

Effects of 0.2 T static magnetic field on human skin fibroblasts

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Abstract

Human skin fibroblasts were exposed to 0.2 T static magnetic field generated by a magnetic resonance tomograph. After 1 h exposure, cell morphology was modified in association with a concomitant decrease in the expression of some sugar residues of glycoconjugates. Study of cell proliferation and mitogenic signal transduction showed a decrease of thymidine incorporation and of second messenger formation. However, cell viability, assessed by colony forming assay, was unaffected. These results demonstrate that the static magnetic field generated by routinely used magnetic resonance tomograph induces alterations on human skin fibroblasts.

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1. Introduction

The biological effects of electromagnetic fields generated by commonly used devices are subject of repeated argument [1]. Among matters of controversy, exposure to static magnetic field yielded conflicting results [2–8]. It is noteworthy that low intensity magnetic resonance tomographs (field intensity below 0.5 T) are increasingly used for diagnostic purposes, based on the assumption that these devices are less harmful than traditional diagnostic equipments using X-rays (i.e. those of classical radiology or computer assisted tomography). Therefore, the objective of this study was to evaluate the biological effects of this type of static magnetic field frequently encountered in medical practice.

Considering that the epidermis is the human tissue closest to the magnetic field generated by the tomograph, we studied its biological effects on human skin fibroblasts. Since the major concern about electromagnetic field exposure is the risk to develop anomalies of cell growth, we studied the alterations of intracellular mitogenic second messengers generated at the plasma membrane, diacylglycerol and inositol phosphates, and of cell proliferation and

viability. We also assumed that charged molecules on the outer part of the cell could be influenced by the static magnetic field. Among these molecules, sialic acid is known to regulate cell to cell adhesion, recognition and proliferation in normal and pathological tissue [9,10] as well as embryonic differentiation [11–13]. Thus, we investigated the effects of static magnetic field on the distribution pattern of sialic acid sugar residues, evaluated by lectin histochemistry [14]. Finally, changes of cell morphology were evaluated by scanning electron microscopic observation.

2. Materials and methods

2.1. Cell lines

The cell line Detroit 550 (human skin fibroblasts) was obtained from the Zooprofylactic Institute of Lombardia and Emilia, Brescia, Italy. Detroit 550 cell line was cultured in minimum essential medium in Earle Salts (GIBCO BRL, Milano, Italy), supplemented with 10% foetal calf serum (Mascia Brunelli, Milano, Italy), 100 U/ml penicillin, 100 µg/ml streptomycin, at 37 °C in a controlled atmosphere with 5% CO₂. Cells were split once a week, or when necessary, at the density 1:4–1:2, using a solution trypsin-EDTA

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(Sigma, St. Louis, MO, USA). Cells were exposed for 1 h to the static magnetic field, 2 days after the seeding in logarithmically growing conditions. One hour exposure was chosen based on the evidence that the average diagnostic examination lasts about 1 h.

2.2. Exposure to static magnetic field

Cultures were exposed for 1 h to 0.2 T static magnetic field generated by sectorial magnetic resonance tomograph used in clinical practice (Esaote, Italy). This tomograph uses an ultra-compact permanent magnet generating a homogeneous magnetic field of 0.2 T intensity that allows formation of diagnostic images. The magnetic unit is small (height, 113 cm; height of the transverse plane passing through the centre of the magnetic field, 65 cm). The culture plates were placed in the isocentre of the magnet, supported by a plastic support (i.e. a polystyrene tube holder) located in the geometric centre of the gantry. Temperature near the culture plates was monitored and no variation was recorded throughout the experiments. Consistently, this type of tomograph for clinical use does not require cooling for the duration of common examinations (45–60 min). Control (sham exposed) cultures received the same treatment (i.e. permanence outside the CO₂ incubator) and were placed in a nearby room with identical temperature (26 °C) and humidity conditions (60%). Experimental conditions for culture exposure were those previously described for other cell types [4,15]. The tomograph was switched off as diagnostic device during the experiments, i.e. there was no radio-frequency generating images.

2.3. Study of cell proliferation and of cell viability

Cell proliferation was monitored as incorporation of [³H]thymidine in duplicating DNA. Cells, cultured in medium with serum as described, were seeded in tissue culture plates at a density of 7×10^4 cells per well. Exposed and control cells were pulse-labelled with [³H]thymidine (10 μCi per well) for 4 h. [³H]radioactivity was measured by liquid scintillation counting. Before pulse labelling, the cell number was reconfirmed, and only if there were no significant differences between experimental points, pulse labelling was performed. The long-term effects of static magnetic field exposure on cell viability were evaluated in clonogenic assay [16]. Briefly, after exposure, cells from control and exposed samples were transferred into a CO₂ incubator and maintained at 37 °C, 5% CO₂, and 100% humidity for 7 days, and thereafter scored for the number of colonies (aggregated containing more than 50 cells) under an inverted microscope. Both plating density (1×10^3 cells/dish) and day 7 time point were established by preliminary experiments. Differences between exposed and control samples were evaluated by the Student's *t*-test.

2.4. Second messenger measurement

Detroit 550 cells were seeded in tissue culture plates at a density of 5×10^5 per well. The level of second messengers (in sham exposed, and in exposed cells) was determined in cells pre-labelled to equilibrium with [³H]glycerol and [³H]inositol for 24 h. After each experiment, a modified Folch extraction was performed by adding ice-cold methanol and chloroform (1/1 v/v) [17,18]. Diacylglycerol and polyphosphoinositides were then extracted and separated by thin-layer chromatography. Inositol phosphates were separated by ion exchange chromatography [18]. Results of diacylglycerol and inositol phosphate measurements are expressed as radioactivity (cpm) associated with each compound. In order to overcome errors due to variability in cell number, labelling procedure or extraction efficiency, we also measured and reported the radioactivity associated with polyphosphoinositides recovered from the thin-layer chromatography plate. Since diacylglycerol and inositol phosphates derive from the hydrolysis of polyphosphoinositides [17,18], recovery of comparable amount of radioactivity associated with polyphosphoinositides from different experimental points (as shown in Table 1) indicated that no significant difference in cell number, labelling procedure or extraction efficiency had occurred. Differences between exposed and control samples were evaluated by the Student's *t*-test.

2.5. Study of cell morphology

Cell morphology was examined by scanning electron microscopy immediately after exposure. For scanning electron

Table 1
Effects of 0.2 T static magnetic field on signalling, proliferation and viability

	Control (cpm)	Exposed (cpm)
IPs	5003 ± 399	3550 ± 271 ^b
DAG	1389 ± 47	958 ± 59 ^b
PIPs	53954 ± 1984	56651 ± 2139 ^a
Thymidine	147503 ± 6973	92925 ± 4932 ^b
Number of colonies	85 ± 9	94 ± 8 ^a

Cell cultures were exposed to 0.2 T static magnetic field for 1 h as described. In experiments designed for measuring inositol phosphates (IPs) formation, lithium chloride (20 mM), was present during the final incubation. DNA for [³H]thymidine, total inositol phosphates (IPs), polyphosphoinositides (PIPs), and diacylglycerol (DAG) were extracted and measured as described. Data for thymidine incorporation, inositol phosphates, diacylglycerol and polyphosphoinositides are expressed as cpm of radioactivity associated with each compound and are mean ± S.E.M. (*n* = 6). Differences between exposed and control samples were evaluated by the Student's *t*-test.

^a Indicates that the difference between control and exposed cells was not statistically significant (i.e. *P* > 0.05).

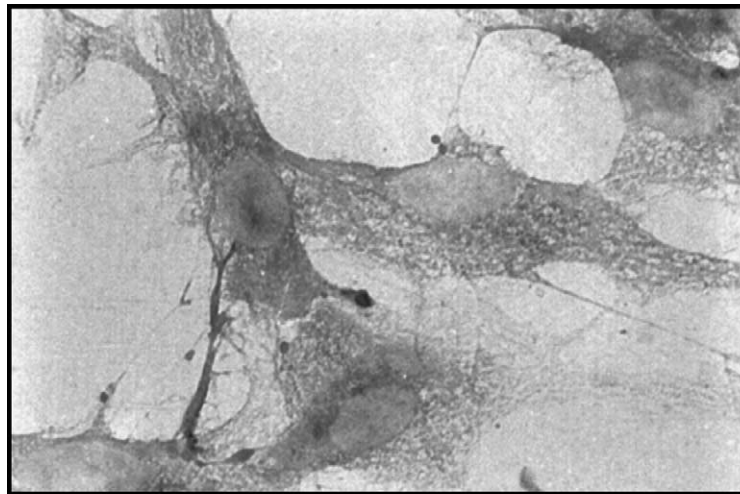
^b Indicates that the difference between control and exposed cells was statistically significant (*P* < 0.02).

microscopic study, the cells, cultured on small glass dishes, were at first fixed in glutaraldehyde 2% in phosphate buffer (pH 7.4), and then postfixed in osmium tetroxide 1% in the same buffer. After this, the cells were dehydrated in graded ethanol and dried in a critical point dryer. Then, the specimens were coated with gold 10%/palladium 90% by means of a sputtering device, and observed by means of a Cambridge Stereoscan 100 microscope, at an accelerating voltage between 15 and 25 kV.

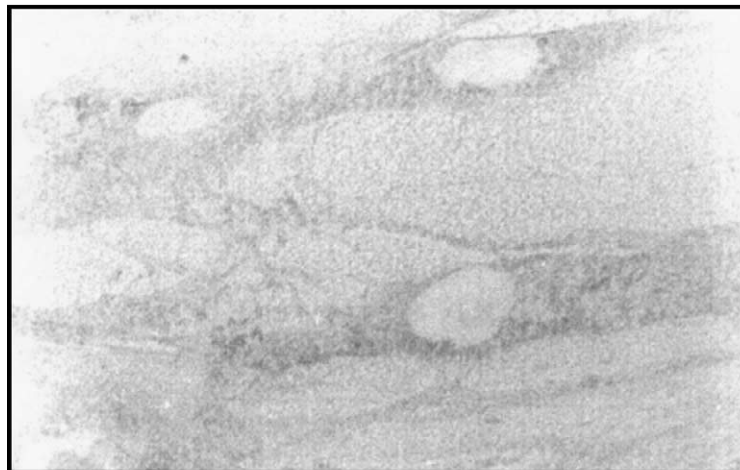
2.6. Lectin histochemistry

Specimens of cultured cells were fixed in Carnoy's fluid for 5 min and, after hydration, the sections were treated with hydrogen peroxide for 10 min to inhibit endogenous peroxidase, rinsed in distilled water and washed with 1% bovine serum albumin (BSA) in 0.1 M phosphate buffered saline

(PBS), pH 7.2. The cells were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-Iectin conjugated) dissolved in 0.1 M PBS containing 0.1 M NaCl, 0.1 mM CaCl₂, MgCl₂, and MnCl₂ and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma), which allowed maximum staining with minimum background was of 20 µg/ml for SBA (*Glycine max*), binding specificity α/β-D-GalNAc > D-Gal and of 25 µg/ml for PNA (*Arachis hypogaea*), binding specificity D-Gal [β1 → 3]-D-GalNAc. Staining of the sites containing bound lectin-HRP was obtained by incubation of the cells with PBS (pH 7.0), containing 3,3'-diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide for 10 min at room temperature. Thereafter, the specimens were rinsed in distilled water; dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount.



(A)



(B)

Fig. 1. Lectin binding affinity of the control cells and of 0.2 T static magnetic field exposed cells. (A) Control cells. Neuraminidase-PNA-HRP. The plasma membrane and cytoplasmic granules show an intense reactivity: 800×; bar = 12.5 µm. (B) 0.2 T static magnetic field exposed cells. Neuraminidase-PNA-HRP. A weak reactivity at the plasma membrane and at cytoplasmic granules is observed: 850×; bar = 12.5 µm.

Controls for lectin staining included: (I) substitution of unconjugated lectins for lectin-HRP conjugates; (II) exposure to HRP and substrate medium without lectin; (III) oxidation with 1% periodic acid for 10 min prior to lectin staining; (IV) exposure of cells to 10/20 $\mu\text{g/ml}$ of each lectin-HRP conjugate containing 0.1 M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl-D-mannopyranoside.

2.7. Sialidase digestion

In some experiments sialic acid was removed by pretreating the sections for 18 h at 37 °C in a solution of sodium acetate buffer 0.25 M, pH 5.5, containing 0.1 U/ml sialidase (neuraminidase type X from *Clostridium perfringens* (Sigma)), 5.0 mM CaCl_2 and 154 mM NaCl, prior to staining with lectin-HRP conjugates. Controls containing the sialidase buffer without the enzyme were also prepared.

3. Results

3.1. Effects of 0.2 T static magnetic field exposure on lectin histochemistry and cell morphology

As far as lectin histochemistry is concerned, the plasma membrane and cytoplasm granules showed reactivity with PNA and SBA, following neuraminidase digestion, thus revealing the presence of sialic acid and the sugar residues D-Gal ($\beta 1 \rightarrow 3$)-D-GalNAc and β -D-GalNAc in subterminal position. The reactivity with these lectins, especially at the plasma membrane level, was clearly reduced in 0.2 T static magnetic field exposed cells (Fig. 1, panel B) when compared with control cells (Fig. 1, panel A).

By scanning electron microscopic observation, the control cells, were isolated or gathered in small groups. The cells were dome-shaped and showed a rather smooth and regular plasmalemma, sometimes with little globular masses,

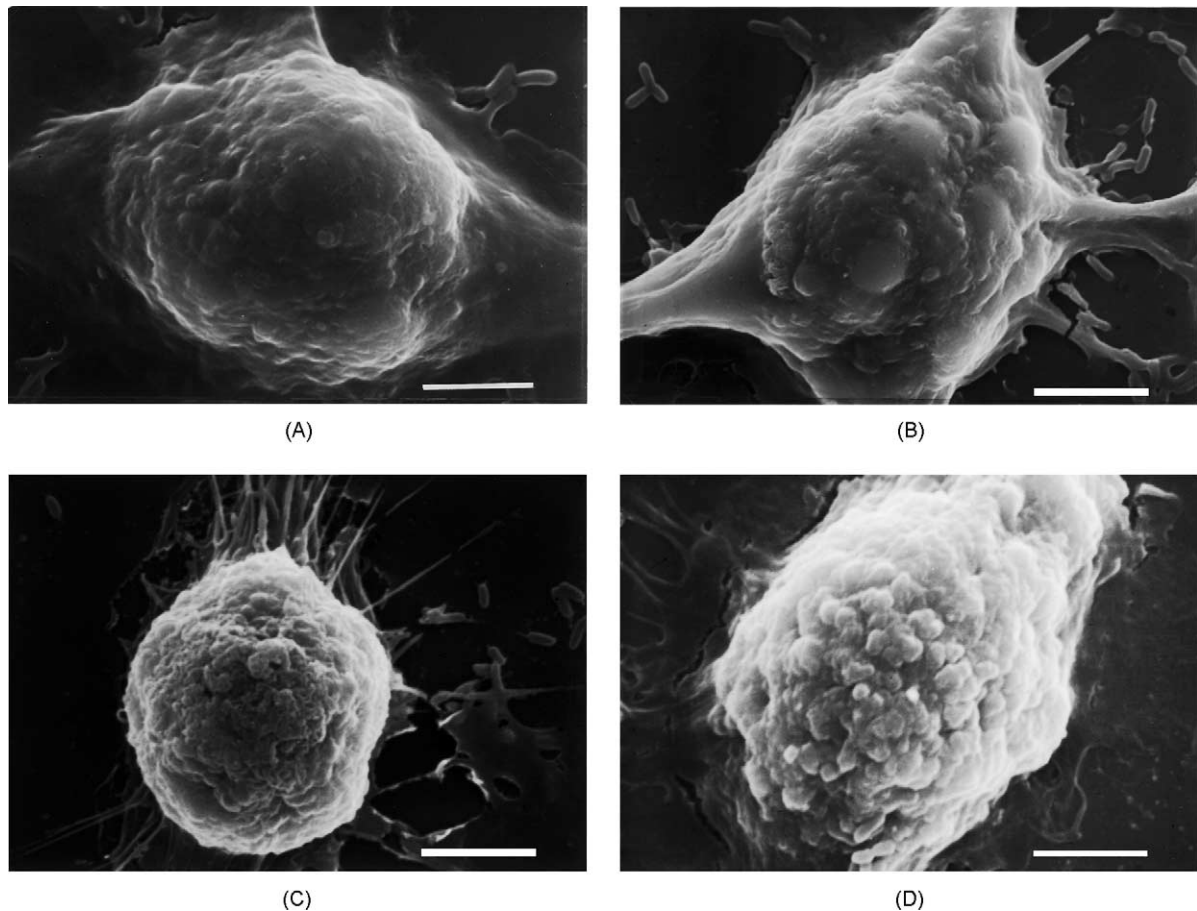


Fig. 2. Scanning electron microscopy of control cells and of 0.2 T static magnetic field exposed cells. (A) Control cell. Cell shows an irregular polygonal outline and a velamentous shape. The cell is dome-shaped and shows a rather smooth and regular plasmalemma, with little globular masses, with the appearance of secretory granules. The basal contour is expanded in few spindle-like expansions or in a velamentous border, probably expression of cell adhesion: 150 \times ; bar = 100 μm . (B) 0.2 T static magnetic field exposed cell. This cell shows a spindle-like shape with poorly branched filaments arising from the cell contour: 150 \times ; bar = 100 μm . (C) 0.2 T static magnetic field exposed cell. This cell shows stiff protrusions with a straight and thin appearance; the velamentous border is no longer present and the adhesion at the substrate is significantly decreased: 150 \times ; bar = 100 μm . (D) 0.2 T static magnetic field exposed cell. Cell shows a jerky shape and a very irregular plasmalemma which arises in numerous deep bulging apical masses: 150 \times ; bar = 100 μm .

with the appearance of secretory granules (Fig. 2, panel A). The basal contour was sometimes expanded in spindle-like expansions or more frequently, in a velamentous border, probably expression of cell adhesion (Fig. 2, panel A). The cellular dimensions could greatly vary, but the large majority showed a diameter near 2–2.5 μm .

In exposed cells the diameter was smaller than in sham exposed cells often less than 2 μm . Cells showed a spindle-like shape with long, straight and stiff protrusions (Fig. 2, panel B); the change of cellular shape was so significant that very often exposed cells appeared surrounded by numerous very thin and sharp cytoplasmic expansions arising from the cell body (Fig. 2, panel C). Cell to cell adhesion signs in the basal portion were often discontinued. The plasmalemma had a more irregular appearance both around the cell contour and on the surface of the cell body where it formed numerous and deep globular masses suggesting severe cell distress (Fig. 2, panel D).

3.2. Effects of 0.2 T static magnetic field exposure on cell growth and second messenger formation

Mitogenic second messengers are generated at the level of the plasma membrane through the hydrolysis of polyphosphoinositides. The products of this hydrolysis, diacylglycerol and inositol phosphates, affect a number of cellular functions by activating protein kinase C and mobilizing intracellular calcium. The level of these intracellular second messengers was significantly decreased in cells exposed to 0.2 T static magnetic field (Table 1). It should be noted that a major hindrance in measuring intracellular second messengers is that metabolic changes may render it difficult to distinguish between significant findings and trivial epiphenomena. In order to ascertain whether diacylglycerol and inositol phosphate decrease were actually due to static magnetic field exposure or just part of altered metabolism and uptake of radioactive precursors, we measured the level of radioactivity associated with polyphosphoinositides and we found no significant difference between control and exposed cell cultures (Table 1). Furthermore, in order to rule out the possibility of a generalized metabolic disorder in the exposed cells, we also determined (NADP)-isocitrate dehydrogenase activity; no significant differences in this enzyme activity were observed between the cell cultures tested (not shown). Cell proliferation, evaluated by thymidine incorporation in exposed cells was significantly decreased, matching the decreased mitogenic messenger formation. However, the effect of 0.2 T static magnetic field on the clonogenic assay did not reveal any significant change in the number of formed colonies between exposed and control cells (Table 1). The apparent discrepancy between the results obtained by labelling the cells with [^3H]thymidine and by counting the number of colonies could be explained as follows. Thymidine labelling measured DNA synthesis immediately after exposure to 0.2 T static magnetic field, and its inhibition could be related to the inhibition of mi-

togenic second messenger formation. However, the number of colonies formed 7 days after exposure assessed cell viability rather than the rate of proliferation. Thus, observation of a similar number of colonies 7 days after exposure seems to indicate that the effect of 0.2 T static magnetic field on DNA synthesis was transient and did not involve permanent damage or harmful effects on cell viability.

4. Discussion

Considering the widespread use of electromagnetic generating devices in medical practice, the implications of any connection between electromagnetic fields and health risks have raised a growing interest for their potential biological effects on cell growth, viability and response to other insults. In the present study we investigated the effects of the 0.2 T static magnetic field generated by a common magnetic resonance tomograph on cultured human skin fibroblasts. Exposure of cultured human cells to this type of static magnetic field caused peculiar morphological changes. The cell shape modification was associated with the significant decrease in the expression of D-Gal ($\beta 1 \rightarrow 3$)-D-GalNAc and β -D-GalNAc and the sialic acid bound to these sugar residues in terminal position in the intra- and extra-cellular cell matrix. It is well known that sialic acid plays an important role in the strengthening membrane stability and in cell to cell adhesion [19]. Like any charged molecule, these carbohydrates may be sensitive to the magnetic field which could modify their spatial arrangement in the cytoskeleton and in the extra-cellular matrix. The different distribution and concentration of these cell components may trigger changes in the cell architecture bringing about the observed changes in the cell shape and adhesion. Alternatively, the variation in the carbohydrate appearance may be a consequence of the general cellular response to the magnetic field effects.

As far as the mechanisms underlying these effects are concerned, a direct action on electrically charged groups may be relevant. Metals such as iron, zinc, manganese, cobalt are sensitive to magnetic fields which may exert their differential effects on proteins, enzymes and cellular components containing these metallic elements. Intracellular shifts of different ions, such as calcium, are alternative targets of magnetic fields that could selectively affect cell orientation, enzyme activity and gene expression leading to the observed changes of cell morphology and function.

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References

- [1] Schoen D. Annals of conflicting results: looking back on electromagnetic field research. *Can Med Assoc J* 1996;155:1443–6.
- [2] Snyder DJ, Jahng JW, Smith JC, et al. C-Fos induction in visceral and vestibular nuclei of the rat brain stem by a 9.4 T magnetic field. *NeuroReport* 2000;11:2681–5.
- [3] Danielyan AA, Mirakyan MM, Grigoryan GY, et al. The static magnetic field effects on ouabain H3 binding by cancer tissue. *Physiol Chem Phys Med NMR* 1999;31:139–44.
- [4] Pacini S, Vannelli B, Barni L, et al. Effect of 0.2 T static magnetic field on human neurons: remodeling and inhibition of signal transduction without genome instability. *Neurosci Lett* 1999;267:185–9.
- [5] Schimmelpfeng J, Dertinger H. Action of a 50 Hz magnetic field on proliferation of cells in culture. *Bioelectromagnetics* 1997;18:177–83.
- [6] Zhao YL, Johnson PG, Jahreis GP, et al. Increased DNA synthesis in INIT/10TI/2 cells after exposure to a 60 Hz magnetic field: a magnetic field or a thermal effect? *Radiat Res* 1999;151:201–8.
- [7] Espinar A, Piera V, Carmona A, et al. Histological changes during development of the cerebellum in the chick embryo exposed to a static magnetic field. *Bioelectromagnet* 1997;18:36–46.
- [8] Salerno S, Lo Casto A, Caccamo N, et al. Static magnetic field generated by a 0.5 T MRI unit affects in vitro expression of activation markers and interleukin release in human peripheral blood mononuclear cells. *Int J Radiat Biol* 1999;75:457–63.
- [9] Damjanov I. Biology of disease. Lectin cytochemistry and histochemistry. *Lab Invest* 1987;57:5–20.
- [10] Zanetta JP, Badache A, Maschke S, et al. Carbohydrates and soluble lectins in the regulation of cell adhesion and proliferation. *Histol Histopathol* 1994;9:385–412.
- [11] Gheri G, Gheri Bryk S, Balboni GC, et al. Identification of sugar residues in human fetal olfactory epithelium using lectin histochemistry. *Acta Anat* 1992;145:167–74.
- [12] Gheri G, Gheri Bryk S, Sgamabati E, et al. Characterization of the glycoconjugates sugar residues in developing chick esophageal epithelium. *Histol Histopathol* 1993;8:351–8.
- [13] Gheri Bryk S, Gheri G, Sgambati E, et al. The nasal respiratory epithelium in human fetus: lectin histochemistry. *Acta Anat* 1994;151:80–7.
- [14] Danguy A, Akif F, Pajak B, et al. Contribution of carbohydrate histochemistry to glycobiology. *Histol Histopathol* 1994;9:155–71.
- [15] Pacini S, Aterini S, Ruggiero C, et al. Influence of static magnetic field on the antiproliferative effect of Vitamin D on human breast cancer cells. *Oncol Res* 1999;11:265–71.
- [16] Fitzgerald TJ, Henault S, Sakakeeny M, et al. Expression of transfected recombinant oncogenes increases radiation resistance of clonal hematopoietic and fibroblast cell lines selectively at clinical low dose rate. *Radiat Res* 1990;122:44–52.
- [17] Ruggiero M, Casamassima F, Magnelli L, et al. Mitogenic signal transduction: a common target for oncogenes that induce resistance to ionizing radiations. *Biochem Biophys Res Commun* 1992;183:652–8.
- [18] Ruggiero M, Wang ML, Pierce JH. Mitogenic signal transduction in normal and transformed 32D hematopoietic cells. *FEBS Lett* 1991;291:203–7.
- [19] Varki A. Biological roles of the oligosaccharides: all of the theories are correct. *Glycobiology* 1993;3:97–130.