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TERMINAL DIFFERENTIATION OF HUMAN FIBROBLASTS IS INDUCED BY RADIATION

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Abstract

In order to analyze the effect of various kinds of radiation on the terminal differentiation processes of fibroblasts in culture, both human skin and lung fibroblasts were irradiated with electromagnetic non ionizing as well as ionizing radiation in clonal and sparse mass culture systems. As analyzed by cell biological (cell type frequencies), biochemical (collagen synthesis) and molecular markers (expression of protein PIVa) human skin and lung fibroblasts are induced to differentiate prematurely into terminal postmitotic cells. Thus, both electromagnetic and ionizing radiation induce terminal differentiation in cultured cells. These data add some new aspects for the interpretation of radiation effects on cells, e.g., in clinical therapy, as well as for the development of normal tissue responses during early and late effects after radiotherapy.

Key Words: Terminal Differentiation, Cultured Fibroblasts, Electromagnetic Field, Ionizing Radiation, Collagen Synthesis.

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Introduction

It has been shown for many years that various kinds of radiation (ionizing, non-ionizing, and electromagnetic field) exert severe effects on cells *in vivo* and *in vitro* which can result in significant biological distortions and physiological alterations. Due to lesions at the DNA-level resulting from the cells exposure to ionizing, e.g., gamma-radiation or UV-light the cell's ability to reproduce is affected and cell death is the ultimate consequence. This consequence represents the basis for the various forms of radiation treatment during radiotherapy of cancer. At present, however, it remains unclear to what extent ionizing radiation causes the so called reproductive cell death by inducing terminal differentiation processes. If terminal differentiation of cells, especially tumor cells, could be achieved by ionizing radiation this would offer some interesting new aspects for developments in radiotherapy.

Over the recent years the effects of electric fields and low frequency electromagnetic radiation has had increasing impact on some forms of bone fracture treatments (2, 6, 7). It could be demonstrated that the behavior of several cell systems *in vitro* are affected by electric fields and/or electromagnetic radiation (4, 8-10, 16, 18). However, despite some speculative hypothesis (see ref. 1) the cellular and molecular biological mechanism(s) of these effects of electromagnetic radiation are still unclear.

In the present study we used human skin and lung fibroblasts to analyze the effects of electromagnetic and ionizing radiation on the differentiation pattern and the expression of molecular markers of these cells. We will provide evidence that both electromagnetic and ionizing radiation induce the terminal differentiation of cells with high mitotic activity into irreversible postmitotic cells which in terms of their physiological properties are quite comparable to the postmitotic cells developing spontaneously *in vivo* and *in vitro*.

Materials and Methods

Cell Cultures

Normal human skin (cell strain HH4) and lung (cell strain WI38) fibroblasts were cultured in Dulbecco's modified Eagles (DME) medium supplemented with 10% fetal calf serum (fcs) and standard amounts of antibiotics (3). At each transfer the cell number was determined and for routine subcultures cells were seeded at a constant density of 2 x 10^4 cells per cm^2. At each passage the cumulative number of population doublings (CPD) were determined. For all radiation experiments the cell lines used were at the CPD level 28-32.

Electromagnetic radiation

A Magnetodyn function generator (W. Kraus, Institut für Medizinische Physik, München, FRG) was used to drive six solenoid coils, which generated the electromagnetic field. Each coil could take up six cell culture petri dishes as it was described in detail elsewhere (16). The electromagnetic wave form applied was a continuously sinusoidal bipolar wave with a frequency of 20 Hz and a maximum magnetic induction of 8.4 mT. The distortion factor of this signal shape was less than 1%, as measured by gaussmeter Bell 640 and a spectrum analyzer HP3582A. The coils were installed in a humidified 95% air: 5% CO_2 incubator at 37°C. In order to prevent an increase in the incubation temperature due to the heat production of the electromagnetic coils, the coils were equipped with a water cooling system, which resulted in a constant incubation temperature of 37 ± 0.3°C.

Cells (WI38) were seeded at densities 1 x 10^3 cells per cm^2 and incubated without further subcultivation for 21 days in the presence or absence of low frequency electromagnetic field (EMF) radiation (sinusoidal, biphasic, 2 x 6 hours per day) as described above and elsewhere (16). The average intensity of the EMF was 6 mT. After 7, 14, and 21 days of incubation the total cell number, the cell type frequencies (see below) and biochemical parameters, i.e., total collagen synthesis and ^{35}S-methionine polypeptide pattern, were analyzed in the control and irradiated cell populations.

Ionizing radiation

Human skin fibroblasts (HH4) were seeded at a density of 50 cells per cm^2 and incubated for 2 days in DME medium supplemented with 20% fcs. Cells were then irradiated with various doses (0-7 Gray, Gy) of ionizing radiation generated by a ^{137}Cs-source. At various time points after irradiation parallel cell cultures were analyzed either for surviving colonies (> 50 cells) or for the frequencies of the various fibroblast cell types (see below).

Table 1: Cell type specific proliferation potential of the mitotically active progenitor cells of the fibroblast cell system.

	Average Proliferation Potential (PD)	Average Doubling Time (hours)
MFI	24 ± 7	27 ± 5
MFII	17 ± 5	30 ± 6
MFIII	5 ± 2	43 ± 5

The average proliferation potential, i.e., the number of population doublings (PD) each cell can go through before differentiating into the next cell type, i.e., MFI to MFII and MFII to MFIII, has been analyzed in subcloning experiments of pure clones types. The average doubling time of each cell type was calculated from the increase in cell number of the corresponding clonal populations between day 3 and 7 of logarithmic growth. Numbers shown represent the mean ± SD (n = 12) using the human skin fibroblast cell strain HH4. Similar results have been obtained using the human lung fibroblast cell strain WI38 (Rodemann, unpublished data).

Cell Type Frequencies

At the time points indicated, 3-5 parallel cultures of control and irradiated cells were washed with phosphate buffered saline and subsequently fixed with 3.7% paraformaldehyde and 70% ethanol (10 min each). Cells were then stained with Coomassie and Giemsa solutions as described elsewhere (3, 11, 15). For determination of the cell type frequencies of the three potentially mitotic fibroblast cell types MFI, MFII, and MFIII and the postmitotic fibroblasts PMFIV, PMFV, and PMFVI (in the data shown taken together as PMF-cells) in the experiments using electromagnetic fields at least 2000 cells in three independent series of experiments were classified according to morphological criteria recently described (3, 11, 15). In the experiments using ionizing radiation, at least 500 cells and 500 clonal colonies were classified according to morphological criteria. In all experiments the percentage of death cells was tested by the fluorescein diacetate/ethidium bromide assay described elsewhere (16).

Collagen Synthesis and ^{35}S-Methionine Polypeptide Pattern

Control and irradiated (EMF) cells were labelled at day 7, 14 and 21 of the experiment with 10 μCi/ml of ^{3}H-proline (40 Ci/mmol) in serum free medium supplemented with sodium-ascorbate and β-amino-proprionitrile (0.1 mg/ml each) for 18 hours as described in detail elsewhere (13). Incorporation of ^{3}H-proline into total pepsin-resistant material consisting, at least, of 80-85% collagen was analyzed according to standard procedures (13).

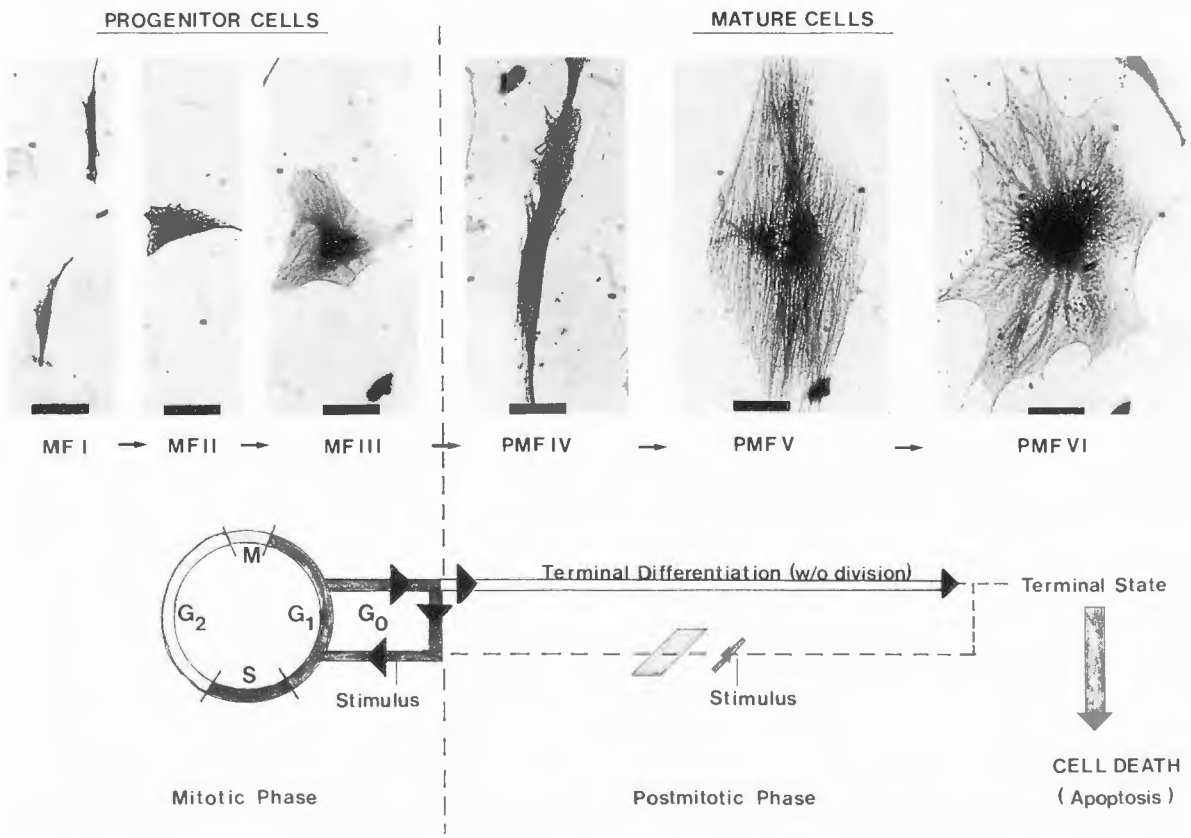

Figure 1. Differentiation lineage of human fibroblasts in culture. The mitotically active progenitor fibroblasts and the postmitotic and maturing fibroblasts differentiate along the sequence indicated by arrows: MFI ⟶ MFII ⟶ MFIII ⟶ PMFIV ⟶ PMFV ⟶ PMFVI. Quiescent MF-type cells arrested in G_1 of the cell cycle can be stimulated by growth factors and/or serum; postmitotic fibroblasts cannot be stimulated to reenter the cell cycle. Pictures are taken from sparse mass cultures of the human skin fibroblast cell strain HH4. The typical morphology of the various potentially mitotical active and irreversible postmitotic fibroblast cell types is shown. Quiescent fibroblasts of the various cell types are of the same morphology. Similar data can be demonstrated for the human lung fibroblast cell strain WI38. M, G_1, G_2, and S are abbreviations of the cell cycle phases: M, mitosis; G_1, gap 1; G_2, gap 2; S, phase of DNA-synthesis. Bar: 75 μm.

Control and irradiated cells were labelled at day 7, 14 and 21 of the experiments with 200 μCi/ml of ^{35}S-methionine (400 Ci/mmol) in methionine-free DME supplemented with 1 μg/ml non-radioactive l-methionine and 10% fcs for 18 hours as recently described (11, 12, 14). Aliqouts of the lysed cell samples containing 500.000 cpm were analyzed by high resolution 2-D-gel electrophoresis (11, 12).

Results

Normal terminal differentiation of the fibroblast cell system

In vitro cultured human skin or lung fibroblasts are heterogeneous populations of potentially mitotic and postmitotic fibroblast cell types (Fig. 1). In the mitotically active fibroblast compartment three cell types MFI, MFII, and MFIII can be distinguished by their morphology, proliferation potential and the expression of cell type specific marker proteins (3, 11, 15). MFI is a small spindle shape cell with a proliferation potential of 20-25 population doublings as analyzed by subcloning experiments (Fig. 1; Table 1). MFI differentiates into the cell type MFII, a fibroblast of epithelioid morphology characterized by a proliferation potential of about 15 CPD (Table 1). The cell MFII comprises the main cell type present in *in vitro* cultured fibroblast populations between CPD level 5-35 (data not shown). MFII differentiates into cell type MFIII, the last mitotically active fibroblast with a pleiomorphic morphology. This cell is characterized by a very reduced proliferation potential of 3-7 CPD (Table 1). After exhaustion of its proliferation potential MFIII then spontaneously differentiates into PMFIV, the first irreversible postmitotic fibroblast (Fig.

1). PMFIV is a very elongated cell with high contractile activity. In culture this cell type is present for 7-12 days and then differentiates into the postmitotic fibroblast PMFV. PMFV is a transition cell type present in culture for only a very short time (3-4) days which then differentiates into the terminal mature cell of the fibroblast cell system, the postmitotic fibroblast PMFVI. This cell is characterized by a large pleiomorphic morphology and an *in vitro* life span of 10-30 weeks. Thus, the fibroblast cell system can be divided into the mitotically active progenitor cell compartment with the cell types MFI, MFII, and MFIII, and into the mature and postmitotic cell compartment with the cell types PMFIV, PMFV, and PMFVI. Quiescent cells of the MF-types, which are identical to proliferating cells in terms of morphology and expression of cell type specific proteins (3, 11), can be stimulated by growth factors to undergo cell divisions whereas PMF cells cannot (Rodemann *et al.* 1991, in preparation). For all of the mitotically active and postmitotic cell types of the terminal fibroblast cell lineage specific intracellular proteins have been described which can be used as differentiation markers (11).

Electromagnetic Field Radiation

The frequencies of the various mitotically active and postmitotic cell types of the fibroblast cell system were analyzed in control and irradiated cell populations after various time of incubation. Fig. 2 A-D shows that normal human lung fibroblasts of the cell line Wi38 which initially comprised 84.6% MFII type cells differentiate upon the exposure to electromagnetic radiation into cell populations predominantly (approximately 81.6%) made up of postmitotic cells (PMF-cells) after 21 days of irradiation. In the non-irradiated control cultures no significant change in the differentiation pattern could be observed after 21 days of culture. As can be judged by the increase in cell number the induction of the terminal differentiation into PMF-type cells by EMF radiation requires approximately 1-2 cell divisions. During the experimental period of 21 days the increase in cell number of the EMF-irradiated cell populations is about 23 fold (maximum cell density $1.5 \pm 0.2 \times 10^5$ cells per dish; data not shown), whereas the cell number of the control cultures increased by about 2-3 fold (maximum cell density $9 \pm 1.1 \times 10^5$ cells per dish; data not shown).

As demonstrated by the analysis of total collagen synthesis (Fig. 3), the EMF-radiation induced postmitotic cells synthesize up to 13x more total collagen as the non-irradiated controls. This reflects the differentiation dependent increase in the synthetic capacity for collagen types I and III in PMFVI type cells. Non-irradiated control cells showed a significant decrease in total collagen synthesis reflecting the density-dependent down regulation of this process (Fig. 3) (16).

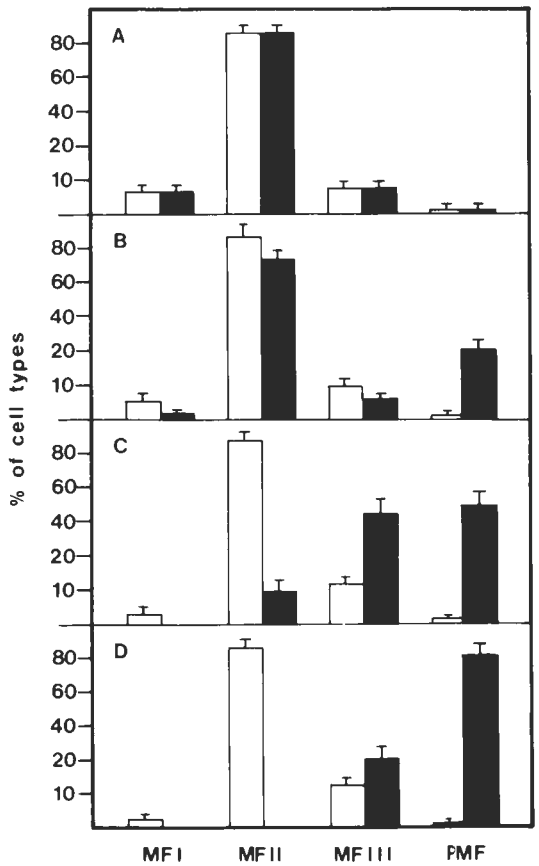

Figure 2. Frequencies of mitotic (MF) and postmitotic (PMF) human lung fibroblast cell types after electromagnetic irradiation. Human lung fibroblasts (cell strain WI38) were seeded at a constant density of 1×10^4 cells per cm^2 and incubated with (closed bars) and without (open bars) exposure to electromagnetic field radiation (sinusoidal, biphasic, 6 mT) for 21 additional days. At 7 days intervals (A = day 0; B = day 7; C = day 14; D = day 21) at least 4 parallel cultures were analyzed for cell type frequencies by classifying at least 2000 cells according to morphological criteria (3, 11, 15). Three independent series of experiments were performed.

Reflecting the time-dependent increase in the amount of PMF-type cells in the EMF-irradiated cell populations, these cultures show a significant increase in the expression of protein PIVa as analyzed by 2-D-PAGE and computerized video densitometry (Fig. 4) (12). This protein has recently been described to be specific for spontaneously or experimentally induced PMF-type cells of human fibroblasts (11).

Ionizing radiation

As demonstrated by Fig. 5 human skin and lung fibroblasts show significant differences in the sensitivity

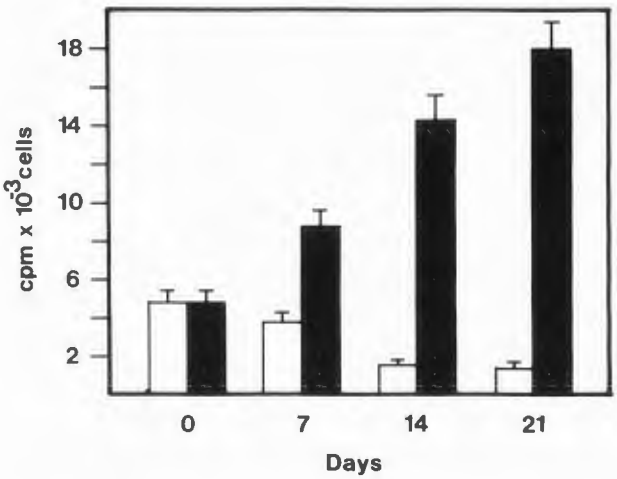

Figure 3. Overall collagen synthesis in human lung fibroblasts (cell strain WI38) after various time points of electromagnetic irradiation. Human lung fibroblasts (cell strain WI38) were labelled as described in Methods. Radioactivity (cpm) of ^{3}H-proline incorporated into pepsin resistant protein consisting of at least 80-85% total collagen was correlated to the cell number determined from parallel cultures. Numbers shown represent the mean of three independent series of experiments (three parallel cultures in each series). Open bars: control cultures; closed bars: irradiated cultures.

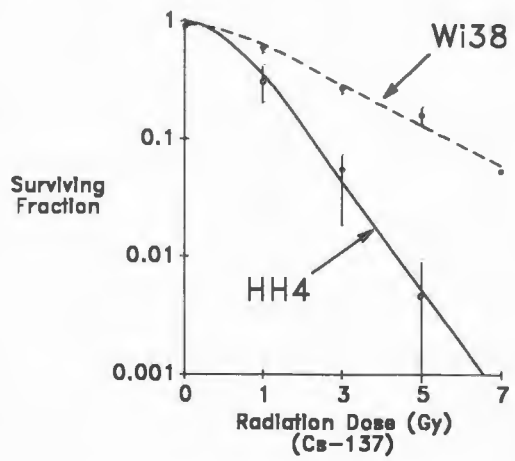

Figure 5. Survival curves of human skin and lung fibroblasts following ionizing radiation. Human skin (cell strain HH4) and lung (cell strain WI38) fibroblasts were seeded as described in Methods and irradiated with the radiation doses indicated. The surviving cell fraction was determined by counting cell colonies bigger than 50 cells 8 days after irradiation. Numbers shown represent the mean ± standard error of mean (SEM) of three parallel cultures.

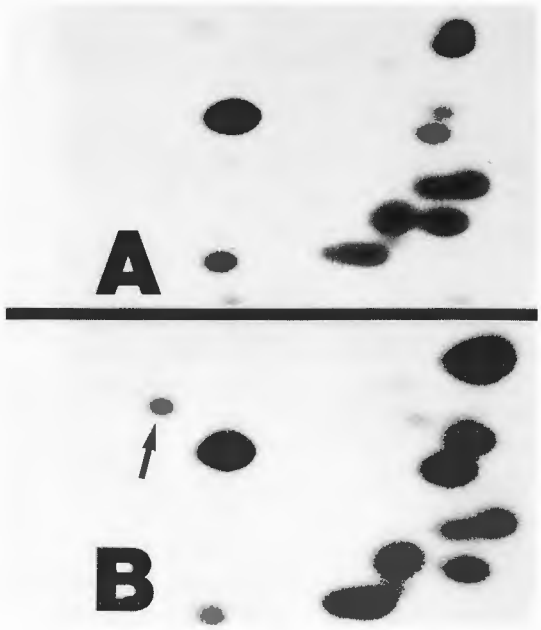

Figure 4: Expression of protein PIVa in postmitotic human lung fibroblasts (cell strain WI38) induced by electromagnetic radiation. The expression of protein PIVa was analyzed by high resolution 2-D-gel electrophoresis. Fluorograms of the 2-D gels were quantified by computerized video-densitometry (11, 12, 14). Fluorograms shown are of the control cells (4A; 21 days of incubation) and of irradiated cells (4B; 21 days of incubation). Arrow head indicates protein PIVa (M_r 33 kDa; P_i 5.0). Acidic end to the right.

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to ionizing radiation. For both cell types the D_O ranges from 1-2 Gy. In order to determine whether this survival curve of human fibroblasts reflects mitotic cell death or rather the induction of terminal differentiation due to ionizing radiation, the frequencies of the various mitotically active MF- and the postmitotic PMF-cell types of the human skin fibroblast cell strain HH4 were analyzed 12 days after irradiating the cells with 1 and 7 Gy, respectively. As shown in Fig. 6 A-D, both radiation doses 1 and 7 Gy induce significantly the terminal differentiation process towards PMF-cell types within 12 days of post-radiation incubation. When the frequencies of the mitotic cell types (MFI/MFII and MFIII) were analyzed in the radiation dose of 1 Gy small amounts of MFI/MFII types cells and fairly high amounts of MFIII type cells were present 12 days post irradiation (Fig. 6C). In the same time period, at the radiation dose of 7 Gy only very low amounts of MFIII type cells were present (Fig. 6D). With both radiation doses the predominant cell types present 12 days after radiation were the PMF-cells (Figs. 6C, 6D). As can be judged from

the total number of cells present 10 days after radiation the amount of cell death was only between 20% (7 Gy) and 40% (1 Gy) (preliminary data). Thus, ionizing radiation at least in higher doses induces the terminal differentiation rather than mitotic cell death of the analyzed human skin fibroblast cell.

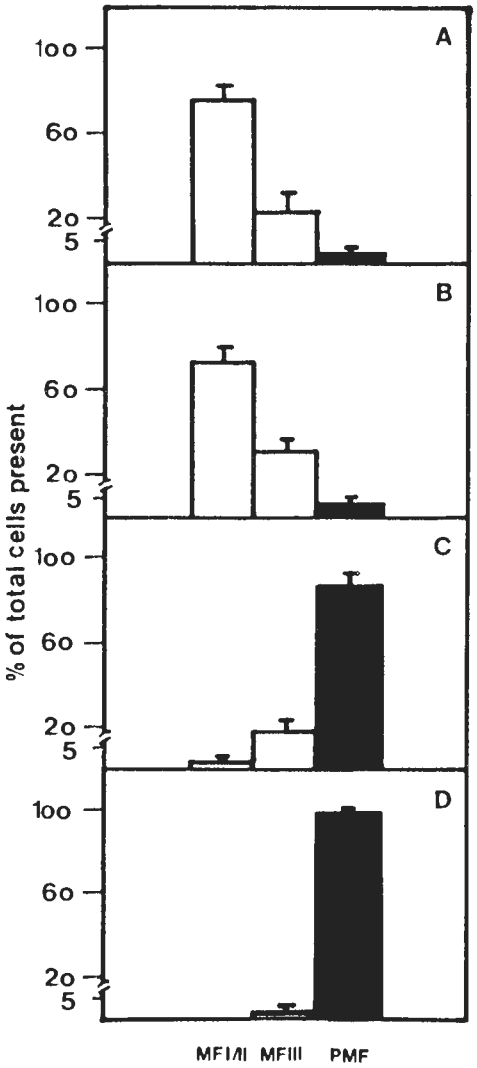
Discussion

The normal differentiation processes of both human skin and lung fibroblasts are characterized by the sequential appearance of three mitotic (MF) cell types in the progenitor compartment and three postmitotic (PMF) cell types in the maturing compartment (Fig. 1). Thus, the fibroblast cell systems differentiates along the terminal cell sequence: MFI-MFII-MFIII-PMFIV-PMFV-PMFVI (Fig. 1; ref. 3, 11, 15). The postmitotic fibroblast PMFVI represents the terminally differentiated and mature cell type of the fibroblast cell system which is most active in exerting the fibroblast specific functions, like synthesis of collagen and other extracellular matrix components (3, 15). For each cell type of the differentiating fibroblast cell system specific morphological criteria and marker proteins have been described (3, 11, 15), which, for example, can be used as markers in experiments analyzing the effects of radiation on the terminal differentiation process of these cells. One of these proteins is protein PIVa (M_r 33 kDa; P_i 5.0) (11). This protein has been postulated to be a regulatory protein, controlling the postmitotic differentiation state of fibroblasts (11). In ongoing experiments the message for PIVa-expression could be demonstrated by *in vitro*-translation experiments. Furthermore, protein PIVa could be induced in MFII type cells by micro-injection of RNA isolated from PMF-type cells (Rodemann *et al.*, unpublished data). Thus, the differentiation processes leading to terminally differentiated postmitotic fibroblasts is most likely genetically controlled and seems to be somehow related to the observable repression of the proto-oncogene c-fos in MFIII and PMF-type fibroblasts (17, Rodemann *et al.* unpublished data).

The results presented in this study support the idea that various kinds of radiation, e.g., electromagnetic and ionizing radiation at certain intensities and doses, induce terminal differentiation of fibroblast cultures predominantly made up of MFII-type cells into post mitotic fibroblast cultures predominantly composed of the cell type PMFVI as summarized in Fig. 7. At least in the case of electromagnetic radiation (sinusoidal, biphasic waveforms) of 6 mT intensity, which is routinely used for bone fracture healing in clinical therapy (6, 7), the induction of terminally differentiated, mature postmitotic fibroblasts correlates with the cell's ability of enhanced total collagen synthesis and the expression of the PMF-type specific protein PIVa. Similar results, indicating a stimulation of the synthesis of total collagen as a result of EMF-radiation, have been reported for short term exposure (7 days) of rabbit bone marrow fibroblasts by Farndale *et al.* (5).

In the present study ionizing radiation (in the dose range of 1 and 7 Gy) also induced terminal differentiation of human skin fibroblast populations mainly composed of MFII type cells into morphologically fully differentiated postmitotic populations of predominantly PMFVI-type cells. At present we are analyzing the expression of postmitotic differentiation markers, like protein PIVa, at the biochemical and molecular level. Interestingly, from the data available it can be speculated that ionizing radiation at low doses (e.g., 1 Gy) induces mitotic cell death at relatively higher rates than high radiation doses (e.g., 7 Gy). Simultaneously, as judged by the cell seeding efficiency, the frequencies of the various cell types present, and the total number of cells present 10 days after radiation with 1 and 7 Gy, respectively, terminal differentiation into PMF-type cells is more efficiently induced by the higher radiation dose. A possible explanation could be given by the mechanism assumed to be involved with radiation induced terminal differentiation (20, 21): Due to continuation of cytoplasmic growth during the mitotic cell cycle delay following exposures to ionizing radiation the cytoplasmic/nuclear mass ratio increases and triggers terminal differentiation. Since the cycle delay increases with dose, an increasing percentage of cells reaches terminal differentiation by molecular mechanisms yet unknown. Thus, the passage through mitosis and the expression of chromosomal injury are prevented and fewer cells die from mitotic cell death. This paradoxical reversion phenomenon has been observed in several cell systems in dose ranges between 2.5 and 50 Gy (20). Therefore, it was unexpected to be detectable between 1 and 7 Gy. In ongoing experiments, the relationship between mitotic cell death and terminal differentiation is studied in more detail.

Taken together, these experiments suggest that both electromagnetic and ionizing radiation induce terminal differentiation in the fibroblast cell system *in vitro*. The molecular mechanisms of the radiation induced terminal differentiation remain to be resolved. One possible mechanism could involve the modulation of Ca^{2+} channels. It could be demonstrated that intracellular Ca^{2+} levels as well as Ca^{2+} fluxes are sensitive to both electromagnetic and ionizing radiation (5, 19). This could in turn induce molecular events leading to the induction of the gene expression characteristic for terminally differentiated postmitotic cells (3, 11, 16, 17). However, studies by Cossarizza *et al.* (4) indicate that the effect of electromagnetic fields on lymphocyte proliferation is not due to the mobilization of intracellular free Ca^{2+} and



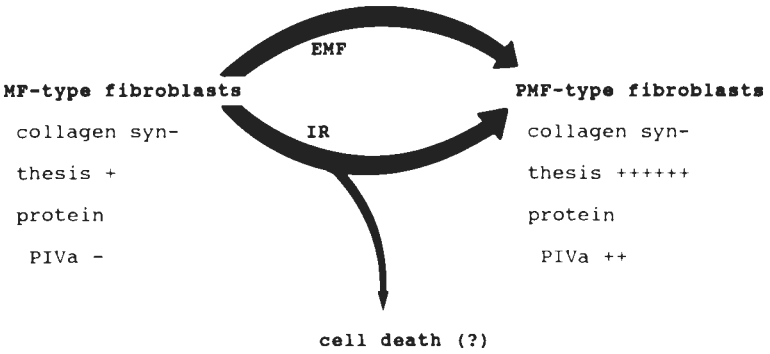


Figure 7 (above). Effects of electromagnetic (EMF) and ionizing (IR) radiation on human fibroblasts in culture.

Figure 6 (at left). Pattern of terminal differentiation of human skin fibroblasts induced by ionizing radiation. Human skin fibroblasts (cell strain HH4) were seeded at a density of 50 cells per cm_2; two days later cells were irradiated with 1 and 7 Gy, respectively. At day 12 the cell type frequencies of the irradiated cultures and non-irradiated controls were determined as described in Methods. 500 cells and 500 clonal colonies were classified according to morphological criteria (3, 11, 15). Numbers shown represent the mean ± SEM of three independent determinations in randomly chosen microscopic fields. **A**: control cultures at day 0 (day of irradiation); **B**: control cultures at day 10; **C**: irradiated (1 Gy) cell cultures at day 10 (after irradiation); **D**: irradiated (7 Gy) cell cultures at day 10 (after irradiation). Open bars: mitotically active cell types MFI/MFII and MFIII; closed bars: postmitotic cell types PMF.

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alterations of Ca^{2+} channel activities. Further molecular biological studies concerning intracellular signal transduction and gene activation mechanisms using the described terminally differentiating fibroblast cell system will shed light onto the underlying molecular mechanisms regulating the cellular responses to radiation.

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Discussion with Reviewers

J.G. Szekely: Were the WI38 cells subcultured during the EMF exposure?

Authors: No, the WI38 cells were not subcultured during the EMF exposure. The experimental design of this study was the following: The cells were seeded at the relative low cell number of 5 x 10^4 cells per 10 cm culture dish. During the following 21 days of exposure to EMF the cultures were not subcultured. In seven day intervals the cell numbers of the unexposed controls and the exposed EMF-cultures were determined by cell counting. Every third day the medium was renewed.

J.G. Szekely: Did the cells reach stationary phase of growth before the postmitotic differentiation event?

Authors: No, the fibroblast cultures exposed to EMF for 21 days did not reach the stationary phase of growth. Within 14 to 21 days the cell number of the EMF-exposed WI38 cultures increased from 5 x 10^4 cells per dish to approximately 1.5 x 10^5 cells per dish whereas in the control cultures the cell number increased to approximately 1 x 10^6 cells per dish. With the culture dishes used confluent WI38 cells in the stationary phase of growth have an average cell number of at least 3-4 x 10^6 cells per dish. All EMF-exposed cultures analyzed after 21 days by specific cell staining were by no means in the state of contact inhibition or in a phase of stationary growth. When the EMF induced postmitotic cells were subcultured and reseeded at low cell densities (5-10 x 10^4 cells per dish) it could be demonstrated that the amount of the cells being of the irreversible postmitotic differentiation state was 100% in all experiments done. Thus, the differentiation into postmitotic fibroblast types was not due to some cellular mechanisms caused by contact inhibition but induced by the treatment with EMF.

J.G. Szekely: Although the low cell number in the experiments using ionizing radiation is necessary for colony counting, I think the cell survival experiments with colony counting should be separate from the differentiation experiments. If colonies were classified, did all the

cells differentiate into non-mitotic cells at the same time?
Authors: On the contrary, we deliberately did the survival experiments together with the differentiation experiments. The survival curves demonstrated in Fig. 5 are based on the counting of colonies with cell numbers bigger than 50 cells. This means that each cell giving rise to a colony of at least 50 cells had to go through at least 5-6 cell divisions within the 12 days after irradiation. This implies that only mitotically very active cells, like cell types MFI or MFII, are able to form colonies of that size. By the criteria underlying a survival curve in general colonies smaller than 50 cells are not counted at all. However, in terms of cell survival this definition, although it is generally agreed on in radiobiology, may lead to a misinterpretation of the cellular events taking place after irradiation. Since we could demonstrate that ionizing radiation induces terminal differentiation into postmitotic cells with high incidence, this fact has to be taken into account in the interpretation of survival curves. A decrease in cell survival with increasing irradiation doses does not necessarily mean that an increasing percentage of the irradiated cells did not "survive" the radiation procedure and have to be considered as "death". The data from our experiments rather clearly indicate that an increasing percentage of the irradiated cells has been triggered into terminal differentiation and that these cells are, as a consequence of this event, not able to form colonies of the expected size. However, by all means, these cells have to be considered as living and biosynthetically active cells. Therefore, we counted and classified both, individual plated cells as well as colonies of cell numbers bigger than 4 cells. In the survival curve, however, as explained in the text, only colonies bigger than 50 cells were plotted. Most of the colonies counted had cell numbers smaller than 50 cells representing colonies made up of cells of the differentiation state MFIII. The time period of the differentiation into postmitotic cells depends on the differentiation state of the cell (e.g., MFI, MFII or MFIII) that is being triggered to terminal differentiation. Thus, the differentiation time into PMF type fibroblasts ranges from 3-9 days depending upon the cell type composition of the starting population.

Z. Somosy: Is there any relationship between the increased ruffling activity of cells, which is reported after short time (few minutes or hours) of ionizing and non-ionizing irradiations and the radiation induced terminal differentiation of fibroblasts?
Authors: We have not analyzed the ruffling activity of the cells in these particular irradiation experiments. From preliminary experiments focusing on differential membrane associated parameters of the spontaneously arising MF- and PMF-cells it seems that the cell types MFI, MFII, and MFIII, do show some ruffling activity, whereas PMF cell types do not. However, at present we have no quantitative information concerning the different cell types of fibroblasts.