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Do Molecular Markers Predict the Electromagnetic Field Treatment of Cancer Through p53 Suppressor Gene?

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Abstract

In recent years there have been enormous studies made toward understanding diagnosis and treatment of cancer. Although there has been a great deal learned about cancer, the treatments available for it have not progressed nearly as much. Attempted removal of the tumor followed by chemotherapy and radiotherapy still prevail as the most effective treatments used. The present study used the electromagnetic fields (4.5 Hz) to treat tumor implanted in mice. The Polymerase Chain Reaction/Restriction Fragment Length Polymorphisms (PCR/RFLPs) technique was selected as a biomarker to evaluate the effect of exposure to electromagnetic fields in implanted Ehrlich tumor of female BALB/C mice. Eighty mice used and divided into four groups (20 each); control, radiated (control exposed to 4.5 Hz), infected (control infected by Ehrlich tumor) and infected exposed (infected exposed to 4.5 Hz). The duration of exposure was for two hours every two days. Electromagnetic field exposure includes group 2 and group 4. DNA genome was extracted and p53 suppressor gene detected (~2130 bp). *Aat*I, *Ban*II, *Eae*I restriction endonucleases did not differentiate between the PCR products (p53 genes) of the four groups (control, radiated, infected and infected exposed mice groups). *Ban*I, *Dra*I, *Dra*III, *Hae*II and *Pst*I differentiated between the four groups. The results approved that the electromagnetic fields could treat the tumor and PCR\RFLPs could be useful diagnostic technique.

Key words: electromagnetic field, cancer, p53 gene, PCR/RFLP.

Introduction

There have been considerable concern and controversy recently about the effects on health from the increasing exposure of populations to extremely low-frequency electromagnetic fields (ELF-EMFs) (Merchant *et al*., 1994). These concerns have centered principally on childhood cancer, but other diseases have been similarly implicated. It is generally accepted that EMFs can exert biological effects; they have been widely used in clinical practice to promote processes such as neural regeneration and bone repair (Bassett, 1993). However, these treatments generally use magnetic field strengths that exceed those encountered in residential and commercial environments, commonly being highfrequency fields of 1 to 100 mT with a pulsed waveform (Lacy-Hulbert *et al*., 1998).

Mutation of the p53 tumor suppressor gene is one of the most frequently reported events in neoplastic cells, occurring in approximately 50% of human cancers (Greenblatt *et al*., 1994 and Hsieh *et al*, 2001). The most prevalent types of p53 mutation found were G:C to A:T transitions and G:C to T:A transversions. Studies have demonstrated that these types of mutations are the most common mutations observed in animals (Belinsky *et al*, 1991; Oreffo *et al*, 1993; Ronai *et al*, 1993; Chang *et al*, 1996). There have been many reports concerning the utility of p53 gene

alterations for tumor diagnosis (Brennan *et al*., 1995 and Partridge *et al*., 2000) and management of cancer patients (Guinn and Mills, 1997; Clahsen *et al*., 1998; Iwao *et al*., 1998; Weller, 1998 and Herr *et al*., 1999). Such studies have come to the fore as our understanding of the pathways involved in apoptotic cell death and the response to DNA damage has increased. Tumors harboring a subset of mutations in the conserved core region, encoded by exons 5–8, have been associated with reduced survival or poor response to therapy, when compared to mutations that lie outside of this region, or wild-type sequence (McBride *et al*., 1986; Lane, 1992; Yeargin *et al*., 1992; Hsiao *et al*., 1994; Harvey *et al*., 1995; Aas *et al*.; 1996; Sherr, 1996; Burke *et al*., 1998; Hussain and Harris, 1998; Iniesta *et al*. 1998; Berns *et al*., 2000 and Temam *et al*., 2000).

Several pilot studies have suggested a role for p53 in determining the responsiveness of a tumor to chemotherapy or radiation therapy (Lowe *et al*., 1994; Rusch *et al*., 1995, Nieder *et al*., 2005 and Sunada *et al*., 2005). Despite many studies examining the role of p53 gene mutations or protein expression as a prognostic factor in human cancer, their use has not been recommended for routine use in clinical practice for several reasons. p53 immunohistochemical staining is not currently performed in any standardized fashion (Sidransky and Hollstein 1996). A variety of antibodies has been used to detect p53 protein accumulation without standardization among antibodies, and the methods used for interpretation of positively differ among various studies. In addition, p53 protein does not accumulate with all types of p53 mutations (Mitsudomi *et al*., 1995 and Carbone *et al*., 1994). Detection of p53 mutations by using direct sequencing is labor-intensive, involves the use of radioactive isotopes or sophisticated software analysis with fluorescent detection, and is thus beyond the capability of almost all clinical laboratories. Moreover, identification of mutations in primary tumors is further complicated by the dilution of neoplastic cells among many control, nonmutated cells. These factors have contributed to the paucity of large prospective studies of sufficient statistical power to provide conclusive evidence of the role of p53 inactivation in predicting patient outcome (Linzer and Levine, 1979; Buchman *et al*., 1988; Levine *et al*., 1991; Weinberg, 1991; Børresen-Dale, 1997; Erber *et al*., 1998; Weller, 1998; Herr *et al*., 1999 and Vogelstein *et al*, 2000).

A rapid, accurate means of identifying p53 mutations in clinical samples would expedite the use of this information in clinical practice as well as facilitate studies further defining its role in the management of patients with cancer. The p53 GeneChip was more sensitive than direct sequencing at detecting p53 mutations, but neither technique was infallible at detecting mutations (Berns *et al*., 1988).

To further understand the effect of electromagnetic field on the tumor and also, to find out good diagnosis for the tumor, we planned to determine the electromagnetic field effect on p53 suppressor gene of control, radiated (control mice exposed to EMF), infected mice (implanted Ehrlich tumor) and infected exposed mice (infected mice exposed to EMF) digesting them by certain restriction endonucleases.

Material And Methods

Biological materials: Eighty female BALB/C mice (16-20 g) were used. The mice housed at The National Cancer Institute, Cairo University, Egypt. The study carried out through four groups of experimental mice as; 20 control mice, 20 control mice exposed to electromagnetic field, 20 implanted Ehrlich tumor and 20 implanted Ehrlich tumor exposed to electromagnetic field. Mice of group three and group four were infected subcutaneously (at thigh region) with 1 x 10 single cell/ml (injection of 0.2 ml for each mouse) suspension isolated from Ehrlich ascites carcinomas.

Electromagnetic field exposure: The whole mice were fixed alive between two electrodes. The animals were exposed to 4.5 Hz amplitude modulated waves square wave form and the wave carrier frequency

was 10 MHz. The exposure started at the 10th day of injection and extended for 21 days. The duration of exposure was for two hours every two days. Electromagnetic field exposure includes group 2 and group 4.

DNA extraction: One gram from each sample of the four groups were homogenized in 500 µl of isotonic solution and centrifuged at 5,000 rpm for 5 minutes. The cell pellet was resuspended in 500 µl of UNSET (Lysis solution; 8M urea, 2% sodium dodecyl sulfate, 0.15M NaCl, 0.001M EDTA, 0.1M Tris pH 7.5); (Hugo *et al.*, 1992 and Awwad 2003). Phenol-chloroform extraction was used two to three times to separate the organic and aqueous phases. To precipitate the nucleic acid, iced absolute ethanol was added (2:1 v/v), and left to incubate at -20^{o}C for 24 to 48 hours. The nucleic acids were recovered by centrifugation at ~5,000 rpm for 15 minutes. The pellet was dried and then resuspended in 40 µl of sterile H_2O. One µl of the resuspended pellet was checked by agarose gel electrophoresis for the presence of DNA, as in figure 1.

Polymerase Chain Reaction Amplification: To amplify the complete p53 gene, one µl of whole-cell DNA template was used plus oligonucleotide primers complementary either to the 5` and 3` ends of the gene (Sense oligo 5'-AATGGATGATTTGATGCTGTCCC-3' and Antisense oligo 5'-CGTGCA-AGTCACAGACTTGGC-3') (Calle- Martin *et al*., 1990). The standard PCR reaction mixture was used (Kessing *et al.*, 1989). The standard polymerase chain reaction program for amplification of p53 gene was: 30-35 cycles; one minute, 94^{o}C; two to three minutes, 45^{o}C; and three minutes, 72^{o}C. Deoxynucleotide triphosphates (dNTP, dATP, dGTP, dTTP, and dCTP) were from Perkin Elmer Cetus, USA. The taq DNA polymerase used for p53 gene amplification was from Boehringer Mannheim Biochemica (Germany) and Gibco/BRL (Gaithersburg, Md., USA). One µl of the PCR products was checked by gel electrophoresis for the presence of p53 gene size (~2130 bp), as in figure 2.

Restriction fragment length polymorphisms (RFLPs): Several restriction enzymes were used in this study; these are *Aat*I (Toyobo Biochemicals); *Ban*I, *Ban*II, *Dra*I, *Dra*III and *Eae*I (Boehringer-Mannheim) and *Hae*II and *Pst*I (Sigma). Restriction endonucleases were used to digest the p53 gene of the 4 groups. Digestion and RFLP analysis were perfo-rmed as described by Vidigal *et al.* (1998).

Results

P53 suppressor gene was obtained from the control, radiated (control mice exposed to electromagnetic field), infected (implanted Ehrlich tumor) and infected exposed (implanted Ehrlich tumor exposed to electromagnetic field) groups obtained from the PCR. The sizes of p53 gene were approximately 2130 bp (Fig. 2).

*Aat*I, *Ban*II, *Eae*I restriction endonucleases did not differentiate between the PCR products (p53 genes) of the four groups (control, radiated, infected and infected radiated mice groups). *Aat*I restriction endonuclease cut the p53 gene of the four groups into two restriction fragments (~840 and ~1290 bp; Fig. 3 and Table 1). Whereas, *Ban*II restriction enzyme digested the p53 gene of the four groups of mice into six restriction patterns (~150, ~160, ~180, ~300, ~460 and ~880 bp; Fig. 4 and Table 2). Also, p53 genes of the four groups did not differentiate when digested with *Eae*I restriction enzyme and gave three restriction bands with all mice groups (~180, ~730 and ~1950 bp; Fig. 5 and Table 3).

The four groups of mice clustered into two clusters when their p53 genes digested with *Dra*I restriction endonuclease (Fig. 6 and Table 4). *Dra*I restriction enzyme cut the control group of mice into two bands (~360 and ~1770 bp; lane 1), whereas, the same restriction endonuclease digested the other three groups of mice (radiated, infected and infected exposure) into three restriction patterns (~360, ~770 and ~1000 bp; lanes: 2, 3 and 4 respectively).

*Ban*I and *Hae*II restriction endonucleases grouped the four groups of mice into two groups (Figures 7 and 8; Tables 5 and

6, respectively). *Ban*I restriction enzyme clustered the control and the radiated in one cluster with three restriction bands (~200, ~700 and ~1230 bp; Fig. 7: lanes 1 and 2; Table 5), while the same restriction enzyme grouped the infected and the infected exposure groups in one cluster with four restriction fragments (~100, ~200, ~600 and ~1230 bp; Fig. 7: lanes 3 and 4; Table 5). Also, *Hae*II restriction endonuclease differentiated the four groups into two groups; the control and the radiated mice groups in one with three restriction patterns (~480, ~690 and ~960 bp; Fig. 8: lanes 1 and 2; Table 6) and the infected and infected exposure mice groups with four restriction fragments (~150, ~430, ~590 and ~960 bp; Fig. 8: lanes 3 and 4; Table 6).

The four groups of mice were differentiated into three groups when their p53 genes digested with *Dra*III restriction enzyme (Fig. 9 and Table 7). *Dra*III restriction endonuclease digested the control group of mice into two fragments (~890 and ~1240 pb; Fig. 9: lane 1 and Table 7) and cut the radiated and infected exposure groups into three restriction bands (~300, ~890 and ~940 bp; Fig. 9: lanes 2 and 4 and Table 7), whereas the same restriction enzyme digested the p53 gene of the infected group of mice into four restriction patterns (~200, ~500, ~650 and ~780 bp; Fig. 9: lane 3 and Table 7).

Also, *Pst*I restriction enzyme clustered the four groups of mice into two groups when digested the p53 genes of the control, radiated and infected exposed groups into three restriction fragments (~130, ~470 and ~1530 bp; Fig. 10: lanes 1, 2 and 4 and Table 8), while the same enzyme cut the p53 gene of the infected group of mice into four restriction patterns (~130, ~470, ~600 and ~930 bp; Fig. 10: lane 3 and Table 8).

Table 1: Length of p53 suppressor gene fragments, resulting from digestion with *Aat*I enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 3)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~840	~1290	……	……	……	……
Radiated	~840	~1290	……	……	……	……
Infected	~840	~1290	……	……	……	……
Infected Exposed	~840	~1290	……	……	……	……

Table 2: Length of p53 suppressor gene fragments, resulting from digestion with *Ban*II enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 4)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~150	~160	~180	~300	~460	~880
Radiated	~150	~160	~180	~300	~460	~880
Infected	~150	~160	~180	~300	~460	~880
Infected Exposed	~150	~160	~180	~300	~460	~880

Table 3: Length of p53 suppressor gene fragments, resulting from digestion with *Eae*I enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 5)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~180	~730	~1950	……	……	……
Radiated	~180	~730	~1950	……	……	……
Infected	~180	~730	~1950	……	……	……
Infected Exposed	~180	~730	~1950	……	……	……

Table 4: Length of p53 suppressor gene fragments, resulting from digestion with *Dra*I enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 6)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~360	~1770	……	……	……	……
Radiated	~360	~770	~1000	……	……	……
Infected	~360	~770	~1000	……	……	……
Infected Exposed	~360	~770	~1000	……	……	……

Table 5: Length of p53 suppressor gene fragments, resulting from digestion with *Ban*I enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 7)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~200	~700	~1230	……	……	……
Radiated	~200	~700	~1230	……	……	……
Infected	~100	~200	~600	~1230	……	……
Infected Exposed	~100	~200	~600	~1230	……	……

Table 6: Length of p53 suppressor gene fragments, resulting from digestion with *Hae*II enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 8)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~480	~690	~960	……	……	……
Radiated	~480	~690	~960	……	……	……
Infected	~150	~430	~590	~960	……	……
Infected Exposed	~150	~430	~590	~960	……	……

Table 7: Length of p53 suppressor gene fragments, resulting from digestion with *Dra*III enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 9)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~890	~1240	……	……	……	……
Radiated	~300	~890	~940	……	……	……
Infected	~200	~500	~650	~780	……	……
Infected Exposed	~300	~890	~940	……	……	……

Table 8: Length of p53 suppressor gene fragments, resulting from digestion with *Pst*I enzyme of the control, radiated, infected and infected exposed groups. (see Fig. 10)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Control	~130	~470	~1530	……	……	……
Radiated	~130	~470	~1530	……	……	……
Infected	~130	~470	~600	~930	……	……
Infected Exposed	~130	~470	~1530	……	……	……

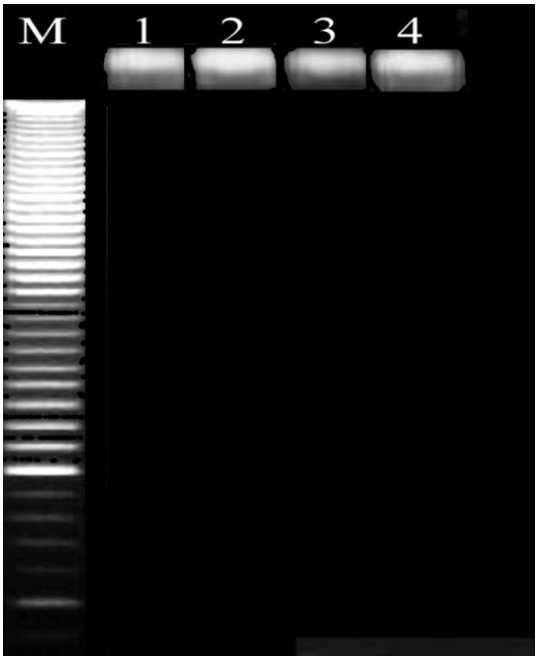

Fig. 1: DNA genome from control, exposed, infected and infected exposed groups of mice. Lane M is the DNA ladder (100-4000 bp). Lanes 1 represents the DNA genome of control group and lanes 2-4 represent the radiated, infected and infected exposed groups of mice, respectively.

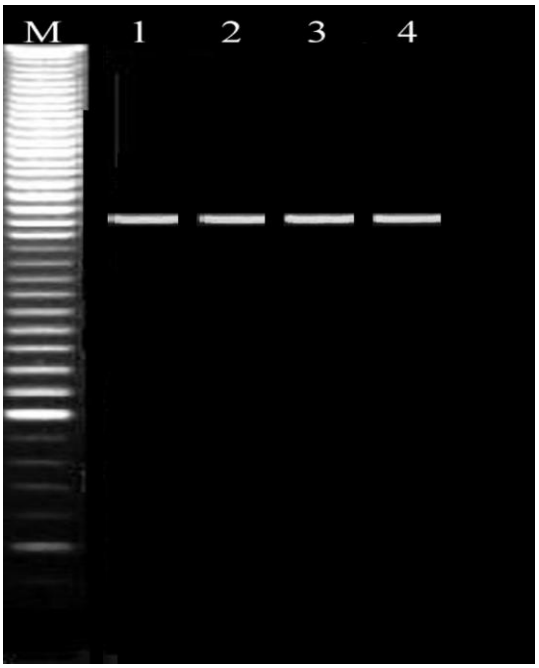

Fig. 2: Symbolized full-segment p53 suppressor gene (~2130 bp) of the control and the other three treated groups. Lane M is the DNA ladder (100-4000 bp). Lane 1 represents p53 suppressor gene of the control group of mice and lanes 2-4 represent the gene of the other three groups (radiated, infected and infected exposed, respectively).

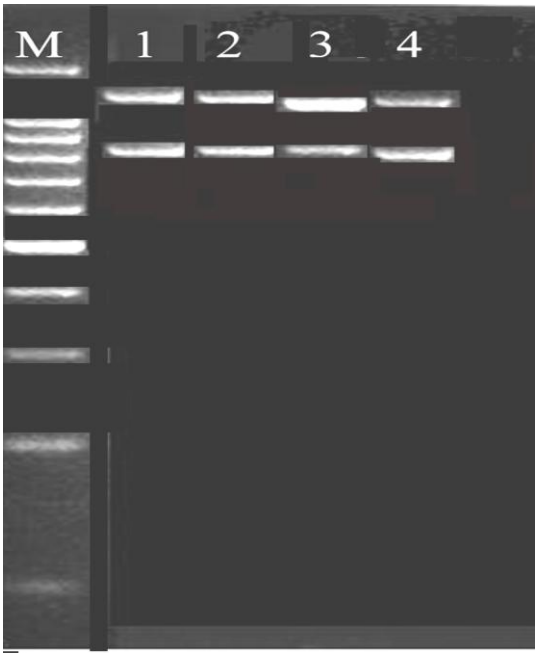

Fig. 3: Representative RFLPs patterns from the control and the three groups with *Aat*I restriction endonuclease, which produced roughly the same fragments (two bands; ~840 and ~1290 bp, for all). Lane M is DNA ladder (100-1500 bp). Lane M is DNA ladder (100-1500 bp).

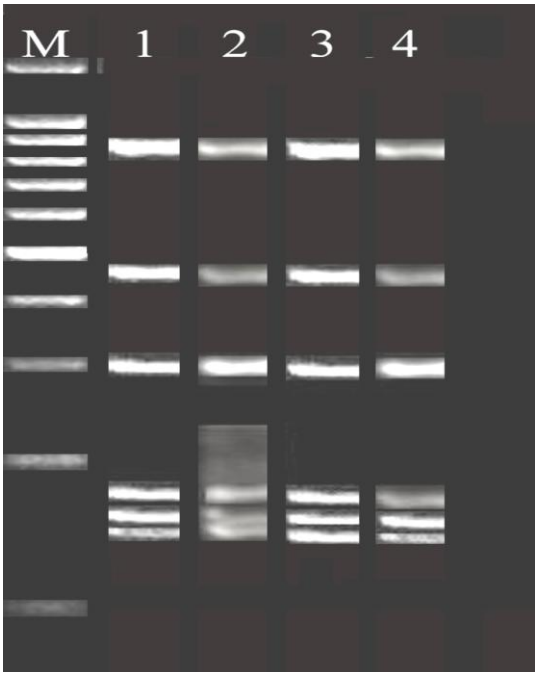

Fig. 4: Representative RFLPs patterns from the control and the three groups with *Ban*II restriction endonuclease, which produced roughly the same fragments (six bands; ~150, ~160, ~180, ~300, ~460 and ~880 bp, for all). Lane M is DNA ladder (100-1500 bp).

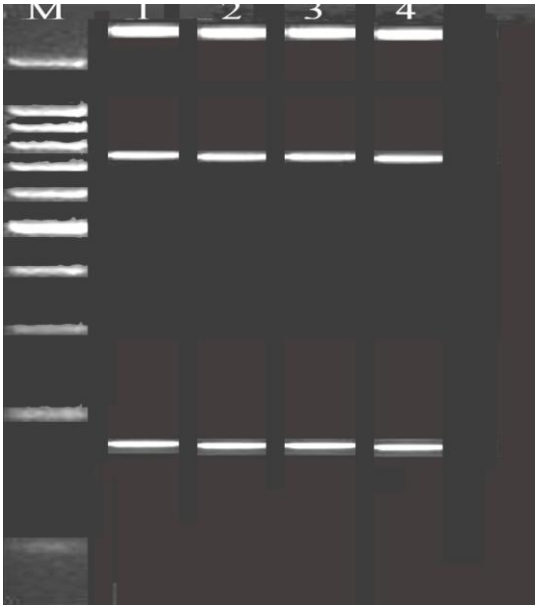

Fig. 5: Representative RFLPs patterns from the unexposed rats and three treated groups with *Eae*I restriction endonuclease, which produced roughly the same fragments (three bands; ~180, ~730 and ~1950 bp, for all). Lane M is DNA ladder (100-1500 bp).

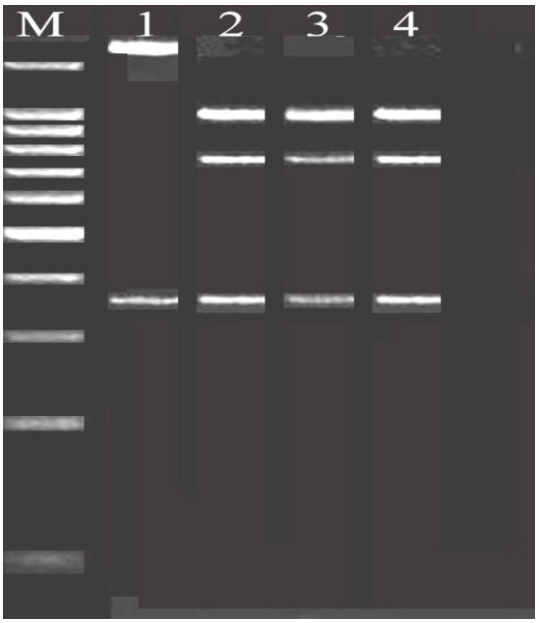

Fig. 6: Representative RFLPs patterns from the control and the three treated groups with *Dra*I restriction endonuclease, which digested the gene of the control group into two restriction fragments (~360 and ~1770 bp; lane 1). Whereas, the gene of the other three groups (radiated group, lane 2; infected group, lane 3 and infected exposed group, lane 4) were digested with the same enzyme into three restriction fragments (~360, ~770 and ~1000 bp). Lane M is DNA ladder (100-1500 bp).

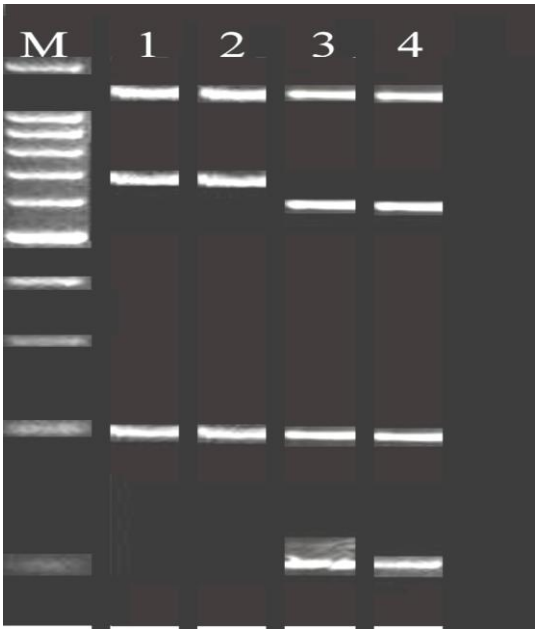

Fig. 7: *Ban*I restriction enzyme digested the p53 suppressor genes of the control and radiated groups into three cuts (~200, ~700 and ~1230 bp; lanes 1 and 2). Whereas, the genes of the infected and infected exposed groups were digested with the same enzyme into four fragments (~100, ~200, ~600 and ~1230 bp; lanes 3 and 4).

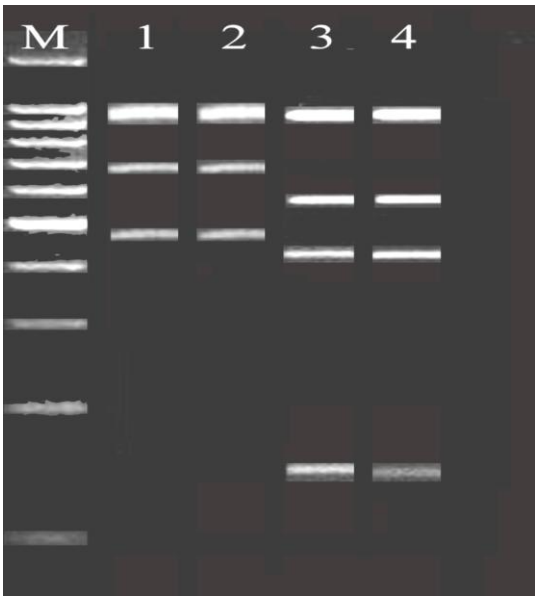

Fig. 8: *Hae*II restriction enzyme digested the p53 gene of the control and radiated groups of mice into three cuts (~480, ~690 and ~960 bp; lanes 1 and 2). Whereas, the gene of infected and infected exposed groups were digested with the same enzyme into four fragments (~150, ~430, ~590 and ~960 bp; lanes 3 and 4). Lane M is DNA ladder (100-1500 bp).

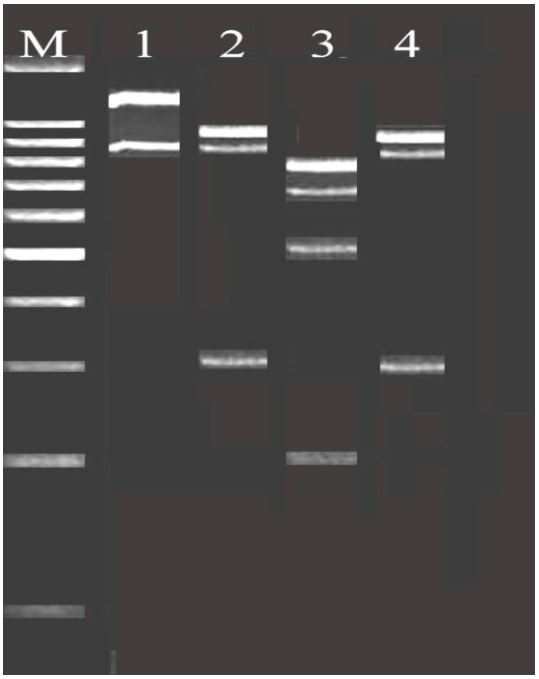

Fig. 9: *Dra*III restriction enzyme digested the p53 suppressor gene of the control group into two restriction cuts (~890 and ~1240 bp; lane 1). Whereas, the genes of radiated and infected exposed groups were digested with the same enzyme into three fragments (~300, ~890 and ~940 bp; lanes 2 and 4). The same endonuclease fragmented the gene of infected group into four fragments (~200, ~500, ~650 and ~780 bp; lane 3). Lane M is DNA ladder (100-1500 bp).

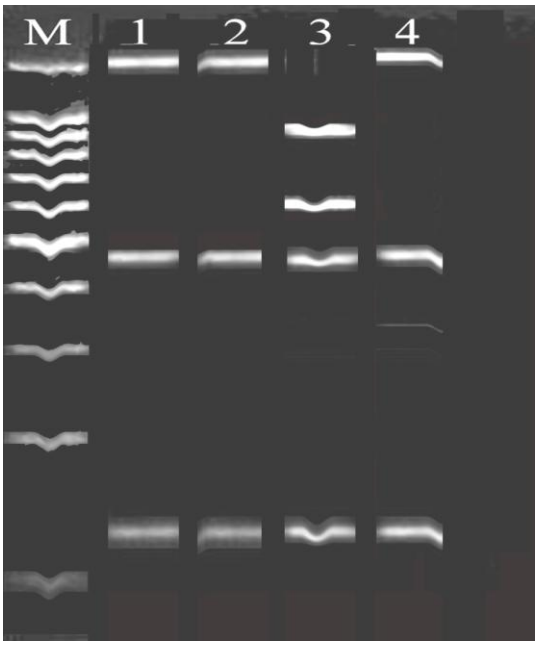

Fig. 10: *Pst*I restriction enzyme digested the p53 suppressor genes of the control, radiated and infected exposed groups into three cuts (~130, ~470 and ~1530 bp; lanes 1, 2 and 4). Whereas, the gene of the infected group was digested with the same enzyme into four fragments (~130, ~470, ~600 and ~930 bp; lane 3). Lane M is DNA ladder (100-1500 bp).

Discussion

p53 is often considered as a classical example of a tumor suppressor. However, tumor has been observed in only about 50% of tumors from patients with germline p53 mutations (Varley *et al*., 1997, 1999 and Sedlacek *et al*., 1998), and there is also evidence of haplo-insufficiency (Venkatachalam *et al*., 1998). And in contrast to other tumor suppressor genes where many of the mutations are nonsense or frameshift alterations leading to absent or truncated protein, most p53 mutations are missense changes. Some substitutions also result in the gain-of-function activity, which can promote transformation and carcinogenesis even in the presence of a wild-type allele (Finlay *et al*., 1989; Dittmer *et al*., 1993). In addition, certain p53 mutants demonstrate a dominantnegative function by, for example, forming oligomeric complexes with the wild-type protein and thus blocking its control functions (Milner & Medcalf, 1991).

Studies on the role and effects of the p53 gene, however, provide a new avenue for possible treatment for cancer that may turn out to be more effective and have fewer side effects than current treatments. The PCR and RFLP analysis of the p53 gene, used here, has proven to be helpful in diagnostic studies of the effect of the electromagnetic field on the tumor and to estimate genetic mutations in their DNA. The molecular data have been confirmed as an effective tool for studying DNA damages (Sachs *et al*., 2004).

PCR/RFLP profile produced high variations between the p53 suppressor gene of control, radiated (control exposed to EMF), infected (implanted Ehrlich tumor) and infected exposure (infected exposed to EMF) mice according to the differences of profiles obtained with the restriction endonucleases *Dra*I, *Ban*I, *Hae*II, *Dra*III and *Pst*I. On the other hand, the molecular observations achieved with PCR/RFLP of p53 gene suggested that *Aat*I, *Ban*II, *Eae*I restriction enzymes did not distinguish the mutations of the genes of all groups based on the similarity of profiles obtained with these restriction endonucleases.

EMF enhances free radical activity in cells, which in turn lead to DNA damage (Lai and Singh, 1997a). Also, EMF exposure caused DNA-protein and DNA-DNA crosslinks (Singh and Lai, 1998) and increased apoptosis and necrosis in cells of the rat (Lai and Singh, 1997b).

Low frequency alternating electromagnetic fields may be useful for cancer treatment. In studies by the late Charles Hannan and his associates, the growth rate of implanted tumors in mice was significantly decreased by exposure to an electromagnetic field (Hannan *et al*., 1994). The field also enhanced the potency of the antitumor compound daunorubicin on implanted multi-drug resistant tumor in mice *in vivo* (Liang *et al*., 1997). More recently, Santi Tofani and his associates (2001) in Italy reported an increase in cell death morpho-logically consistent with apoptosis in two transformed cell lines (WiDr human colon adenocarcinoma and human breast adeno-carcinoma) exposed to electromagnetic.In addition, nude mice bearing WiDr tumors subcutaneously treated with daily exposure of electromagnetic fields showed a significant tumor growth inhibition.

The present study shows that PCR/RFLP is a simple and rapid technique representing an important progress in studies on the effect of electromagnetic fields on the tumors. The study demonstrated that p53 suppressor gene contains useful genetic markers for the diagnosis and follow up the treatment of tumor by the electromagnetic fields.

On the other hand, the relationship between EMF exposure and cancer is not clear. It will be very important in the future to continue investigating the EMF effects in tumors from different points of view to give an answer to the established problems and benefits about the tumor treatment by EMF exposure.

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هل يمكن للمؤشرات الجزيئية أن تتنبأ بعلاج السرطان بواسطة المجال الكهرومغناطيسى عن طريق الجين المثبط ب53؟

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تم في السنوات الأخيرة إجراء العديد من الدراسات لفهم تشخيص و علاج السرطان.و رغم أنة تم معرفة الكثير عن السرطان, فإن علاجاتة المتاحة لم تتقدم بنفس القدر فما زال التخلص من الورم و الذي يعقبة العلاج الكيميائي والإشعاعي هو العلاج الأكثر استخداما. أستخدم المجال الكهرومغناطيسى فى الدراسة الحالية لعلاج ورم تم زرعه فى الفئران .تم اختيار تقنية تفاعل أنزيم البلمره المتسلسل/ و اختلافات أطوال القطع الناتج عن إنزيمات القصر كمؤشر لتقييم تأثير التعرض للمجالات الكهرومغناطيسية على ورم إيرليش فى إناث الفئران من السلالة (BALB/C).

أستخدم ثمانون فأرا تم تقسيمهم إلى أربعة مجموعات (20 فأرا لكل مجموعه) هى الطبيعية و المشعة (الطبيعية التى تعرضت ل 4.5 هرتز) و المصابة (طبيعية مصابة بورم إيرليش) و المصابة المشعة (المصابة و التى تعرضت ل 4.5 هرتز).تم التعريض لمدة ساعتين كل يومين و لمدة 21 يوم . تم إستخلاص دنا (د ن ا) الفئران و تحديد الجين المثبط ب53(حوالى 2130 زوج قاعدى).لم ينتج عن الهضم بإنزيمات القصر *Aat*I, *Ban*II, *Eae*I إختلافات بين نواتج تفاعل إنزيم البلمرة المتسلسل فى المجموعات الأربعة . ميزت بين المجموعات الأربع إنزيمات القصر *Ban*I, *Dra*I, *Dra*III, *Hae*II , *Pst*I .

أشارت النتائج إلى إمكانية إستخدام المجالات الكهرومغناطيسية لعلاج الأورام و أن تقنية تفاعل أنزيم البلمرة المتسلسل / و إختلافات أطوال القطع الناتجة عن أنزيمات القصر يمكن أن تمثل تقنية تشخيصية مناسبة.