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Molecular Biological Studies on the Effect of the Electromagnetic Fields on ETS-1 Oncogene

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Abstract

ETS-1 is the founding member of the ETS family of transcription factors. ETS factors have important roles in oncogenesis, signal transduction and development. In human tumors, ETS-1 is expressed in endothelial cells and fibroblasts of the tumor stroma and is proposed to play a role in tumor vascularization and invasion by upregulating expression of matrix-degrading proteases. In human carcinomas, ETS-1 is also expressed by neoplastic cells, but little is known about the functional implications of this observation. The present study aimed to detect the tumor by using electromagnetic fields through ETS-1 oncogene. The detection of point mutations correlated with diseases is currently performed by digestion of PCR products (PCR/RFLP) by using restriction endonucleases. It has been described here a method based modified on primers during the PCR, and using some restriction endonucleases (*Aat*I, *Ban*I, *Ban*II, *Dra*I, *Dra*III, *Eae*I, *Pst*I and *Sac*II) which create a restriction fragment length polymorphism (RFLP) indicative of the studied mutation. The present study used the electromagnetic fields (4.5 Hz); 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 were used and divided into four groups (20 each); normal, exposed (exposed to 4.5 Hz), infected (normal infected by Ehrlich tumor) and infected exposed (infected exposed to 4.5 Hz). DNA genome was extracted and ETS-1 oncogene detected (~4460 bp). *Aat*I, *Ban*II and *Eae*I restriction endonucleases did not differentiate between the PCR products (ETS-1 genes) of the four groups (normal, exposed, infected and infected exposed mice groups). *Dra*III, *Sac*II, *Pst*I, *Ban*I and *Dra*I differentiated between the four groups. The results proved that the electromagnetic fields could treat the tumor and PCR\RFLPs were able to be a useful diagnostic technique.

Key words: electromagnetic field, cancer, ETS-1 oncogene, PCR/RFLP.

Introduction

Studies of electromagnetic field effects on cells have been carried out in three major domains of high, low, and extremely low electromagnetic fields. The application of high-pulsed electromagnetic fields to cells has been associated mostly with the phenomenon of electroporation (Neumann and Rosenheck, 1972; Zimmermann *et al*., 1974 and Kinosita and Tsong, 1977). Its employment has gained wide use in the areas of cell biology and biotechnology, since it supplied an efficient physical tool to permeabilize cells by opening hydrophilic pathways in the cell membrane, through which molecules could diffuse along their electrochemical gradients (Teissie, 2002; Gehl, 2003 and Weaver, 2003). Exposure of cells to extremely low electromagnetic fields has been associated with environmental exposures of humans to low-frequency electromagnetic and also, with possible

application of medical therapeutic devices (Lacy-Hulbert *et al*., 1998; Adair, 1999 and Ahlbom *et al*., 2001). The underlying mechanisms of these very weak electromagnetic fields are still ill understood and highly debated (Adair 1999; Weaver *et al*., 1999; 2000 and Foster, 2003). The intermediate domain of low electromagnetic fields has been routinely applied in electrophysiological studies of ion transport through channels. It was realized quite early that physiological electric fields in tissues are at the lower end of this range (Jaffe, 1966; Nuccitelli and Jaffe, 1974 and Borgens *et al*., 1977). These endogenous electric fields were associated with processes of development, regener-ation and wound healing (Nuccitelli, 2003).

ETS proteins comprise a family of transcription factors that share a unique DNA binding domain, the ETS domain (Ghysdael and Boureux, 1997; Sharrocks *et al*., 1997; Dittmer and Nordheim, 1998 and Graves and Petersen, 1998). The name "ETS" stems from a sequence that was detected in an avian erythroblastosis virus, E26, where it formed a transforming gene together with Δgag and *c-myb* (Nunn *et al*., 1983 and Blair and Athanasiou, 2000). The newly discovered sequence was called E26 transformation specific sequence or ETS. Later, a cellular homologue to the viral ETS (*v-ETS*) gene, *c-ETS-1*, was found suggesting that *v-ETS* derived from *c-ETS-1* (Watson *et al*., 1985 and Ghysdael *et al*., 1986).

The c-ETS-1 protein is closely related to c-ETS-2. It is believed that these two proteins derived from the same ancestor gene by duplication (Lautenberger *et al*., 1992 and Albagli *et al*., 1994). In humans, the *ETS-1* and *ETS-2* genes are located on two distinct chromosomes, *ETS-1* on chromosome #11, *ETS-2* on chromosome #21 (Watson *et al*., 1985).

ETS-1 is produced by a variety of solid tumors, including epithelial tumors, sarcomas and astrocytomas. Depending on the tumor type, ETS-1 expression is either increased or exclusively found in invasive higher grade tumors. High ETS-1 levels in breast, ovarian and cervical carcinoma correlate with poorer prognosis (Davidson *et al*., 2001; Fujimoto *et al*., 2002; Span *et al*., 2002 and Takai *et al*., 2002). ETS-1 was found to be an independent prognostic marker of breast cancer that was not linked to other tumor markers, such as nodal status, tumor size, histological grade or estrogen receptor status (Span *et al*., 2002). In lung, colorectal and squamous cell carcinoma, ETS-1 expression was associated with a higher incidence of lymph node metastasis (Pande *et al*., 1999; Saki *et al*., 2001 and Tokuhara *et al*., 2003). In endometrial and ovarian cancer, the presence of ETS-1 correlated with a higher histological grade (Takai *et al*., 2000 and Takai *et al*., 2002). In addition to advanced solid tumors, high ETS-1 expression has also been found in leukemic T-cells (Sacchi *et al*., 1988).

ETS genes are transcription factors expressed in different tissues. Biochemical pathways interfering with the intracellular calcium ion concentration are known to influence members of this gene family. Considering experimental observations and previous reports, Romano-Spica and Mucci (2003) proposed a hypothetical model of interaction between EMF and ETS, based on possible interference in pathways involving calcium as a second messenger. Mucci *et al.* (2001) and Romano-Spica *et al.* (2000) observed an increase in ETS-1, mRNA and protein expression, but a decrease in ETS-2 protein levels when the rat was exposed to the electromagnetic field.

Several techniques were observed the mutations of ETS-1 gene and its expressions such as; Western blot (Lu *et al*., 2004), GeneChip (Deneen *et al*., 2003) and Restriction Fragment Length Polymorphisms (Kerckaert *et al*., 1989).

To further understand the effect of electromagnetic fields on tumors and also, to effectively diagnose them, the present study endeavored to determine the electromagnetic field effect on ETS-1 oncogene of normal, exposed (normal mice exposed to EMF), infected (implanted Ehrlich tumor) and infected exposed mice (infected mice exposed to EMF) digesting them by certain restriction endonucleases.

Material And Methods

Animals and animal husbandry: Four groups of female BALB/C mice (20 mice each; 16-20 g) were used and housed at The National Cancer Institute, Cairo University, Egypt. The four groups of experimental mice were grouped as; 20 normal mice, 20 exposed mice (normal mice exposed to electromagnetic field), 20 infected mice (implanted Ehrlich tumor) and 20 infected exposed mice (implanted Ehrlich tumor exposed to electromagnetic field). The third and fourth mice groups were injected at their thigh region by 0.2 ml each of 1 x 10 single cell/ml suspension isolated from Ehrlich ascites carcinomas.

Exposure setup: The whole mice were fixed alive between two electrodes. The experimental mice were exposed to 4.5 Hz amplitude modulated square waves form and the wave carrier frequency was 10 MHz. The exposure started on the 10^{th} 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. At the end of the twenty first day of exposure, the tumor size of the exposed infected group reduced.

***DNA extraction*:** One gram from each sample of the four groups was 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 (figure 1).

ETS-1 oncogene detection: To amplify the complete ETS-1 gene, one µl of whole-cell DNA template was used in addition to oligonucleotide primers complementary either to the 5` and 3` ends of the gene (ETS-1:a 5'-ACCCAGAT GAGGTG-GCCAGGAGATG-3' and ETS-1:b 5'-AGCCCCTTCAGTGCCATCAC TCGTC-3'). The standard PCR reaction mixture was used (Kessing *et al.*, 1989). The standard polymerase chain reaction program for amplification of ETS-1 oncogene was: 35-40 cycles; one minute, at 94^{o}C; two to three minutes, at 45^{o}C; and three minutes, at 65^{o}C. Deoxynucleotide triphosphates (dNTP, dATP, dGTP, dTTP, and dCTP) were from Perkin Elmer Cetus, USA. The taq DNA polymerase used for ETS-1 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 ETS-1 gene size (~4460 bp) .

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 *Pst*I and *Sac*II (Sigma). Restriction endonucleases were used to digest the ETS-1 gene of the four groups. Digestion and RFLP analysis were performed as described by Vidigal *et al.* (1998).

Results

ETS-1 oncogenes were obtained for normal, exposed, infected and infected exposed groups from the PCR products. The sizes of ETS-1 genes were approximately 4460 bp (Fig. 2).

*Aat*I, *Ban*II and *Eae*I restriction endonucleases did not clarify the differences between the normal, exposed, infected and infected exposure mice ETS-1 oncogene. *Aat*I restriction enzyme cut the four mice gene groups into three restriction fragments (~140, ~570 and ~3750 bp, Fig. 3 and Table 1). ETS-1 oncogene of the four

groups digested into four digested fragments (~320, ~730, ~1470 and ~1940 bp) when treated with *Ban*II restriction endonuclease (Fig. 4 and Table 2). Also, *Eae*I restriction enzyme restricted the ETS-1 gene into four restriction fragments (~330, ~710, ~1540 and ~1880 bp; Fig. 5 and Table 3) but into different sizes than *Ban*II restriction enzyme when digesting the same gene.

*Dra*III and *Sac*II restriction endonucleases clustered the gene of the normal group in one cluster and the gene of the other three groups in another. *Dra*III enzyme digested ETS-1 oncogene of the normal group into two restriction fragments (~670 and ~3790 bp; Fig. 6, lane 1 and Table 4) while cut the exposed, infected and infected exposed gene into three restriction bands (~670, ~1500 and ~2290 bp; Fig. 6, lanes 2, 3 and 4 and Table 4). Also, *Sac*II restriction endonuclease fragmented the normal ETS-1 oncogene into two bands (~2030 and ~2430 bp; Fig. 7, lane 1 and Table 5) whenever the same restriction enzyme digested the gene of the exposed, infected and infected exposed into four restriction fragments (~500, ~730, ~1500 and ~1730 bp; Fig. 7, lanes 2, 3 and 4 and Table 5).

*Pst*I restriction enzyme differentiated the oncogene of the four groups into two groups; the gene of normal and exposed mice in one group and the other two in another group (Fig. 8 and Table 6). *Pst*I restriction endonuclease digested the gene of normal and exposed gene into four bands (~130, ~710, ~1410 and ~2210 bp; lanes 1 and 2) whereas it cut the gene of infected and infected exposed gene of mice into five restriction bands (~130, ~500, ~710, ~1410 and ~1710 bp; lanes 3 and 4).

Three clusters were differentiated when *Ban*I restriction enzyme digested their gene (Fig. 9 and Table 7). *Ban*I restriction endonuclease digested the oncogene of normal and exposed mice into three cuts (~740, ~1160 and ~2560 bp; lanes 1 and 2) while it digested the gene of infected mice into five restriction fragments (~300, ~440, ~1000, ~1160 and ~1560 bp; lane 3) and infected exposed mice into four bands (~300, ~440, ~1160 and ~2560 bp; lane 4).

*Dra*I restriction endonuclease differentiated between ETS-1 oncogene of the four groups (Fig. 10 and Table 8). ETS-1 gene of the normal mice was digested into two fragments (~100 and ~4360 bp; lane 1) when treated with *Dra*I enzyme. *Dra*I restriction fragment digested the gene of exposed mice into three fragments (~100, ~1600 and ~2760 bp; lane 2). The same restriction endonuclease cut the oncogene of infected mice into five bands (~100, ~600, ~1000, ~1200 and ~1560 bp; lane 3) and the gene of infected exposed mice into four restriction fragments (~100, ~600, ~1000 and ~2760 bp; lane 4).

Table 1: Length of ETS-1 oncogene fragments, resulting from digestion with *Aat*I enzyme of the normal,exposed, infected and infected exposed groups. (see Fig. 3)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~140	~570	~3750	……	……	……
Exposed	~140	~570	~3750	……	……	……
Infected	~140	~570	~3750	……	……	……
Infected Exposed	~140	~570	~3750	……	……	……

Table 2: Length of ETS-1 gene fragments, resulting from digestion with *Ban*II enzyme of the normal,exposed, infected and infected exposed groups. (see Fig. 4)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~320	~730	~1470	~1940	……	……
Exposed	~320	~730	~1470	~1940	……	……
Infected	~320	~730	~1470	~1940	……	……
Infected Exposed	~320	~730	~1470	~1940	……	……

Table 3: Length of ETS-1 gene fragments, resulting from digestion with *Eae*I enzyme of the normal, exposed, infected and infected exposed groups. (see Fig. 5)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~330	~710	~1540	~1880	……	……
Exposed	~330	~710	~1540	~1880	……	……
Infected	~330	~710	~1540	~1880	……	……
Infected Exposed	~330	~710	~1540	~1880	……	……

Table 4: Length of ETS-1 gene fragments, resulting from digestion with *Dra*III enzyme of the normal ,exposed, infected and infected exposed groups. (see Fig. 6)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~670	~3790	……	……	……	……
Exposed	~670	~1500	~2290	……	……	……
Infected	~670	~1500	~2290	……	……	……
Infected Exposed	~670	~1500	~2290	……	……	……

Table 5: Length of ETS-1 gene fragments, resulting from digestion with *Sac*II enzyme of the normal, exposed, infected and infected exposed groups. (see Fig. 7)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~2030	~2430	……	……	……	……
Exposed	~500	~730	~1500	~1730	……	……
Infected	~500	~730	~1500	~1730	……	……
Infected Exposed	~500	~730	~1500	~1730	……	……

Table 6: Length of ETS-1 gene fragments, resulting from digestion with *Pst*I enzyme of the normal, exposed, infected and infected exposed groups. (see Fig. 8)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~130	~710	~1410	~2210	……	……
Exposed	~130	~710	~1410	~2210	……	……
Infected	~130	~500	~710	~1410	~1710	……
Infected Exposed	~130	~500	~710	~1410	~1710	……

Table 7: Length of ETS-1 gene fragments, resulting from digestion with *Ban*I enzyme of the normal, exposed, infected and infected exposed groups. (see Fig. 9)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~740	~1160	~2560	……	……	……
Exposed	~740	~1160	~2560	……	……	……
Infected	~300	~440	~1000	~1160	~1560	……
Infected Exposed	~300	~440	~1160	~2560	……	……

Table 8: Length of ETS-1 gene fragments, resulting from digestion with *Dra*I enzyme of the normal, exposed, infected and infected exposed groups. (see Fig. 10)

Groups	Band #1	Band #2	Band #3	Band #4	Band #5	Band #6
Normal	~100	~4360	……	……	……	……
Exposed	~100	~1600	~2760	……	……	……
Infected	~100	~600	~1000	~1200	~1560	……
Infected Exposed	~100	~600	~1000	~2760	……	……

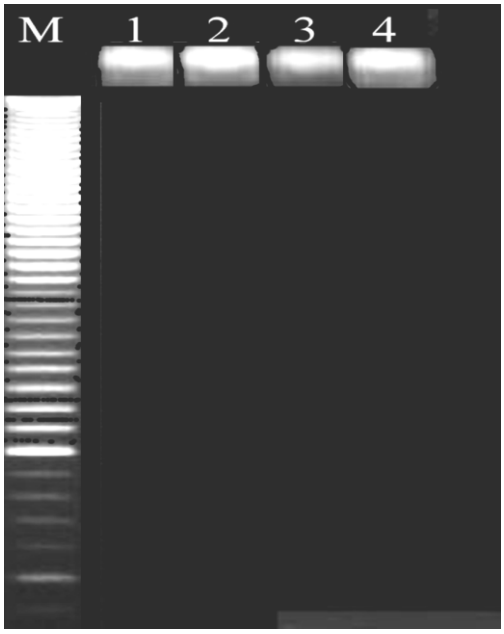

Fig. 1: DNA genome from normal, exposed, infected and infected exposed groups of mice. Lane M is the DNA ladder (100-4000 bp). Lanes 1 represents the DNA genome of normal group and lanes 2-4 represent the exposed, infected and infected exposed groups of mice, respectively.

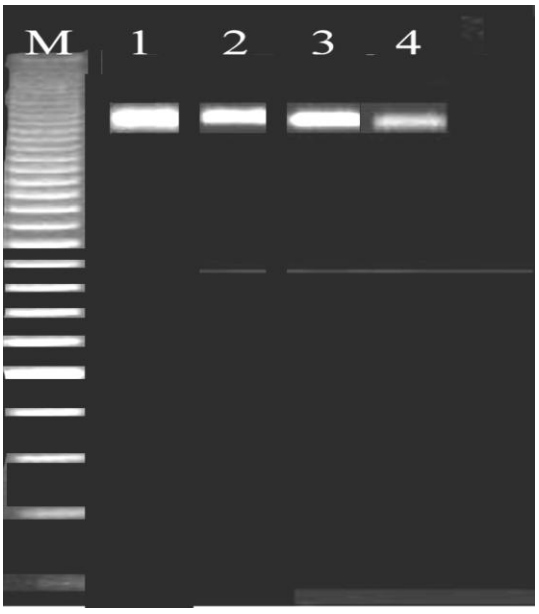

Fig. 2: Symbolized full-segment ETS-1 oncogene (~4460 bp) of the normal and the other three treated groups. Lane M is the DNA ladder (200-6000 bp). Lane 1 represents EST1 gene of the normal group of mice and lanes 2-4 represent the gene of the other three groups (exposed, infected and infected exposed, respectively).

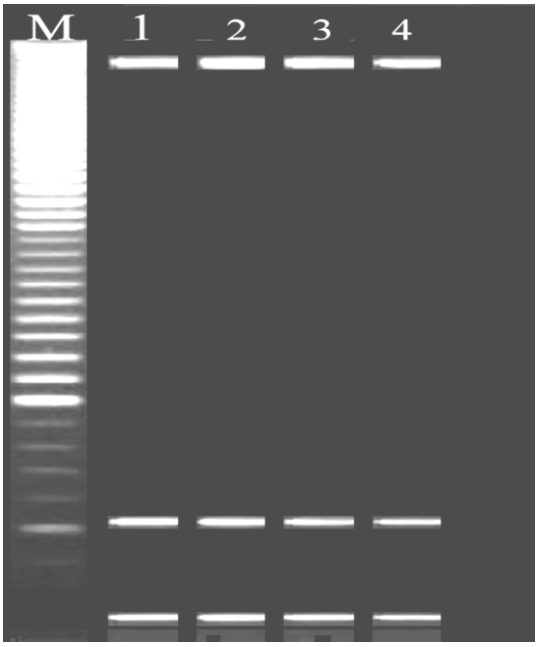

Fig. 3: Representative RFLPs patterns from the normal and the three groups with *Aat*I restriction endonuclease, which produced roughly the same fragments (three bands; ~140, ~570 and ~3750 bp, for all). Lane M is DNA ladder (100-4000 bp).

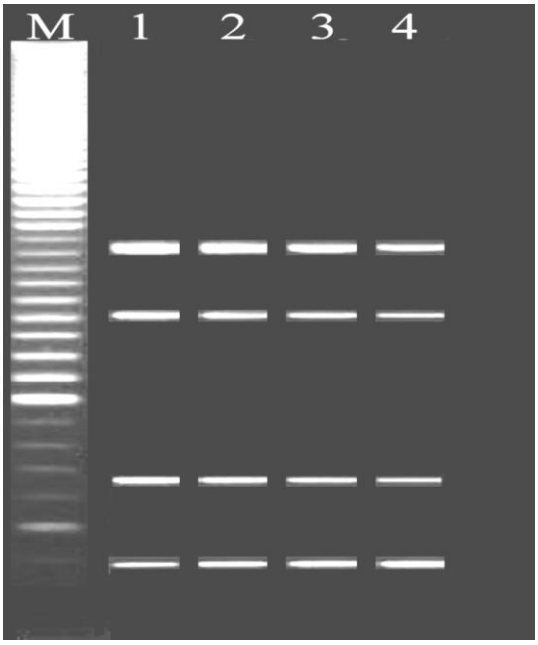

Fig. 4: Representative RFLPs patterns from the normal and the three groups with *Ban*II restriction endonuclease, which produced roughly the same fragments (four bands; ~320, ~730, ~1470 and ~1940 bp, for all). Lane M is DNA ladder (100-4000 bp).

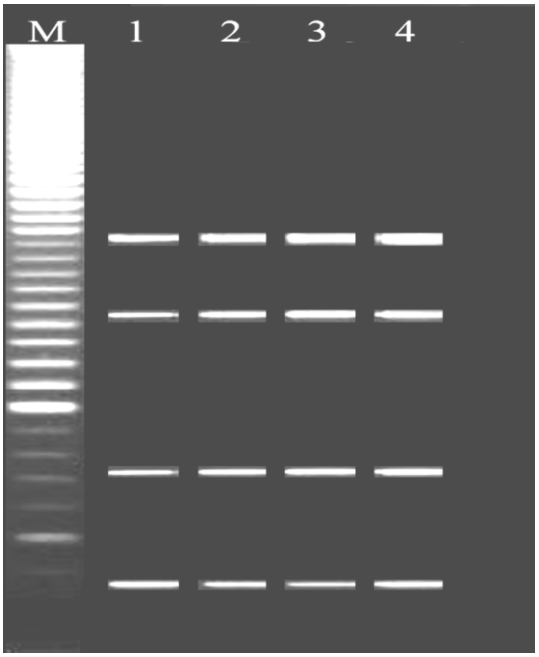

Fig. 5: Representative RFLPs patterns from the unexposed rats and three treated groups with *Eae*I restriction endonuclease, which produced roughly the same fragments (four bands; ~330, ~710, ~1540 and ~1880 bp, for all). Lane M is DNA ladder (100-4000 bp).

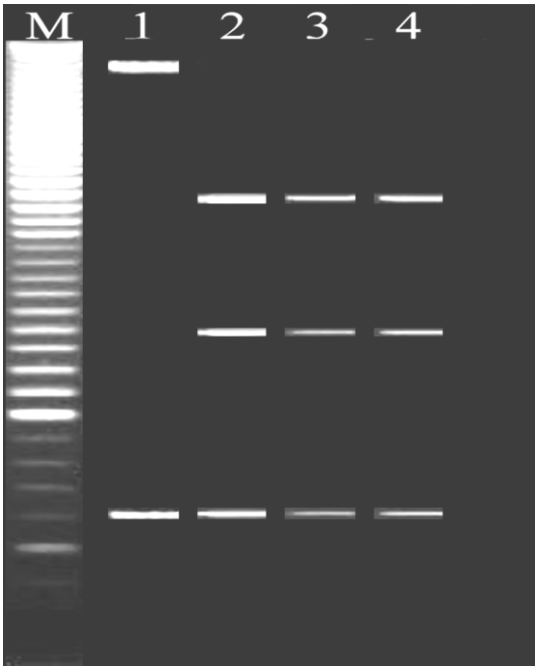

Fig. 6: Representative RFLPs patterns from the normal and the three treated groups with *Dra*III restriction endonuclease, which digested the gene of the normal group into two restriction fragments (~670 and ~3790 bp; lane 1). Whereas, the gene of the other three groups (exposed, lane 2; infected, lane 3 and infected exposed, lane 4) were digested with the same enzyme into three restriction fragments (~670, ~1500 and ~2290 bp). Lane M is DNA ladder (100-4000 bp).

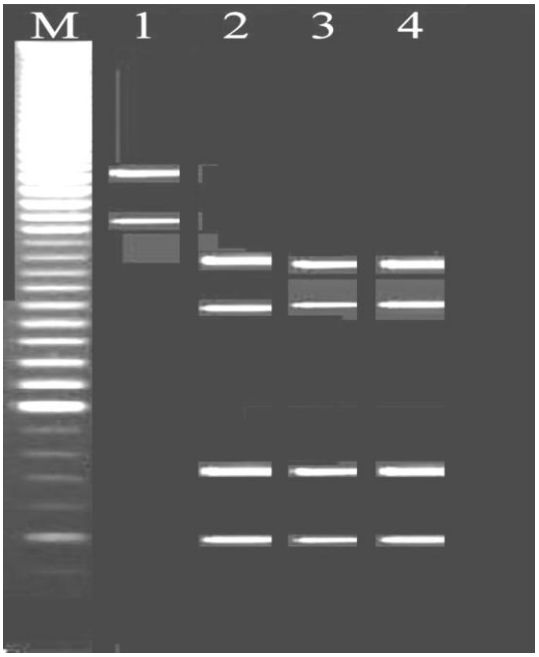

Fig. 7: *Sac*II restriction enzyme digested the ETS-1 genes of the normal group into two cuts (~2030 and ~2430 bp; lane 1). Whereas, the genes of the exposed, infected and infected exposed groups were digested with the same enzyme into four fragments (~500, ~730, ~1500 and ~1730 bp; lanes 2, 3 and 4).

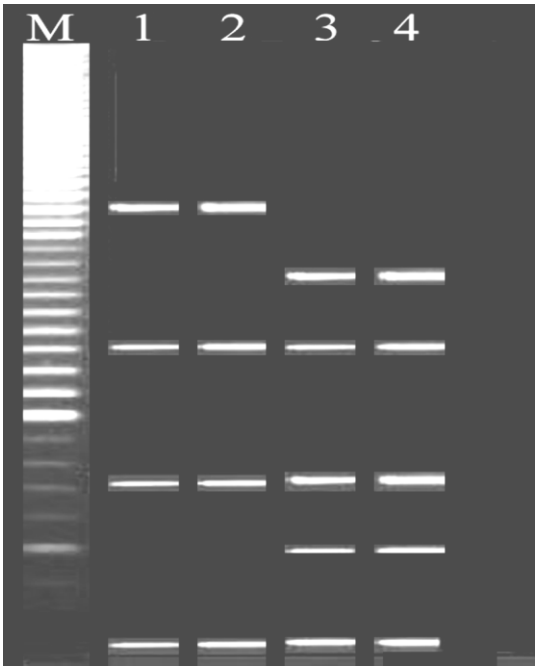

Fig. 8: *Pst*I restriction enzyme digested the ETS-1 gene of the normal and exposed groups of mice into four cuts (~130, ~710, ~1410 and ~2210 bp; lanes 1 and 2). Whereas, the gene of infected and infected exposed groups were digested with the same enzyme into five fragments (~130, ~500, ~710, ~1410 and ~1710 bp; lanes 3 and 4). Lane M is DNA ladder (100-4000 bp).

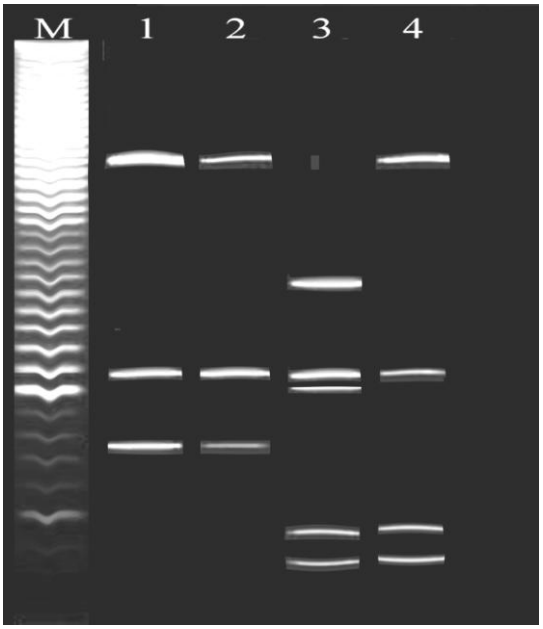

Fig. 9: *Ban*I restriction enzyme digested the ETS-1 gene of the normal and exposed groups into three restriction cuts (~740, ~1160 and ~2560 bp; lanes 1 and 2). Whereas, the gene of infected group was digested with the same enzyme into five fragments (~300, ~440, ~1000, ~1160 and ~1560 bp; lane 3). The same endonuclease fragmented the gene of infected exposed group into four fragments (~300, ~440, ~1160 and ~2560 bp; lane 4). Lane M is DNA ladder (100-4000 bp).

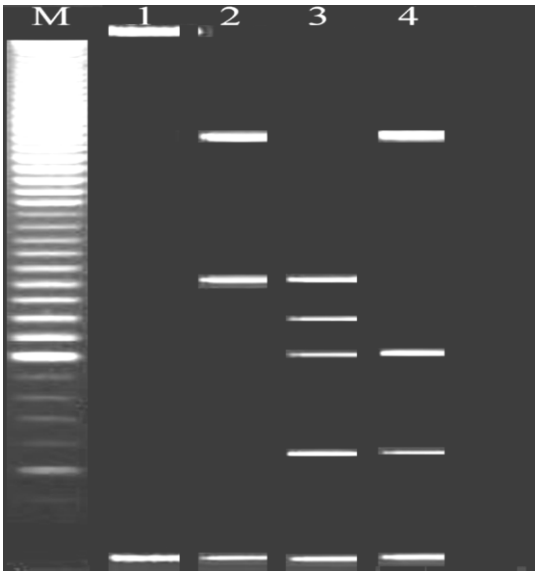

Fig. 10: *Dra*I restriction enzyme digested the ETS-1 genes of the normal group into two cuts (~100 and ~4360 bp; lane 1). Whereas, the gene of the exposed group was digested with the same enzyme into three fragments (~100, ~1600 and ~2760 bp; lane 2). The same restriction endonuclease cut the oncogene of infected mice into five bands (~100, ~600, ~1000, ~1200 and ~1560 bp; lane 3) and the gene of infected exposed mice into four restriction fragments (~100, ~600, ~1000 and ~2760 bp; lane 4).Lane M is DNA ladder (100-4000 bp).

Discussion

The ETS family genes and their products have been implicated in several malignant diseases and pathological genetic disorders. For instance, ETS-1, ETS-2, and ERG have been shown to act as protooncogenes in that they can transform NIH 3T3 cells *in vitro*, and the subsequent injection of these cells into nude mice results in tumor formation (Seth *et al.*, 1989; Seth and Papas, 1990; Topol *et al.*, 1992; Teitell *et al.*, 1999 and Kawagoe *et al.*, 2004). Furthermore, the FLI1 and ERG genes have been shown to be translocated and express chimeric fusion transcripts in almost all Ewing's sarcomas as well as in a large number of other primitive neuroectodermal tumors (Delattre *et al.*, 1992; Sorensen *et al.*, 1994 and Wang *et al.*, 2005). Thus, these findings are strongly suggestive of a basic role for these genes in the genesis of these types of tumors. Recently, the overexpression of ETS-2 in transgenic mice has been shown to cause skeletal abnormalities phenotypically reminiscent of those seen in Down syndrome, in which ETS-2 genes are known to be present in triplicate (Sumarsono *et al.*, 1996 and Rainis *et al.*, 2005).

Several studies on ETS-1 gene treated with electromagnetic fields and waves may be of help in the treatment of cancer (Romano-Spica *et al.*, 2000 and 2003). PCR/RFLPs profile of ETS-1 oncogene produced high variations between the normal, control, infected and infected exposure mice according to the differences of profiles obtained with the restriction endonucleases *Dra*III, *Sac*II, *Pst*I, *Ban*I and *Dra*I. On the other hand, the molecular results obtained with PCR/RFLP of ETS-1 gene suggested that *Aat*I, *Ban*II and *Eae*I restriction endonucleases did not clarify the differences between the normal, exposed, infected and infected exposure mice ETS-1 oncogene based on the similarity of profiles obtained with these restriction endonucleases.

The study may prove that the electromagnetic fields could mutate the mutated ETS-1 oncogene. In addition, the present study shows that the electromagnetic fields could be a suitable source for treating tumors. Also, the PCR/RFLP is a simple and rapid technique representing an important progress in studies on the effect of electromagnetic spectrum on cancer.

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دراسات بيولوجية جزيئية على تأثير المجالات الكهرومغناطيسية على الجين المسرطن ETS-1

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يعتبر المورث ETS-1 العامل الرئيسي لعائلة عوامل النسخ ETS. تلعب مورثات ETS دورا هاما فى عمليات التسرطن الجيني وانتقال الإشارات وكذلك النمو. يعبر الجين ETS-1 عن نفسه في الخلايا الطلائية الداخلية والليفية فى الأورام السرطانية البشرية ويعتقد أنها تلعب دورا في نمو الأورام وانتشارها بزيادة تعبير إنزيمات التحلل البروتيني. كذلك يعبر عن ETS-1 فى الخلايا النامية للأورام الغدية في الإنسان. إلا أنه يعرف القليل عن التداعيات الوظيفية لهذه الملاحظة.

تهدف الدراسة الحالية إلي التعرف على الأورام وعلاجها باستخدام المجالات الكهرومغناطيسية من خلال المورث المسرطن ETS-1. يتم تحديد الطفرات الموضعية المرتبطة بالأمراض عن طريق هضم نواتج تفاعل إنزيم البلمرة المتسلسل واستخدام بعض الإنزيمات المحددة (*Sac*II و*Aat*I, *Ban*I, *Ban*II, *Dra*I, *Dra*III, *Eae*I, *Pst*I) والتي ينشأ اختلاف في أنماط أطوال القطع الناتجة عن الهضم (RFLP) والتي تشير إلى الطفرة موضع الدراسة.

تم فى الدراسة الحالية استخدام مجالات كهرومغناطيسية (4.5 هيرتز) وتقنية تفاعل إنزيم البلمرة المتسلسل واختلاف أطوال القطع الناتجة عن الهضم كمؤشر لتقييم تأثير التعرض للمجال الكهرومغناطيسي على ورم إيرليش الذي تم زرعه في إناث الفئران من سلالة BALB/C. استخدم ثمانون فأرا تم تقسيمهم الى أربع مجموعات هي المجموعة الطبيعية والمجموعة الضابطة (المعرضة الى 4.5 هيرتز) والمجموعة المصابة والمعرضة (مصابة ومعرضة إلى 4.5 هيرتز). تم عزل الدنا وتحديد الجين المسرطن ETS-1 (حوالي 4460 زوج من القواعد النيتروجينية). الإنزيمات المحددة *Eae*I, *Ban*II, *Aat*I لم يميزوا بين نواتج تفاعل إنزيم البلمرة المتسلسل (جين ETS-1) للمجموعات الأربع. الإنزيمات *Dra*I, *Ban*I, *Pst*I, *Sac*II, *Dra*II ميزت بين المجموعات الأربع. أثبتت النتائج أنه يمكن علاج الأورام بالمجالات الكهرومغناطيسية وأن التقنية المستخدمة يمكن استعمالها كتقنية تشخيصية.