A New Mechanism for DNA Alterations Induced by Alpha Particles Such as Those Emitted by Radon and Radon Progeny

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The mechanism(s) by which alpha (α) particles like those emitted from inhaled radon and radon progeny cause their carcinogenic effects in the lung remains unclear. Although direct nuclear traversals by α-particles may be involved in mediating these outcomes, increasing evidence indicates that a particles can cause alterations in DNA in the absence of direct hits to cell nuclei. Using the occurrence of excessive sister chromatid exchanges (SCE) as an index of DNA damage in human lung fibroblasts, we investigated the hypothesis that α-particles may induce DNA damage through the generation of extracellular factors. We have found that a relatively low dose of α-particles can result in the generation of extracellular factors, which, upon transfer to unexposed normal human cells, can cause excessive SCE to an extent equivalent to that observed when the cells are directly irradiated with the same irradiation dose. A short-lived, SCE-inducing factor(s) is generated in α-irradiated culture medium containing serum in the absence of cells. A more persistent SCE-inducing factor(s), which can survive freeze–thaw and is heat labile is produced by fibroblasts after exposure to the α-particles. These results indicate that the initiating target for α-particle-induced genetic changes can be larger than a cell's nucleus or even a whole cell. How transmissible factors like those observed here *in vitro* may extend to the *in vivo* condition in the context of a-particle-induced carcinogenesis in the respiratory tract remains to be determined. — *Environ Health Perspect* 105(Suppl 5):1095–1101 (1997)

Key words: alpha particles, radon/radon progeny, indirect radiation effects, high LET radiation, DNA damage, extracellular factors, sister chromatid exchange, human lung fibroblasts

Introduction

Alpha (α) particles, which consist of doubly charged helium nuclei generated during radioactive decay, are classified as a type of high linear energy transfer (LET) or densely ionizing radiation because of their marked ionization power. Exposure of the respiratory tract to these poorly penetrating particles can occur through a variety of sources. These include the radioactive decay of inhaled particulate uranium, plutonium, thorium, and radium, ^{210}polonium contained in cigarette smoke, and the inhalation of α-emitting radon and its α-emitting decay daughters, ^{214}polonium and ^{218}polonium, as attached and unattached fractions with ambient aerosols (*1,2*). Although high doses of virtually all types of ionizing radiation are capable of causing mutations and cancer in various organisms, practical concern over radiation exposure presently focuses primarily on the carcinogenic potential to humans after exposure to very low doses, such as those that approach background levels. In this regard, and in terms of the absorbed dose-equivalent to humans, environmental exposure to naturally occurring radon and radon progeny is by far the largest single contributor to ionizing radiation dose to the general public (*3*).

Epidemiologic studies conducted on individuals exposed to high concentrations of radon and radon daughter products, e.g., uranium miners, suggest a dose–response relationship for the induction of lung cancer (*4,5*). Based largely on extrapolations of these findings to lower doses, recent estimates suggest that inhalation exposure to indoor radon/radon progeny may be responsible for as many as 24,000 new cases of lung cancer in the United States yearly (*6,7*). However, as is generally the case for genotoxic agents, the precise relationship between dose and the biological effects of these α-emitters has yet to be measured directly over the low-dose range of interest to the general population, thus forcing relevant estimates of cancer induction to be made largely on the basis of biophysical modeling. It becomes vital, therefore, for such models to realistically reflect the essence of underlying cellular mechanisms; otherwise, they may be of dubious practical use and even misleading in terms of the setting of exposure standards and risk assessment analyses.

The mechanism(s) by which α-particles cause lung cancer has not been elucidated. Even so, a variety of genetic lesions, including dose-dependent chromosomal damage, have been associated with the DNA-damaging effects of α-particles [e.g., references (*8–14*)]. α-Particles can give rise to mutations and malignant transformation (*15*), perhaps because the DNA double-strand breaks they can produce when traversing cell nuclei frequently are not repaired or are misrepaired (*16*). Additionally, Kadhim et al. (*17,18*) have recently obtained evidence *in vitro* that suggests that α-irradiation can induce a state of genomic instablity by an as-yet-to-be elucidated mechanism, which presumably could contribute to the ultimate emergence of cancerous phenotypes (*19*). Most investigators have assumed that α-particles inflict their DNA-damaging effects when traversing cell nuclei. Indeed, a substantial amount of information has been obtained about the number of α-particle traversals through the nucleus that is required to kill a cell (*20–23*), as well as information about the effect of size, shape, and/or thickness of the nucleus with respect to a cell's susceptibility to reproductive inactivation with α-irradiation (*24*). Even so, we (*25*) and other investigators (*26,27*) have obtained recent evidence that suggests

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Abbreviations used: α-particles, alpha particles; cGy, centigray; FBS, fetal bovine serum; HFL1, human lung fibroblasts; LET, linear energy transfer; MEM, minimum essential medium; SCE, sister chromatid exchange(s).

α-particles can mediate DNA-damaging effects by extranuclear events.

In this paper, we describe our initial evidence obtained *in vitro* that indicates that DNA damage by α-particles can be induced by the generation of extracellular factors. Should these new findings extend to the *in vivo* condition, existing microdosimetric and cancer risk assessment models for inhaled radon/radon progeny, as well as existing mechanistic models by which α-emitters may induce lung cancer, will require reconsideration.

Methods

Cell Culture

Normal human lung fibroblasts (HFL1, CCL 153, American Type Culture Collection, Rockville, MD) were routinely cultured in 75 cm^2 tissue culture flasks in minimum essential medium (MEM) (GIBCO-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT). All cell cultures were incubated at 37°C in humidified 5% CO_2/95% air. Cells were harvested from the flasks by trypsinization and seeded in 1.5-μm thick Mylar-bottomed, approximately 30-mm diameter culture dishes (*28*) at an initial density of 2×10^5 cells per dish 5 days prior to use, with a change to fresh culture medium on the second day after plating. All the irradiations and other treatments in this study were performed with density-arrested cells.

Exposure of Cells and Culture Media to α-Particles

Unless otherwise indicated, all but residual culture medium was removed from the Mylar dishes prior to the α- or sham-irradiations of cell cultures and replaced immediately after exposure. Confluent HFL1 were exposed to α-particles at room temperature. Exposure to the α-particles was performed using a ^{238}Pu α-particle collimated exposure system that has been described in detail elsewhere (*24,29,30*). Most of the experiments described in this report were performed with α-particles delivered at an 8.4-cGy dose. Cells exposed to this dose of α-particles served as positive controls in experiments designed to assess for α-particle-induced extracellular factors as mediators of excessive sister chromatid exchanges (SCE), whereas cells that were sham-irradiated or that received sham-irradiated culture medium served as routine negative controls. In some experiments, various culture media preparations in cell-free Mylar dishes were exposed to 8.4 cGy of α-particles or they were sham-irradiated before being added to otherwise untreated, confluent cultures of HFL1.

SCE Assay and Data Analyses

The SCE assay used in this study has been described in detail elsewhere (*25*). Briefly, after exposure to α-radiation, sham-irradiation, or the addition of various culture media preparations, culture dishes containing confluent HFL1 were placed in an incubator for a period of 24 hr. The HFL1 were then harvested by trypsinization and replated in 75 cm^2 flasks in 15 ml medium containing 5 mM bromodeoxyuridine (Sigma Chemical Co., St. Louis, MO) at a density of 5×10^5 cells per flask. The flasks were incubated at 37°C in 5% CO_2/95% air in the dark for 48 hr. Four hours before harvesting the cells, 0.1 mg/ml colcemid (GIBCO BRL, Grand Island, NY) was added to the flasks. The cells were collected by trypsinization, suspended in hypotonic potassium chloride (0.075 M) for 15 min at room temperature, and then fixed in 3:1 methanol–glacial acetic acid overnight. Fixed cells were dropped onto cold, wet glass slides and then stained for SCE analyses using the fluorescence-plus-Giemsa staining method (*31,32*).

The numbers of SCE per cell were scored blindly for each sample cell population, and data from 50 to 60 metaphase cells per sample were obtained. It should be pointed out that SCE occur even in unirradiated cells, and baseline levels can vary from experiment to experiment for presently unclear reasons. Therefore, the experiments had matched positive and negative controls. For statistical comparisons, Student's *t*-test and analysis of variance were performed (*33*) where indicated. Probability values less than 0.05 were considered to denote significant differences. All results described herein are representative of data obtained from two or more replicate experiments. Values shown in the figures represent means ± SEM.

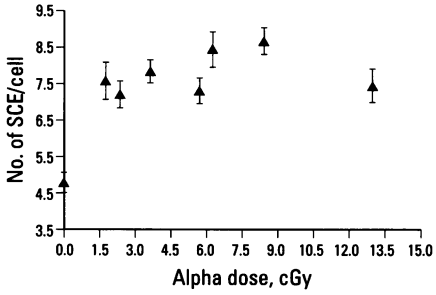

Figure 1. Number of SCE per cell after sham-irradiation (0 cGy) and exposure to α-particles at doses ranging from 1.8 to 12.9 cGy (*25*).

Results

Occurrence of Excessive SCE in Response to α-Irradiation

As we have described previously (*25*), the numbers of SCE per cell that occur in HFL1 are increased significantly after exposure to α-particles administered over low doses ranging from 1.8 to 12.9 cGy (Figure 1), with little indication of a dose response within this range. The percentages of cells that show excessive SCE over this dose range are also closely similar (Table 1). These collective findings, in conjunction with nuclear and whole cell hit information obtained from nuclear and whole-cell morphometry, α-particle dose and fluence, and target theory (*25*), suggest that the mechanism by which α-particles induce excessive SCE in HFL1 becomes maximally operational at low doses in a manner that is independent of nuclear or maybe even whole cell hits (Table 1).

SCE-inducing Extracellular Factor(s) after Exposure to α-Particles

Based on the previous findings, an experiment was performed to determine whether

Table 1. Percentages of HFL1 that received one or more nuclear and whole-cell hits by α-particles compared to the percentages of cells that showed excessive SCE.

Alpha dose, cGy	Cells receiving one or more nuclear hits, %	Cells receiving one or more whole-cell hits, %	Percentage of cells showing excessive SCE, %
1.8	12.1	59.3	36.4
1.9	13.4	63.3	45.0
2.3	15.9	69.8	44.6
3.6	23.4	84.2	47.4
5.7	34.3	94.6	37.7
6.2	36.9	95.9	53.2
8.4	46.2	98.6	56.4
12.9	61.5	99.9	38.1

α-particles can induce the generation of extracellular factors that may mediate DNA alterations. With all but residual culture medium removed from the Mylar dishes, confluent HFL1 were irradiated with 8.4 cGy of α-particles, and the original culture medium was immediately added back to the dishes. The dishes were placed in an incubator, and, either immediately or at intervals of 0.5, 2, and 24 hr after exposure, the medium from the cells was transferred onto other unirradiated, confluent HFL1, also grown on Mylar dishes. After 24 hr of incubation following the media transfers, these unirradiated cells were harvested and further cultured for SCE analysis, as described earlier. Control cells received medium that was obtained from sham-irradiated HFL1 at the same time intervals as above. A significant increase in the mean number of SCE per cell above control levels ($p < 0.001$, t-test) was observed with the cell supernatants that were obtained immediately after exposure to the α-particles (Figure 2). Moreover, the presence of SCE-inducing activity continued to be present in cell supernatants that were harvested up to 24 hr after irradiation. Notably, the levels of excessive SCE induced with the cell supernatants at all postexposure times were similar to those observed with cells that were actually exposed to the α-particles (Figure 1). These results are consistent with the possibility that an SCE-inducing factor was generated from the HFL1 essentially immediately after exposure to α-particles, and that such a factor may be stable up to 24 hr after exposure. The results, however, are also consistent with the possibility that SCE-inducing factor(s) were generated by α-particles interacting with one or more components in the residual culture medium that remained in the Mylar dishes at the time of exposure.

Further Analyses of SCE-inducing Extracellular Factor(s) after Exposure to α-Particles

To assess for SCE-inducing factor(s) that may be generated by interactions of α-particles with culture medium constituents alone, medium removed from untreated, confluent HFL1 (~3 ml/dish) was transferred to blank, cell-free Mylardishes and then irradiated with 8.4 cGy of α-particles. After the irradiations, the α-irradiated medium was immediately added to untreated, confluent HFL1 or placed into an incubator until it was added to other untreated HFL1 at 0.5, 2, and 24 hr after the medium was initially irradiated. Control samples consisted of medium obtained from unirradiated cells. After the media transfers, the unirradiated cells were incubated for 24 hr, harvested, and further cultured and processed for SCE analysis. In parallel with this experiment, we additionally repeated the previous experiment summarized in Figure 2.

Compared to unirradiated medium, the SCE-inducing activities of irradiated medium alone and medium obtained from irradiated cells were equivalently present ($p < 0.001$) when these media were placed on untreated HFL1 immediately after exposure to the α-particles (Figure 3). However, unlike with medium obtained from irradiated cells at subsequent postexposure times, significant increases in the number of SCE per cell ceased when the irradiated medium was maintained in an incubator for 30 min or longer before being added to otherwise untreated cells. Hence, exposure of culture medium to α-particles in the absence of cells can result in the generation of a relatively short-lived SCE-inducing factor(s), whereas exposure of cells along with residual medium present at the time of exposure results in the production of SCE-inducing factor(s) that may be cell-derived and possibly longer lived. Importantly, both factors when actively present are capable of causing excessive SCE at levels comparable to those observed when cells are exposed to an identical dose of α-particles.

In another set of experiments, we set out to obtain more direct evidence for a cellular origin for SCE-inducing factor(s) in response to the α-particles. Confluent HFL1 were exposed to 8.4 cGy of α-particles after removal of all but residual culture medium from the dishes, as before. Immediately after the irradiations, the culture medium that had been removed from the dishes (~3 ml/dish) before the exposure was used to wash (2 times) the irradiated cells. The recovered wash fluid was then immediately transferred onto unirradiated, confluent HFL1, and the cells were incubated for 24 hr before further subculturing and processing for SCE analysis. SCE-inducing activity observed in the wash fluid presumably would be representative of factors generated in the residual medium during irradiation and possibly cell-derived SCE-inducing factor(s). The dishes with cells that had been originally exposed to the α-particles and rinsed were refilled with unirradiated medium obtained from other, unirradiated confluent HFL1 cultures. These dishes were then incubated for 24 hr. Thereafter, the medium from these dishes was transferred onto still other unirradiated confluent HFL1, which were incubated for an additional 24 hr before subculturing and SCE analysis. Since previous experiments suggested that irradiated cells released a persistent factor into culture medium after exposure to α-particles, the presence of SCE-inducing activity observed in this latter transferred medium would serve both to confirm a cellular origin of SCE-inducing factor(s) and to demonstrate that its production by cells can occur after exposure as opposed to only during exposure to α-particles. Negative control cultures in this experiment received medium from sham-irradiated cells. Additionally, a routine positive control consisted of cells exposed to 8.4 cGy of α-particles. As

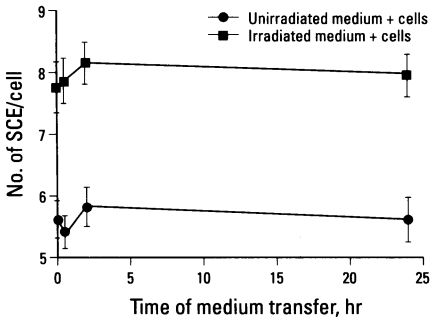

Figure 2. Numbers of SCE per cell observed after HFL1 were exposed for 24 hr to culture medium harvested at various times (immediately to 24 hr) from sham-irradiated HFL1 (0 cGy) and α-irradiated HFL1 (8.4 cGy).

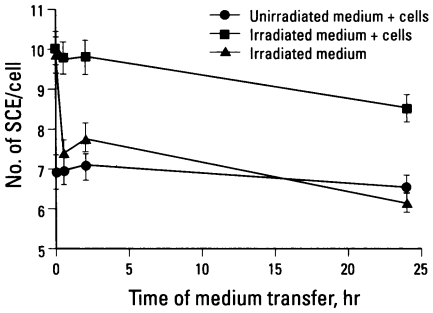

Figure 3. Number of SCE per cell observed after HFL1 were incubated for 24 hr in culture medium harvested at various times (immediately to 24 hr after exposure) from sham-irradiated (0 cGy) HFL1, α-irradiated (8.4 cGy) HFL1, or α-irradiated (8.4 cGy) conditioned medium, i.e., medium that was exposed to α-particles after removal from untreated, confluent HFL1.

illustrated in Figure 4, cells that were immediately washed after exposure to the α-particles (Condition III), cells that received the immediate wash fluid (Condition IV), and those cells that received the subsequent postwash, 24-hr medium (Condition V) all showed significantly increased levels of mean SCE per cell relative to the negative controls (Condition I) ($p < 0.001$). Again, there were no significant differences between the elevated numbers of SCE per cell obtained with these sets of treatment conditions and the increased numbers of SCE per cell obtained with cells that were directly irradiated with the α-particles (Condition II, Figure 4). Such results indicate that cells can produce SCE-inducing factor(s) after exposure to α-particles, and that exposure of unirradiated cells to factor(s) present in the residual medium atop irradiated cells can transferrably induce the production of SCE-inducing factor(s) from the unirradiated cells.

Culture Medium Constituents and α-Particle-induced SCE Activity

Subsequent experiments were performed to narrow the source of the short-lived SCE-inducing activity present in irradiated culture medium. Conceivably, serum components in the medium may serve as the source of a SCE-inducing short-lived reactive species, e.g., reactive lipid peroxidation products, that may be generated directly upon interaction with α-particles or indirectly after reaction with other radiolytic radical products (*34–37*). Additionally, because our previous investigations of the SCE-inducing effects of α-irradiated medium used conditioned media harvested from confluent HFL1 and thereby may have contained some detached cells or cell-derived components that potentially could have contributed to our results, we performed these experiments with the inclusion of fresh media preparations. Conditioned or freshly prepared MEM containing 10% FBS and MEM without serum samples were separately irradiated at 8.4 cGy in cell-free Mylar dishes. Immediately after irradiation, the media were transferred to dishes containing confluently growing HFL1 after the removal of their medium. The cells were then incubated for 24 hr, harvested, and subcultured for SCE analysis. Other cell cultures treated with sham-irradiated MEM–10% FBS served as negative controls. Incubation of HFL1 in α-irradiated medium lacking serum resulted in no significant increase in the numbers of SCE per cell beyond control, baseline levels (Figure 5, Condition IV vs Condition I). However, an unequivocal increase in SCE relative to control values occurred when serum was included as a component of the α-irradiated fresh or conditioned media samples (Conditions II and III). Hence, the SCE-inducing activity caused by exposing culture medium to α-particles appears to involve direct interactions of serum components with α-particles and/or indirect interactions between serum components and α-particle-induced reactive radiolytic products such as hydroxyl radicals. Moreover, these results showed that detached cells or other cell-derived constituents that may have been present in cell-conditioned media used in our previous experiments were not required for the occurrences of excessive SCE initiated by exposure to the α-particles.

Stability of the Cell-derived SCE-inducing Factors(s)

A series of experiments was undertaken to obtain some preliminary information about the stability of the cell-derived SCE-inducing factor(s). After the removal of residual medium, confluent HFL1 were irradiated at 8.4 cGy, their medium was replaced, and the cells were incubated with the medium for 8 hr. Then the medium was removed from the dishes and frozen at –20°C for 16 hr. The medium was then thawed, transferred onto confluent, unirradiated HFL1, and the cells were incubated for an additional 24 hr. The HFL1 were then further processed for SCE analysis. Two types of negative controls were used in this experiment (Figure 6). One set of controls consisted of treating HFL1 with

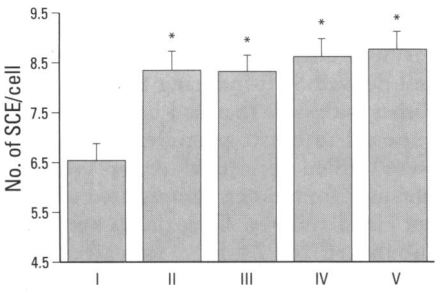

Figure 4. SCE-inducing activities in media obtained from sham-irradiated (0 cGy) and α-irradiated (8.4 cGy) cells or media. (I) Number of SCE per cell obtained after HFL1 were incubated for 24 hr with sham-irradiated medium (negative control). (II) Number of SCE per cell obtained with α-irradiated (8.4 cGy) HFL1 (positive control). (III) Number of SCE per cell obtained with α-irradiated (8.4 cGy) HFL1 that were rinsed twice with normal conditioned medium and subsequently maintained in unirradiated conditioned medium. The medium used to rinse the irradiated HFL1 was used for Condition IV. Supernatants finally harvested from these cells (24 hr after washing and incubation in unirradiated medium) were used for Condition V. (IV) Number of SCE obtained after untreated HFL1 were treated with the initial rinse fluid from Condition III. (V) Number of SCE per cell obtained after HFL1 were treated with conditioned medium that was removed 24 hr from cells used in Condition III. *Significantly higher than Condition I.

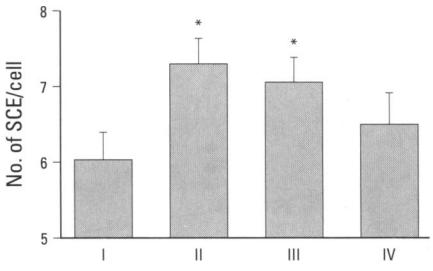

Figure 5. Culture medium composition and SCE-inducing activity after exposure to α-particles. (I) HFL1 cultured for 24 hr in sham-irradiated medium (MEM with 10% FBS), i.e., negative control. (II) HFL1 cultured for 24 hr in α-irradiated (8.4 cGy) conditioned medium that was transferred onto the cells immediately after they were irradiated, i.e., positive control. (III) HFL1 cultured for 24 hr in α-irradiated, fresh, unconditioned medium containing FBS; the medium was added to the cells immediately after they were exposed to α-particles. (IV) HFL1 cultured for 24 hr in α-irradiated, fresh, unconditioned medium lacking FBS; the medium was added to the cells immediately after they were exposed to α-particles.

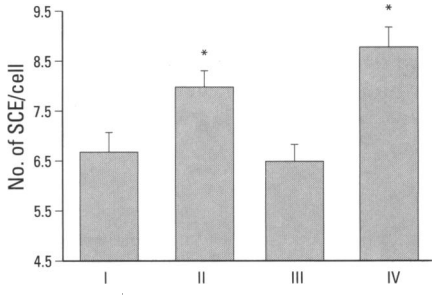

Figure 6. SCE-inducing activities in media from α-irradiated and sham-irradiated HFL1, and in irradiated medium after freeze–thaw. The media were harvested 8 hr after the exposures and subjected to one freeze–thaw cycle where indicated. (I) SCE per cell obtained from HFL1 exposed to freeze-thaw conditioned medium from sham-irradiated cells. (II) SCE per-cell obtained from α-irradiated HFL1 (positive control, no freeze–thaw). (III) SCE per cell obtained from HFL1 exposed to α-irradiated conditioned medium alone (i.e., no cells present at the time of irradiation) that had been subjected to a freeze–thaw cycle; this medium was also obtained 8 hr after α-irradiation. *Significantly higher than Conditions I and III.

medium from sham-irradiated cells that also had been subjected to one freeze–thaw cycle (Condition I). For the other negative control, medium was removed from confluent HFL1; it was exposed to α-particles (8.4 cGy) in cell-free Mylar dishes and maintained in an incubator for 8 hr before freeze–thaw and subsequent incubation with HFL1 (Condition III). A positive control for the experiment consisted of confluent HFL1 that were irradiated with 8.4 cGy of α-particles followed by 24 hr of incubation before further processing for SCE analysis (Condition II). The SCE-inducing activity in medium obtained from irradiated cells was found to have survived freeze-thaw treatment (Condition IV), and, consistent with previous results, i.e., the SCE-inducing activity in irradiated medium is short-lived, i.e., no excessive SCE occurred when the HFL1 were treated with medium that had been irradiated in the absence of cells prior to freeze–thaw (Condition III).

In other analyses, we also have obtained some information about the lability of the cell-derived SCE-inducing factor(s) upon heating (Figure 7). The SCE-inducing activity in medium obtained from HFL1 8 hr after α-irradiation continued to be present after heating the medium at 56°C for 30 min. Heating the medium to 93°C (boiling point of water at the altitude of Los Alamos) for 1 min, however, results in a loss of activity (Condition II).

Discussion

High-LET α-particles such as those emitted by inhaled radon/radon progeny cause cytogenetic alterations in the form of SCE by a mechanism(s) that does not solely involve direct interactions of α-particles with cell nuclei (*25,26*). Indeed, in our previous study (*25*) we found no clear evidence with normal human cells for any role of nuclear traversals by α-particles in mediating the induction of SCE. In the present investigation, we have found that a relatively low dose of α-particles results in the generation of extracellular factors, which, upon transfer to unexposed normal human cells, can cause alterations in DNA in the form of excessive SCE.

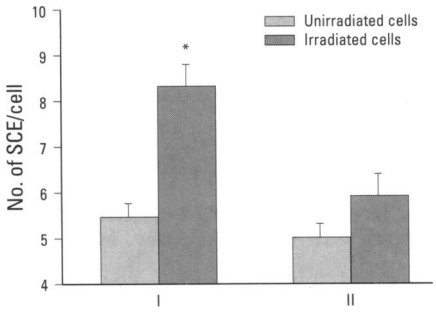

Figure 7. SCE-inducing activities in media from α-irradiated (8.4 cGy) and sham-irradiated HFL1 after heat treatment. (I) Number of SCE per cell obtained from cells exposed to conditioned medium from sham-irradiated and α-irradiated HFL1 after heating at 56°C for 30 min. (II) Number of SCE per cell obtained from HFL1 exposed to conditioned medium from sham-irradiated and α-irradiated HFL1 after heating at 93°C. *Significantly higher than unirradiated control condition.

One source for α-particle-induced factor(s) is serum-containing culture medium, whether harvested as conditioned medium from cells or freshly prepared. Of significance, the extent of excessive SCE induction by the medium-derived factor(s) is quantitatively indistinguishable from that produced when cells are directly exposed to the same dose of the α-particles. Our findings have further indicated that the active specie(s) in the medium is short-lived, and it requires the presence of one or more constituents in serum. While the medium-derived factor(s) has yet to be identified, the short-lived nature of the SCE-inducing activity in irradiated medium suggests a process involving free radicals. It is tempting to speculate that such a factor or factors similarly may be generated in the lung upon interactions between α-particles emitted by radon and radon progeny and airway lining fluids that, like serum, also contain a rich mixture of chemical species.

A second source of extracellularly present SCE-inducing factor(s) observed in our study is derived from α-irradiated cells. As with the medium-derived factor(s), the addition of supernatants from α-exposed cells to unirradiated cells induces SCE to a level that is virtually identical to that observed when cells are directly irradiated. The cell-derived factor(s), however, distinctly differs from the medium-derived factor(s) in several ways. First, the activity of the medium-derived factor(s) diminishes shortly after exposure to α -particles, whereas SCE-inducing activity remains persistently present in cell supernatants for at least up to 24 hr after exposure. Second, the medium-derived factor(s) is produced essentially immediately upon exposure to the α-particles, whereas the cell-derived factor(s) can be produced after exposure to the α-particles. And, unlike the short-lived medium-derived factor(s), the SCE-inducing activity in the cell supernatants survives freeze–thaw and is heat labile in a manner that suggests the possibility that the factor(s) may be proteinaceous in nature.

That extracellular mediators can cause DNA damage is deduced from several observations. For example, conditioned media from cells from subjects with the cancer-prone disorders ataxia–telangiectasia, Bloom's syndrome, and Fanconi's anemia cause chromosomal aberrations and increases in SCE (*38,39*). Clastogenic activity also has been observed in the plasma of individuals after they were accidentally exposed to very high, whole-body doses of ionizing radiation and after therapeutic exposure to X-rays (*40–44*). Such activity additionally has been observed in plasma that was irradiated *in vitro* (*45*). Various chemical species have been proposed to function as clastogenic factors, e.g., aldehydic breakdown products of lipid peroxidation, tumor necrosis factor alpha, and inosine nucleotides (*36,46,47*); however, aside from being associated with superoxide anions because they often can be inhibited by superoxide dismutase (*36*), the exact identification of such factors at play in the above conditions as well as in response to exposure to α-particles remains to be determined.

Finally, how the findings of our study may ultimately extend to the *in vivo* condition remains to be determined. Numerous microdosimetric models, including the recent Human Respiratory Tract Model for Radiological Protection (*48*), have been developed for assessing α-radiation doses to sensitive airway cells in the lower respiratory tract and for estimating cancer risks due to the inhalation of radon and radon progeny (*49–52*). An underlying assumption shared by these models is that traversals of α-particles through the nuclei of target cells, e.g., basal and secretory cells, along the conducting airways alone are of primary concern in terms of being cancer causing. The results from our investigation suggest that an initiating target for α-particle-induced genetic changes can be larger than cell nuclei or even whole cells. With regard to inhaled radon/radon progeny and other α-emitters, accordingly, it remains possible that diffusible factors like those observed here *in vitro* may also communicably play a mechanistic role in α-particle-induced carcinogenesis in the respiratory tract because of the initial interactions of internally deposited a-particles with airway lining and other lung fluids and cells. *In vivo* support for such a transmissible

mechanism, however, is presently limited. Nagarkatti and co-workers (*53*), for example, have recently reported that inhalation exposure of mice to radon can cause alterations in extrapulmonary splenic, thymic, and peripheral lymph nodal lymphocytic subpopulations. Such findings are consistent with the possibility that exposure to α-particles can result in the generation of biologically active, diffusible products that can affect unirradiated cells.

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