempt to elucidate the effects of NO on the cellular thermosensitivity, we examined the thermosensitivity of A-172 cells in various media at 44°C (Fig. 6 and Table 1). A-172 cells in the conditioned medium by preculture of A-172/mp53 cells for 10 h after heating at 44°C for 15 min (CM-Δ) were more thermoresistant than those in fresh growth medium (GM) or the conditioned medium by preculture of A-172/mp53 cells for 10 h without heating (CM; Fig. 6).

In GM, the Tₐ₀ of A-172 cells was 11.3 min. In CM, the thermosensitivity of A-172 cells scarcely changed; the Tₐ₀ of A-172 cells was 13.8 min. However, in the CM-Δ, the Tₐ₀ of A-172 cells was 20.0 min (Table 1). The thermal dose modifying ratios in Tₐ₀ were 1.2 and 1.8 for CM and CM-Δ, respectively. The reduction of thermosensitivity in CM-Δ was similar to that of the thermotolerant A-172 cells prepared by preheating at 44°C for 15 min 10 h before challenge heating, which is represented by the dashed line in Fig. 6. The results in Fig. 6 and Table 1 indicate that NO released from the heated A-172/mp53 cells can induce thermoresistance in the nontreated A-172 cells through intercellular signal transduction.

**DISCUSSION**

In summary, we found that: (a) the accumulation of hsp72 and p53 was induced in NO-recipient cells by cocultivation with the NO-donor cells; (b) the accumulation of these proteins was also induced by exposure of cells to the conditioned medium by preculture of the NO-donor cells; (c) the accumulation of these proteins was completely blocked by the addition of NO scavenger to the conditioned medium; and (d) the cellular thermosensitivity was reduced in the conditioned medium by preculture of NO-donor cells as compared with fresh growth medium. Our findings demonstrate that NO released from the donor cells can induce hsp72 and p53 accumulation in the cocultivated NO-recipient cells through intercellular signal transduction without cell-to-cell interactions such as gap junctions. Previously, we reported that the cellular content of wtp53 increased after heating in human glioblastoma cells having wt p53 gene (29). Therefore, the fact that the accumulation of iNOS was observed only in mp53 cells after heating suggests that wtp53 accumulated after heating may suppress iNOS synthesis. These results confirm previous studies that wtp53 transrepressed iNOS expression through inhibition of the iNOS promoter in a variety of cells in vitro (12) and in vivo (32). Malyshchev et al. (33) reported that NO generated from a chemical agent induces hsp72 accumulation. Forrester et al. (12) have also reported that NO generated from a chemical agent induces wtp53 accumulation. It is well known that a half-life of NO is extremely short (about 6 s), and NO can react immediately with oxygen or reactive oxygen intermediates (34). Therefore, our findings suggest at least three possible pathways of the intercellular signal transductions after hyperthermia, which can be initiated by NO: (a) reaction products of NO, which released from the heated A-172/mp53 cells, may induce directly accumulation of hsp72 and p53 in nonstressed A-172 cells; (b) NO itself and/or reaction products of NO may activate GMP cyclase; subsequently, cGMP may induce the accumulation of hsp72 and wtp53 in nonstressed A-172 cells; and (c) NO itself and/or reaction products of NO may induce secretion of certain cytokines and/or growth factors in the heated A-172/mp53 cells; subsequently they may induce accumulation of hsp72 and p53 in nonstressed A-172 cells. Thus, we demonstrated the intercellular signal transduction initiated by NO production in the donor cells to effect responses in the recipient cells.

**Table 1.** Thermosensitivities of A-172 cells in various media

<table>
<thead>
<tr>
<th>Medium</th>
<th>T₀ (min)</th>
<th>Tₐ₀ (min)</th>
<th>Thermal dose modifying ratio in Tₐ₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh growth medium</td>
<td>11.3</td>
<td>12.3</td>
<td>1.00</td>
</tr>
<tr>
<td>CM⁴</td>
<td>13.8</td>
<td>13.0</td>
<td>1.22</td>
</tr>
<tr>
<td>CM-Δ⁵</td>
<td>20.0</td>
<td>13.6</td>
<td>1.77</td>
</tr>
</tbody>
</table>

⁴ The conditioned medium prepared by culturing of A-172/mp53 cells for 10 h without heating.
⁵ The conditioned medium prepared by culturing of A-172/mp53 cells for 10 h after heating at 44°C for 15 min.

Fig. 5. Accumulation of hsp72 and p53 in A-172 cells exposed to SNAP. A-172 cells were incubated at 37°C for 10 h in the absence (Lane 1) and in the presence of 1 μM (Lane 2), 5 μM (Lane 3), or 10 μM (Lane 4) SNAP, followed by Western blot analysis.

Fig. 6. Thermosensitivity of A-172 cells in the conditioned media of A-172/mp53 cells. The thermosensitivity of A-172 cells was analyzed in control growth medium (C), in the conditioned medium by preculture of A-172/mp53 cells at 37°C for 10 h (Δ), and in the conditioned medium by preculture of A-172/mp53 cells at 37°C for 10 h after heating at 44°C for 15 min (Δ) by a colony formation assay. ---, thermosensitivity of thermotolerant A-172 cells prepared by preculture at 37°C for 10 h after heating at 44°C for 15 min.

Fig. 6. Thermosensitivity of A-172 cells in the conditioned media of A-172/mp53 cells. The thermosensitivity of A-172 cells was analyzed in control growth medium (C), in the conditioned medium by preculture of A-172/mp53 cells at 37°C for 10 h (Δ), and in the conditioned medium by preculture of A-172/mp53 cells at 37°C for 10 h after heating at 44°C for 15 min (Δ) by a colony formation assay. ---, thermosensitivity of thermotolerant A-172 cells prepared by preculture at 37°C for 10 h after heating at 44°C for 15 min.
recipient cells. However, it is still unknown what mediates the signal transduction. In addition, the intercellular signal transduction initiated and mediated by NO and its reaction products may link the intracellular signal transduction induced by not only extracellular stress such as heat shock and UV rays but also intracellular stress such as production of oxidants and changes in metabolism (35).

It is well known that hsps, including hsp72, contribute to cellular thermoresistance and thermostolerance through their functions as molecular chaperones, in which they play an important role in the protein repair process for certain heat-denatured cellular proteins (20). Likewise, wt53 plays an important role in safeguarding the genomic integrity of mammalian cells in response to cellular injury (23). Cellular injury can trigger an accumulation of wt53, resulting in p53-mediated increases in expression of growth-regulatory genes and GI growth arrest (21). Previously, we reported that wt53 induces GI arrest after heating (29). During the GI arrest induced by wt53 after heating, the induced and accumulated hsps may repair heat-denatured proteins, thereby enhancing survival of cellular injury induced by heat and may block heat-induced apoptosis (36). Recently, Gansauge et al. (37) reported that endogenous production of NO revealed a GI arrest in all of the tested cells using human pancreatic carcinoma cell lines. Thus, the thermoresistance of the NO-recipient cells is brought about by hsp72 and wt53 accumulation induced by NO from the donor cells through the intercellular signal transduction pathway.

Previously, to maintain a high plating efficiency of cells, a γ-irradiated or antibiotic-treated cell feeder layer was often used in colony formation assays for measurement of cellular sensitivity to radiation, chemicals, or hyperthermia (38, 39). Recently, however, it has been reported that the cell survival and growth of the target cells are greatly affected by the presence of a feeder layer of irradiated cells, and that the feeder layer suppresses apoptosis induced in the target cells by certain treatments (40, 41). Our results indicate a possible mechanism of the feeder layer effects and suggest that the experimental results obtained using a feeder layer may not represent the intrinsic cellular sensitivity to radiation, chemicals, or hyperthermia. Furthermore, we consider that our results may indicate a possible mechanism of the bystander effects in cancer gene therapy. When a fraction of the cancer cells in a tumor was transfected with wt53 or the TNF α gene, overall growth suppression, agiogenesis suppression, and stimulation of apoptosis were observed in the tumor (42–44). NO induces apoptosis in a p53-dependent manner in many types of cells (45, 46). Therefore, an intercellular signal transduction pathway mediated by NO, without cell-to-cell interactions, may be involved in the mechanisms of bystander effects in cancer gene therapy. Finally, our results suggest that elucidation of the effects of the microenvironmental conditions in tumors on cellular responses after treatments is very important in the fundamental research concerning various cancer therapies, including gene therapy.

REFERENCES


Announcements

(Requests for announcements must be received at least three months before publication.)

2000 ANNUAL MEETING

The AACR’s Annual Meeting is one of the largest and most important annual gatherings of scientists engaged in cancer research worldwide. The next Annual Meeting will take place in San Francisco, CA, April 1–5, 2000. The Chairperson of the Annual Meeting is Peter A. Jones, of the Norris Comprehensive Cancer Center. Co-Chairpersons are Carlos L. Arteaga, Vanderbilt University; Franco M. Muggia, Kaplan Comprehensive Cancer Center; Geoffrey M. Wahl, Salk Institute; and Alice S. Withermore, Stanford University.

The Program Committee will invite outstanding scientists in the field to organize plenary sessions, symposia, controversial sessions, and meet-the-expert sessions. It will also review proffered papers submitted by members of the cancer research community and will schedule acceptable ones in minisymposia, poster discussion sessions, and poster sessions. The deadline for submission of abstracts will be November 1, 1999. Further program information can be found on the AACR’s Website (http://www.aacr.org).

FUTURE ANNUAL MEETINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

2000 April 1–5, San Francisco, CA
2001 March 24–28, New Orleans, LA
2002 April 6–10, San Francisco, CA

CALL FOR NOMINATIONS FOR 2000 AACR-PEZCOLLER INTERNATIONAL AWARD FOR CANCER RESEARCH

The AACR-Pezcoller International Award for Cancer Research is given annually to a scientist who has made a major scientific discovery in the field of cancer. The Pezcoller Foundation was established in 1982 by Professor Alessio Pezcoller, a dedicated Italian surgeon who has made important contributions to medicine during his career and who, through his foresight, vision, and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Over the last decade the Pezcoller Foundation, in collaboration with the ESO-European School of Oncology, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 15,000 members in more than 60 countries who are experts in basic, clinical, and translational cancer research. The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results, in a variety of forums, including publications, meetings, and training and education programs. Because of their joint commitment to scientific excellence in cancer research, the Foundation and the AACR collaborate on the presentation of this award. This has strengthened their already well-established relationship and facilitates international collaborations and interactions. Because of the successful implementation of this joint program in 1998, this award is now given on an annual basis.

The awardee will be selected by an international committee of AACR members appointed by the AACR President with the agreement of the Council of the Pezcoller Foundation. Although normally the Award will be presented to a single investigator, in exceptional cases two individuals may be selected to share the award when their investigations have resulted in related prizeworthy work. The committee will make its selection solely on the basis of the awardee’s scientific accomplishments without regard to race, gender, nationality, geographic location, or religious or political views. The winner will give an award lecture during the AACR Annual Meeting in San Francisco, CA (April 1–5, 2000) and will receive the award in a ceremony at the Foundation’s headquarters in Trento, Italy, right after the Annual Meeting. The award consists of a honorarium and a commemorative plaque.

The Foundation and the AACR are now soliciting nominations for the 2000 Award. Nominations can be made by any scientist who is now or has been affiliated with an institution engaged in cancer research. Institutions or organizations are not eligible for this award, and candidates may not nominate themselves.

There is no official application form for this award. The nomination package should consist of the following:

- the candidate’s curriculum vitae
- an indication of the most important references in the candidate’s curriculum vitae and list of publications
- a letter of recommendation in English (500 words, maximum) describing the candidate’s major scientific achievements and explaining the impact of these achievements on progress in cancer research

Nominators are asked to maintain the confidentiality of the nomination process and to refrain from informing the candidate about the nomination.

The deadline for receipt of nominations by the AACR is October 1, 1999, for consideration for the 2000 Award. The names of the members of the Awards Committee will be published in a later announcement. Questions about the nomination process should be directed to the AACR Office via FAX at (215) 440-9322 or Email at aacr@aacr.org. Nominators should submit the original plus 12 copies of their nominations and supporting materials to:

AACR-Pezcoller International Award for Cancer Research

c/o American Association for Cancer Research, Inc.
Public Ledger Building, Suite 826
150 South Independence Mall West
Philadelphia, PA 19106-3483
USA

CALL FOR NOMINATIONS FOR AACR AWARDS

The AACR presents the following awards to distinguished scientists at its Annual Meeting. The Awards Committee, consisting of separate subsections, will recommend candidates for each award to the AACR Executive Committee. Following is a description of each award. Members wishing to make nominations should provide a short description of the candidate’s accomplishments; the subcommittee involved will obtain further documentation on suitable candidates. Nominations may be directed to the Association Office for forwarding to the appropriate subcommittee. This material should be received no later than September 1, 1999.

Clowes Memorial Award. The Eli Lilly Company established the Clowes Memorial Lecture to honor Dr. G. H. A. Clowes, who was a founding member of the AACR and a research director of Eli Lilly. The purpose of the award is to give "recognition of outstanding research accomplished in some recent period." The Clowes Award should recognize outstanding recent accomplishments in basic cancer research, and the Board of Directors construes this to mean both laboratory research and epidemiological investigations.

Joseph H. Burchenal AACR Clinical Research Award. Bristol-Meyers Squibb has established this award to recognize outstanding achievements in clinical cancer research. It is named for Dr. Joseph H. Burchenal, past president and honorary member of the AACR and a leading figure in the field of cancer chemotherapy. There are no restrictions on the age or geographic location of the awardee.

Richard and Hinda Rosenthal Foundation Award. The Rosenthal Foundation has established this award to "recognize research which has made or promises the offer of soon making a notable contribution to improved clinical care in the field of cancer." The Foundation wishes to honor and provide incentive to young investigators relatively early in their careers. It has, therefore, stipulated that the Association restrict the award to individuals who are engaged in the practice of medicine, who reside in the Americas, and who will not be more than 50 years of age at the time of the award (April 2000).

Rhoads Memorial Award. In 1979, an anonymous doctor established this award in memory of Dr. Cornelius P. Rhoads, a founder and the first Director of the Sloan-Kettering Institute for Cancer Research. This annual award is
intended to give recognition to an “individual on the basis of meritorious achievement in cancer research.” In accordance with the doctor’s wishes, the awardee must be a young investigator; therefore, the Board of Directors of the Association has stipulated that the recipient must not have reached his or her 41st birthday by the time of the award (April 2000).

Cain Memorial Award. The Warner-Lambert Company instituted this award to honor the memory of Dr. Bruce F. Cain of New Zealand for his work in the “design, synthesis, and biological evaluation of potential anticancer drugs.” The purpose of the award is to “give recognition to an individual or research team for outstanding preclinical research that has implications for the improved care of cancer patients.” Examples of such research include discovery of a significant new anticancer agent and major contributions to a compound’s application as an antitumor agent. The award will recognize outstanding contributions in the fields of medicinal chemistry, biochemistry, or tumor biology as related to drug discovery; will encompass anticancer, antiviral, and antifungal agents; will have no age limit; and will be international in scope.

American Cancer Society Award. The American Cancer Society sponsors this award to honor outstanding achievements in the fields of epidemiology, biomarkers, and prevention. There are no age or geographical restrictions on the awardee.

APPLICATIONS AVAILABLE FOR THE AACR GERTRUDE B. ELION CANCER RESEARCH AWARD

Accepting Applications from Assistant Professors throughout the World Supported by a Grant from Glaxo Wellcome Oncology

The AACR Gertrude B. Elion Cancer Research Award fosters Basic, Clinical, and Translational research by non-tenured, tenure-tracked Assistant Professors or equivalent throughout the world. This one-year $30,000 grant is supported by an educational grant from Glaxo Wellcome Oncology. Travel to the AACR Annual Meeting to accept the award is also provided.

Candidates must be nominated by an AACR member and submit a detailed application. Individuals holding the rank of adjunct professor or instructor, tenured faculty, permanent national government employees, and employees of private industry are not eligible. The application deadline is Wednesday, December 15, 1999.

The application form and complete guidelines can be downloaded from the AACR Website: www.aacr.org. Hard copies of these materials can also be requested; contact: Preston Moritz • AACR • Public Ledger Building, Suite 826 • 150 South Independence Mall West • Philadelphia, PA 19106-3483 • ph: (215) 440-9300 • f: (215) 440-9372 • E-mail: moritz@aacr.org

APPLICATIONS NOW AVAILABLE FOR AACR RESEARCH FELLOWSHIPS

For Postdoctoral or Clinical Fellows throughout the World

The AACR Research Fellowships in Clinical, Translational, Prevention, and Basic research foster cancer research by young scientists currently at the postdoctoral or clinical research fellow level. AACR Fellowships provide a one-, two-, or three-year grant of $30,000 per annum plus travel to the Annual Meeting. Beginning in 2000, AACR will offer a three-year translational colon cancer research fellowship. AACR Fellowships are sponsored by Amgen, Inc., Bristol-Myers Squibb Oncology, Cancer Research Foundation of America, Kimmel Foundation for Cancer Research, and the AACR, among others.

Candidates must be nominated by a member of the AACR and must be an AACR Member or have applied for membership by the time the fellowship application is received in the AACR Office. Fellowship Candidates must have been fellows for at least two years but no more than five years prior to July 2000. Academic Faculty holding the rank of assistant professor or higher, students, medical residents, permanent national government employees, and employees of private industry are not eligible. The application deadline is Wednesday, December 15, 1999.

The application form and complete guidelines can be downloaded from the AACR Website: www.aacr.org. Hard copies of these materials can also be requested; contact: Preston Moritz • AACR • Public Ledger Building, Suite 826 • 150 South Independence Mall West • Philadelphia, PA 19106-3483 • ph: (215) 440-9300 • f: (215) 440-9372 • E-mail: moritz@aacr.org

AACR SPECIAL CONFERENCES IN CANCER RESEARCH

A number of meetings are now being organized in the AACR’s series of smaller scientific meetings. Following are the topics, dates, locations, and program committees for these meetings. When full details of each meeting are available, AACR members will be the first to receive complete brochures and application forms for participation in these important conferences. Nonmembers may receive this information by sending their names and addresses to Meetings Mailing List, American Association for Cancer Research, Public Ledger Building, 150 South Independence Mall West, Suite 826, Philadelphia, PA 19106-3483. Up-to-date program information is also available via the Internet at the AACR’s website (http://www.aacr.org/confrnc.html).

MOLECULAR ASPECTS OF METASTASIS

September 22–26, 1999
Snowmass Village Resort, Snowmass, CO

Chairpersons
Ruth J. Muschel, Philadelphia, PA
Patricia S. Steeg, Bethesda, MD

THE MOLECULAR AND GENETIC BASIS OF CHEMOPREVENTION AND EARLY DETECTION OF CANCER

October 6–10, 1999
Sheraton Bal Harbour Beach Resort, Bal Harbour, FL

Chairpersons
David Sidransky, Baltimore, MD
Frank L. Meyskens, Jr., Orange, CA

GENETIC AND FUNCTIONAL CONSEQUENCES OF CELL CYCLE ALTERATIONS IN CANCER

October 20–24, 1999
Sheraton San Diego Hotel & Marina, San Diego, CA

Chairpersons
Steven I. Reed, La Jolla, CA
Joan V. Ruderman, Boston, MA
MOLECULAR TARGETS AND CANCER THERAPEUTICS: DISCOVERY, DEVELOPMENT, AND CLINICAL VALIDATION
Co-sponsored by the National Cancer Institute and the European Organisation for Research and Treatment of Cancer
November 16–19, 1999
Washington Hilton Hotel, Washington, DC

Chairpersons:
Daniel D. Von Hoff, San Antonio, TX
Robert E. Wittes, Bethesda, MD
Jean-Claude Horiot, Dijon, France

DNA REPAIR DEFECTS
January 14–18, 2000
San Diego Hilton Beach & Tennis Resort, San Diego, CA

Chairperson
Richard D. Kolodner, La Jolla, CA

TRANSCRIPTION FACTOR PATHOGENESIS OF CANCER AT THE MILLENNIUM
January 26–30, 2000
Marriott Laguna Cliffs Resort, Dana Point, CA

Chairpersons
Peter K. Vogt, La Jolla, CA
Frank J. Rauscher, III, Philadelphia, PA

PROGRAMMED CELL DEATH: CLINICAL APPLICATIONS
February 27–March 2, 2000
Hyatt Regency Lake Tahoe Resort, Lake Tahoe, NV

Chairpersons
John C. Reed, La Jolla, CA
Junying Yuan, Charlestown, MA

RECENT DEATH
We regret to report the death of Dr. Samuel Schwartz of Minneapolis, MN. Dr. Schwartz was an Emeritus Member of the American Association for Cancer Research.

CALENDAR OF EVENTS
World Congress of Epidemiology: Epidemiology for Sustainable Health, August 31–September 4, 1999, Florence, Italy. Contact: Scientific Secretariat, Department of Statistics, University of Florence, viale Morgagni 59, I-50134 Florence, Italy. Phone: 39 55 4237 244; Fax: 39 55 414277; Email: iea99@stat.ds.unifi.it; Website: http://iea99.ds.unifi.it.


Seventh International Conference on Human Antibodies & Hybridomas, September 8–10, 1999, The Royal College of Physicians, Edinburgh, Scotland. Contact: John Herriot, HAH ’99, Meetings Management, Hart House, No. 4 The Hart, Farnham, Surrey, GU9 7HA, United Kingdom. Phone: (44) 125 272 6066; Fax: (44) 125 272 3303; E-mail: jherriot@meetingsmgmt.u-net.com.

International Conference on Oxidative Stress in Skin Biology and Medicine, September 13–16, 1999, International Institute of Anticancer Research, Kapan-driti, Attiki, Greece. Contact: Dr. Michael Rallis, University of Athens, School of Pharmacy, Div. of Pharmaceutical Technology, Panepistimioiupolis, 15771 Athens, Greece. Phone: 00301–727-4675; Fax: 00301–724-4191; Email: rallis@pharm.uoa.gr.

3rd Annual International Conference of Novel Targets in the Treatment of Pain, September 16–17, 1999, Hilton Washington Embassy Row, Washington, DC. Contact: IBC, 225 Turnpike Road, Southborough, MA 01772-1749. Phone: (508) 481-6400; Fax: (508) 481-7911; Email: reg@ibcus.com; Website: http://www.ibcus.com/2387.


Cytoines, Hormones and Immunity, September 25–30, 1999, Castelvecchio Pascoli, Italy. Contact: Dr. Josip Hendekovic, European Science Foundation, 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France. Phone: +33 3 88 76 71 35; Fax: +33 3 88 36 69 87; Email: euresco@esf.org; Website: http://www.esf.org/euresco.

AAI Annual Meeting, September 27–29, 1999, Towsey Center, Ann Arbor, MI. Contact: Joyce Robertson, Registrar, Dept. of Medical Education, P.O. Box 1157, Ann Arbor, MI, 48106-1157. Phone: (734) 763-1400 or (800) 800-0666; Fax: (734) 936-1641.

10th Anniversary International Symposium on Advances in Anticoagulant, Antithrombotic and Thrombolytic Drugs, October 3–7, 1999, The Fairmont Copley Plaza, Boston, MA. Contact: IBC USA Conferences Inc., 225 Turnpike Road, Southborough, MA 01772-1749. Phone: (508) 481-6400; Fax: (508) 481-7911; E-mail: reg@ibcus.com; Website: http://www.ibcus.com/2198.

23rd Annual Physicians Cancer Symposium, October 14–16, 1999, La Jolla Hyatt Regency Hotel, La Jolla, CA. Contact: Ruthanne Crawford, Stevens Cancer Center, Scripps Memorial Hospital, 9888 Genesee Ave., La Jolla, CA 92037. Phone: (619) 626-6756; Fax: (619) 626-6793; Email: racrawford@chw.edu.

19th Annual Nurses Cancer Symposium, October 14–16, 1999, La Jolla Hyatt Regency Hotel, La Jolla, CA. Contact: Ruthanne Crawford, Stevens Cancer Center, Scripps Memorial Hospital, 9888 Genesee Ave., La Jolla, CA 92037. Phone: (619) 626-6756; Fax: (619) 626-6793; Email: racrawford@chw.edu.

Blood and Marrow Transplanting: Immunotherapy in the 21st Century, October 22, 1999, Towsey Center, Ann Arbor Michigan. Contact: Joyce Robertson, Registrar, Dept. of Medical Education, P.O. Box 1157, Ann Arbor, MI, 48106-1157. Phone: (734) 763-1400 or (800) 800-0666; Fax: (734) 936-1641.

International Conference on Immunology: Antigens, Antigen-presenting Cells and T Cells, October 23–26, 1999, Shanghai, China. Contact: Xuetao Cao, M.D., Ph.D., Chairman and Professor, Department of Immunology, Second Military Medical University, 800 Xiang Yin Road, Shanghai 200433, China. Phone: +86–21-65382502; Email: caoxt@public3.sta.net.cn.

9th North American ISSX Meeting, October 24–28, 1999, Opryland Hotel, Nashville, TN. Contact: ISSX/ACS-DCT Meeting, P.O. Box 3, Cabin John, MD 20818. Phone: (301) 983-5357; Email: lolahan@exec.issx.org; Website: http://www.louisville.edu/medschool/biochemistry/ISSX-ACS.

Education of Physicians in End-of-Life Care, October 28–29, 1999, Renaissance Cleveland Hotel, Cleveland, OH. Contact: The Cleveland Clinic Foundation. Phone: (216) 444-5695, (800) 862-8173; Fax: (216) 445-9406.

International Conference on Palliative Care Nursing. October 29, 1999, Renaissance Cleveland Hotel, Cleveland, OH. Contact: The Cleveland Clinic Foundation. Phone: (216) 444-5695, (800) 862-8173; Fax: (216) 445-9406.
inhibitor, aminoguanidine, to the medium.

was suppressed by the addition of a specific inducible nitric oxide synthase cocultivated with heated mp53 cells, and the accumulation of these proteins protein 72 (hsp72) and p53 was observed in nontreated wtp53 not of the wild-type p53 cells. The accumulation of heat shock not of the wild-type p53 cells. The accumulation of heat shock protein 72 (hsp72) and p53 was observed in nontreated wtp53 cells cocultivated with heated mp53 cells, and the accumulation of these proteins was suppressed by the addition of a specific inducible nitric oxide synthase inhibitor, aminoguanidine, to the medium.”

Erratum

In the article by H. Matsumoto et al., entitled “Nitric oxide is an initiator of intercellular signal transduction for stress response after hyperthermia in mutant p53 cells of human glioblastoma,” which appeared in the July 1, 1999 issue of Cancer Research (pp. 3239–3244), the fourth and fifth sentences are incorrect and should read: “The accumulation of inducible nitric oxide synthase was caused by heat treatment of the mp53 cells but not of the wild-type p53 (wtp53) cells. The accumulation of heat shock protein 72 (hsp72) and p53 was observed in nontreated wtp53 cells cocultivated with heated mp53 cells, and the accumulation of these proteins was suppressed by the addition of a specific inducible nitric oxide synthase inhibitor, aminoguanidine, to the medium.”

International Conference on Advances in Cancer Immunotherapy, March 2–4, 2000, Princeton, NJ. Abstract deadline: October 1, 1999. Contact: Lois Gillespie, Garden State Cancer Center, 520 Belleville Avenue, Belleville, NJ 07109. Phone: (973) 844-7007; Fax: (973) 844-7020; Email: gscancer@att.net.

First International Conference on Translational Research and Pre-Clinical Strategies in Radio-Oncology, March 5–8, 2000, Palazzo dei Congressi Conference Center, Lugano Switzerland. Contact: Dr. Jacques Bernier, Cantonal Department of Radio-Oncology, Sand Giovanni Hospital, CH-6504 Bellinzona, Switzerland. Phone: 41 91 820 9157; Fax: 41 91 820 90 44; jbernier@cschs.ch; Website: http://www.osg.ch/ictr2000.html.

Annual Meeting of the American Society for Blood and Marrow Transplantation, March 29–April 1, 2000, Anaheim, CA. Contact: IBMT/ABMTR Statistical Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. Fax: (414) 456-6530. Website: http://www.asbmt.org.

IX International Symposium on Luminescence Spectrometry in Biomedical and Environmental Analysis – Spectroscopic and Imaging Detection Techniques, May 15–17, 2000, CORUM, Centre des Congrès, France. Contact: Dr. Dan A. Lerner, Chairman, University of Montpellier, École Nationale Supérieure de Chimie 8, Rue de l’Ecole Normale, F-34296 Montpellier cedex 5, France. Phone: 33–04 6714 4323; Fax: 33–04 6714 4349; Email: lerner@enscm.fr.

Erratum
Nitric Oxide Is an Initiator of Intercellular Signal Transduction for Stress Response after Hyperthermia in Mutant \( p53 \) Cells of Human Glioblastoma

Hideki Matsumoto, Sachiko Hayashi, Masanori Hatashita, et al.