Analysis of genomic instability in patients with breast cancer
تحليل عدم الثبات الجيني في مرضى سرطان الثدي

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Abstract

Genomic instability resulting in multiple molecular events believed to be a driving force in the carcinogenic process. In this study, the random amplified polymorphic DNA (RAPD) technique, a simple polymerase chain reaction (PCR) based DNA polymorphism assay system was used for analyzes genomic instability in blood and tissues obtained from five breast cancer patients. DNAs from breast cancer tissues and corresponding DNAs from blood samples were amplified by RAPD with five different 10-bases arbitrary primers. The ability to detect genomic instability in five cancer tissues by each single primer ranged from 60 to 100 percent. Samples 3, and 4 represented the highest genomic instability since it detected by all primers used. Changes in the genome that were revealed by RAPD included deletion or insertion, and allelic losses or gains. The most important finding that emerged from the RAPD analysis, is that deletion was also observed in blood DNAs which revealed by the absence of amplified DNA fragments from blood DNAs, while the corresponding fragments were present in the tumor DNA. Our results display that an insertion of a 468bp amplified fragment was observed in 3 of 5 tumor samples (two fragments using primer OPA-03 and the third fragment using primer OPA-10) whereas, the same 468bp amplified fragment was deleted in 2 of 5 blood samples one using the primer OPA-03 and the second fragment using primer OPA-13. Genomic instability analyzed by RAPD is important to understand the molecular events in breast cancer.

المستخلص

يؤدي عدم الثبات المجيني إلى آحداث جزيئية متعددة يعتقد أنها القوة المحركة في عملية التسرطن . في هذه الدراسة جرى التحري عن عدم الثبات المجيني في دنا خمس عينات مأخوذة من نسيج الورم لمريضات سرطان الثدي وخمس عينات دم أُخذت من المريضات أنفسهن ، واستعملت تقنية التضاعف العشوائي المتعدد الأشكال لسلسلة الدنا (RAPD) المعتمدة على التفاعل التضاعفي لسلسلة الدنا (PCR) لانجاز التحري عن عدم الثبات المجيني باستعمال خمس بادئات عشرية القواعد اعتباطية التعاقب . تراوحت قدرة كل بادئ على التحري عن عدم

الثبات المجيني في عينات نسيج الورم الخمس ما بين (60 إلى 100)% . أظهرت العينات 3 و 4 أعلى عدم ثبات مجيني لكونها أعطت عدم ثبات مجيني مع كل بادئ من البادئات التي استعملت في هذه الدراسة . تضمنت التغيرات في المجين والتي أظهرتها نواتج التضاعف العشوائي المتعدد الأشكال حذف أو إضافة ، وتضمنت هذه التغيرات أيضا الفقدان أو الاكتساب الاليلي . لوحظ الحذف أيضا في بعض عينات الدنا المأخوذ من الدم ، في حين كانت نظيراتها موجودة في دنا الورم المأخوذ من المريض نفسه . أظهرت نواتج تحليل التضاعف العشوائي المتعدد الأشكال إضافة لقطعة دنا متضاعفة ذات وزن جزيئي 468 زوج قاعدة (bp) في ثلاث عينات نسيج من أصل خمس عينات ، حزمتين لوحظت باستعمال البادئ OPA-03 والحزمة الثالثة باستعمال البادئ OPA-10 ، في حين لوحظ فقدان القطعة المتضاعفة نفسها بالوزن الجزيئي 468 زوج قاعدة في عينتي دم من أصل خمس عينات مرة باستعمال البادئ OPA-03 وأخرى باستعمال البادئ OPA-13 . يعد تحليل عدم الثبات المجيني باستعمال تقنية ال RAPD مهم في فهم الأحداث الجزيئية في سرطان الثدي .

Introduction

Progress in molecular cancer genetic has facilitated the detection of genomic alteration in cancer [1]. Gene discovery has been greatly facilitated by molecular cytogenetic technologies identifying chromosomal regions associated with various stages and outcomes. Furthermore, high throughput, genome-wide approaches and the complete sequencing of the human genome have accelerated the large-scale discovery of cancer-related genes and pathways [2].

RAPD is a PCR-based DNA fingerprinting technique that uses an arbitrary chosen oligonucleotide primer for the amplification of genomic or cDNA fragments [3,4]. RAPDs produce DNA profiles of varying complexity, depending on primer and template used. Each amplification product is expected to result from the existence of two annealing sites in inverted orientations, 3 ends facing other within amplifiable distance [3]. In this study RAPD analysis was used to analyze genomic instability in patients with breast cancer.

Materials and Methods

DNA Samples

Breast cancer tissues and corresponding acid citrate dextrose (ACD) peripheral blood were obtained from five breast cancer patients who underwent surgery at Baghdad Teaching Hospital. DNAs were extracted from cancer tissues and corresponding blood samples using the standard phenol / chloroform extraction and ethanol precipitation method [5]. The samples were incubated with extraction buffer (1 ml Tris. Cl pH 8.0), 20 ml EDTA (pH 8.0), with 0.5 g of sodium dodecyl sulfate) at 37 Cº for 10 min. proteinase k was added in a final concentration of 100 µg/ ml. the suspension of lysed cells was incubated in a water bath at 50 Cº for 3 hour. The digested lysate was subjected to two further extractions with an equal volume of phenol / chloroform. After centrifugation, DNA was precipitated from the aqueous phase by two volumes of cold absoluted ethanol.

RAPD Analysis

Five arbitrary primers (Operon technologies) were used for RAPD analysis. The numbers and sequences of theses primers were; (i) OPA-0.: 5' AGT CAG CCAG; (ii) OPA-10: 5' GTG ATC GCAG; (iii) OPA-13: 5' CAG CAC CCAC; (iv) OPD-20: 5' ACC CGG TCAC; (v) OPE-07: 5' AGA TGC TGCC. Amplification reactions were performed in

volumes of 25 µl containing 10 mM Tris. Cl pH (8.3); 50 mM KCl, 15 mM $MgCl_2$, and 0.001 % gelatin (Roche. Mol. Biochem), 200 mM dNTPs (Boehriger Mannheim), 10 pmol primer, 25 ng of genomic DNA, and 1.5 U of Taq DNA polymerase (Roche. Mol. Biochem).

The reaction program included one cycle at 94 Cº for 2 min. for initial denaturation of template DNA. Then, 40 cycles of amplification were done; each cycle included 1 min at 94 Cº for template denaturation, 1 min at 36Cº for annealing of primers, and 2 min at 72 Cº for final extension. Amplification products were analyzed by electrophoresis in 1.2% agarose gel and detected by staining with ethidium bromide.

Results

Thirty four DNA fragments were amplified in the patients samples. While most of the fragments were common to both blood and tumor samples of a patient, 13 fragments were amplified in one patients' sample but not the other, and/or exhibited reduction or enhancements in the intensity which indicate genomic instability.

Samples 3 and 4 of the breast cancer tissues showed the highest genomic instability, because they were detected by all primers used in RAPD reaction. Other samples also displayed high genomic instability; since they were detected by three primers used Table (1). The detectibility of genomic instability by each primer was different: the highest belonging to primers OPA-10 and OPA-13 (100%), and primer OPE-07 being the lowest 60%, and the average detectibility of these primers was 85 percent Table (1). Primer OPD-20 failed to reveal the polymorphism, for this reason it is excluded from RAPD analysis.

Table (1): Genetic instability in breast cancer tissues detected by RAPD analysis

	Breast cancer tissues					
Primer number	1	2	3	4	5	primer detectibility(%)
OPA-03	–	+	+	+	+	80
OPA-10	+	+	+	+	+	100
OPA-13	+	+	+	+	+	100
OPE-07	+	–	+	+	–	60

+, Genetic instability detected; –, no genetic instability detected

Based on separation of amplified products on the agarose gel, we demonstrate the analysis of RAPD for each sample. Regarding sample (1), primer OPA-10 revealed single amplified DNA fragment in blood DNA sample, the corresponding amplified DNA fragment in tumor DNA sample was absent Figure (2). Primer OPA-13 led to three amplified DNA fragments, there was a missing amplified DNA fragment with a molecular weight 468 bp in tumor DNA sample compared to blood DNA sample Fig.(3). Single amplified DNA fragment was observed in DNAs of blood and tumor samples with primer OPA-07. Summary of presence (+), and absence (–), of amplified DNA fragments in tumor DNA sample compared to blood DNA sample of sample 1 is presented in Table (2) reductions and enhancements in the intensity of some of the amplified fragments were

observed in blood and tissue samples Figure (2) A 13. On the other hand, primer OPA-03 revealed smear for blood sample with no band in tumor sample Figure (1).

Table (2): Number of bands detected by RAPD with primers OPA-10, OPA-13 and OPE-07 in the blood and tumor DNAs for sample 1

Primer number	OPA-10		OPA-13		OPE-07	
Band number	B	T	B	T	B	T
1	+	–	+	+	+	+
2			+	+		
3			+	–		

B, Blood; T, Tumor; +, band present; –, band absent

Sample (2), primer OPA-03 displayed 5 amplified DNA fragments, one DNA fragment with a molecular weight 468 bp was missing in the blood DNA in comparison with tissue DNA. Only single amplified DNA fragment was observed with primers OPA-10 and OPA-13 in both blood and tumor DNA samples Table (3). There were differences in the intensity of these bands Figures (1, 2, 3). Primer OPE-07 failed to reveal reliable DNA amplified products Figure (4).

Table (3): Number of bands detected by RAPD with primers OPA-03,OPA-10 and OPA-13 in the blood and tumor DNAs for sample 2

Primer number	OPA-03		OPA-10		OPA-13	
Band number	B	T	B	T	B	T
1	+	+	+	+	+	+
2	+	+				
3	+	+				
4	+	+				
5	–	+				

B, Blood; T, Tumor; +, band present; –, band absent

Sample (3), with primer OPA-03, there were 5 amplified DNA fragments with a 468 bp missing band in blood DNA sample in comparison with tumor DNA sample. Only one amplified DNA fragments was observed with primers OPA-10 and OPE -07 Table (4). The heightened intensity of the amplified DNA fragments were found in tumor sample Figure (1,2). Primer OPA-13 revealed three amplified DNA fragments in blood DNA corresponding to smear in the tumor DNA Figure (3).

Table (4): Number of bands detected by RAPD with primers OPA-03, OPA-10 and OPE-07 in the blood and tumor DNAs for sample 3

Primer number	**OPA-03**		**OPA-10**		**OPE-07**	
Band number	**B**	**T**	**B**	**T**	**B**	**T**
1	+	+	+	+	+	+
2	+	+				
3	+	+				
4	+	+				
5	–	+				

B, Blood; T, Tumor; +, band present; –, band absent

Sample (4), three amplified DNA fragments were obtained with primer OPA-03 with no band missing or reductions or enhancements in the intensity of these bands. One amplified DNA fragment with 468 pb was absent in blood DNA sample among two amplified fragment were noted with primer OPA-10 with enhancement in the intensity of the amplified fragments that belonging to the tumor DNA. Primer OPE-07 led to single amplified DNA fragment without change in the intensity of the amplified fragments in blood or tissue DNAs. Table (5) Figure (1,2,4) A smear was observed with primer OPA-13 in tumor DNA corresponded to only one amplified DNA fragment in blood DNA Figure (3).

Table(5): Number of bands detected by RAPD with primers OPA-03, OPA-10 , and OPE-07 in the blood and tumor DNAs for sample (4)

Primer number	OPA-03		OPA-10		OPE-07	
Band number	B	T	B	T	B	T
1	+	+	+	+	+	+
2	+	+	–	+		
3	+	+				

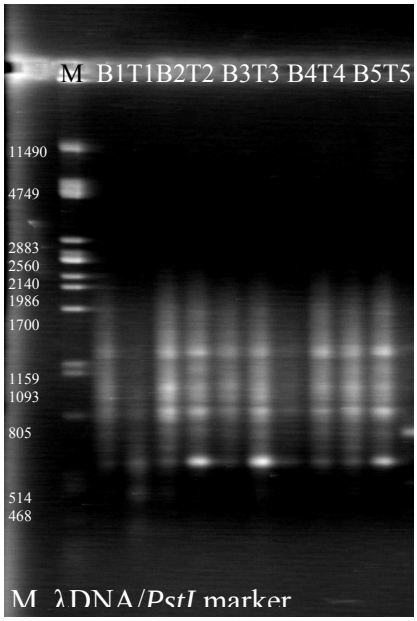
B, Blood; T, Tumor; +, band present; –, band absent

Sample (5), with primer OPA-03 in comparison with blood four amplified DNA fragment with molecular weighs 1225, 1027, 805, 468 bp were absent in tumor DNA sample. Whereas one amplified DNA fragment with a molecular weight 560 pb was absent in blood DNA sample in comparison with tumor DNA sample Table(6) Fig. (1). Primer OPA-13 resulted in four amplified DNA fragments. Three with molecular weights (1766, 1700, 1159) bp were missing in tumor DNA sample compared to blood DNA sample. There were also changes in the band intensity in the blood DNA Figure (3). There was only single amplified DNA fragment in blood DNA sample with a smear in tumor DNA sample with primer OPA-10 Figure (2). Primer OPE-07 failed to display reliable RAPD products Figure (4).

Table(6): Number of bands detected by RAPD with primers OPA-03 and OPA-13 in the blood and tumor DNAs for sample (5)

Primer number	OPA-03		OPA-13	
Band number	B	T	B	T
1	+	–	+	–
2	+	–	+	–
3	+	–	+	–
4	–	+	+	+
5	+	–		

B, Blood; T, Tumor; +, band present; –, band absent



Figre (1): RAPD patterns of 5 breast cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPA-03

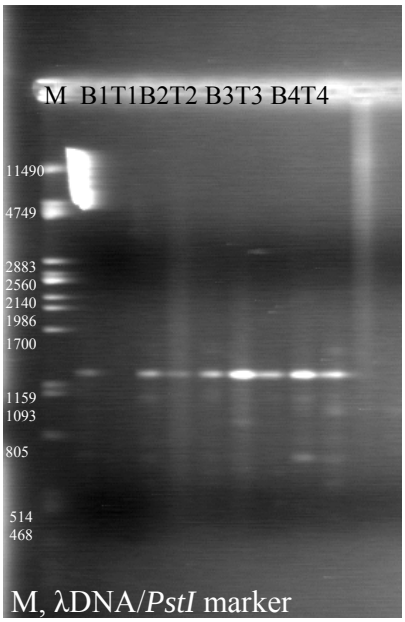


Fig (2): RAPD patterns of 5 breast cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPA-10

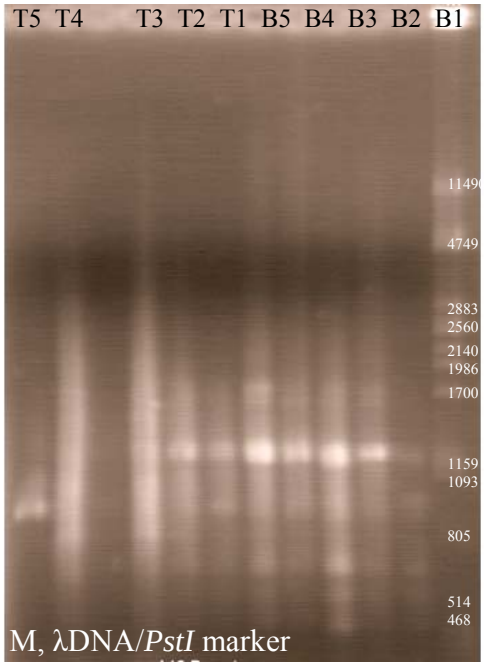


Fig (3): RAPD patterns of 5 breast cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPA-13

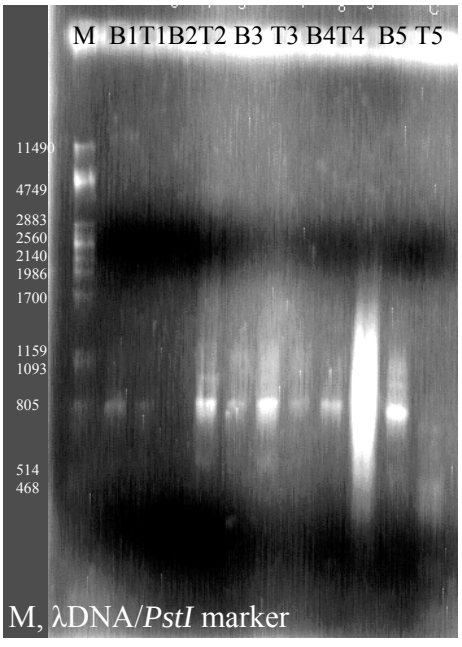


Fig (4): RAPD patterns of 5 breast cancer tissues (T) and corresponding blood DNA samples
(B) obtained with primer OPE-07

Discussion

The emergent focus in our research was used random amplified polymorphic DNA (RAPD) to generate fingerprinting that detect genomic alterations in human breast cancer. RAPD priming approach provides several advantages; it is the only method that permits the cloning, in a single step, of DNA sequences that have undergone the two most common alterations in the cancer cell genome: loss of heterozygosity and gain of extra gene sequence [6]. The difference in RAPD fingerprints arise from nucleotide substitutions that create or abolish primer sites and from deletion, insertion or inversion of priming sites or of a fragment between sites [7].

In our experiments – due to exclude OPD–20 – (80%) of all primers used revealed genetic alterations which indicate genomic instability. The ability to detect genetic instability by each primer was different, the highest belonging to primers OPA–10 and OPA–13 (100%). This indicates that some loci in the DNA or chromosome underwent changes of nucleotide sequences like the sequences in primers OPA–10 and OPA–13. On the other hand, samples (3, 4) represent the highest genomic instability since it is detected by all the used primers this reveals that a lot of DNA damages or changes had happened during the evolution of cancer [8].

Changes in the genome that were observed in this study included deletions or insertions, indicated by the absence or presence of amplified DNA fragments, and allelic losses or gain indicated by difference in the intensity of amplified DNA fragments in tumor and blood samples. The disappearance (deletion) or appearance (insertion) of an amplified DNA fragment might be associated with genetic rearrangements, or unequal mitotic recombination [9]. The ability of random priming to detect moderate gains of chromosomal fragments, which cannot be identified by restriction fragment length polymorphism (RLFR) and microsatellite allelotyping, underlines the potential of the method in tumor studies [10].

The most important finding that emerged from the present study is that an insertion of a 468bp amplified fragment was observed in 3 of 5 tumor samples two fragments using primer OPA – 03 and the third using primer OPA – 10, whereas, the same 468bp amplified fragment was deleted in 2 of 5 blood samples one fragment using the primer OPA – 03 and the second fragment using primer OPA – 13. This shows that there are different genomic alterations and mutations that participate in the cancer development [11]. Based on the RAPD analysis, it seems likely that the appearance / or disappearance of the 468bp amplified fragment in the genome of breast tumors is most likely the result of mutation or a genetic alteration commonly associated with breast tumors and , presumably, is specific for the breast tumors occurring at the OPA–03, OPA-10, and OPA–13 primers binding sites. In addition, the insertion of more than one amplified DNA fragment in the genome of sample (5) refers to that there are accumulated genetic alterations may lead to breast cancer.

An enhancement of the single intensity of an amplified DNA fragment may be related to localized overamplification of that gene locus in the genome, or could result from changes at the chromosome level, such as trisomy or tetrasomy. Similarly, a reduction in intensity could be a result of aneuploidy. Any of these events could play an important role in the development of breast cancer or they could occur during the clonal expansion of the genetically unstable tumor cells [9]. Moreover, data suggest that the molecular nature of the flanking region of the target site determines the relative intensity of the RAPD bands [12]. Primers OPA–03, OPA–10, OPA–13 led to smears or no band arrays with three tumor DNA samples whereas, only one DNA blood sample revealed smear using the OPA–03 primer. This can only be explained on the basis of mismatch between the primer and the DNA template [3].

The high genomic instability detected in breast cancer by our RAPD analysis is in line with results of [1] who suggest that the genomic damage in breast cancer is significantly higher than in Uveal melanoma, also our results agree with previous studied published by [9].

Finally, this study has revealed that RAPD – PCR fingerprinting is extremely useful for assessing genomic damage in breast cancer.

References

1. Papadopoulos, S., Benter, T., Anastassiou, G., Pape, M., Gerhard, S., Bornfeld, N., Ludwig, W.D., and Dorken, B. (2002): Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. Int. J. Cancer, 99: 193-200.
2. Baak, J.P., Path, F.R., Hermsen, M.A., Meijer, G., Schmidt, J., and Janssen, E.A. (2003): Genomic and proteomics in cancer. Eur.J. Cancer, 39: 1199-1215.
3. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18:6531-6535.
4. Welsh, J. Rampino, N., McClelland, M., and Perucho, M. (1995): Nucleic acid fingerprinting by PCR-based methods: Applications to problems in aging and mutagenesis. Mut. Res., 338: 215-229.
5. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989): Molecular Cloning: a laboratory manual, 2nd edn. Coldspring Harbor Laboratory Press.
6. Peinado, M.A., Malkhosyan, S., and Velazquez, A. (1992): Isolation and characterization of allelic losses and gains in colorectal tumors by arbitrary primed polymerase chain reaction. Proc. Natl. Acad. Sci. USA., 89:10065-10069.
7. Caetano-Anolles, G., Bassam, B.J., and Gresshoff, P.M. (1991): DNA amplification fingerprinting: A strategy for genome analysis. Plant Mol. Biol. Rep., 9: 294-307.
8. Jianxun, W., Qianwen, W., and Feng, Y. (2002): Genetic instability in cancer tissues analyzed by random amplified polymorphic DNA. Chin. Med. J., 115: 430-432.

9. Singh K.P., and Roy, D. (2001): Identification of novel breast tumor-specific mutation(s) in the q11.2 region of chromosome 17 by RAPD/PCR fingerprinting. Gene, 269:33-43.

10. Malkhosyan, S., Yasuda, J., Soto, J.L., Takao, S., Yokota, J., and Perucho, M. (1998): Molecular karyotype (amplotype) of metastatic colorectal cancer by unbiased arbitrary primed PCR DNA fingerprinting. Proc. Natl. Acad. Sci. USA., 10170-10175.

11. Jasim, S.L. (2004): Genetic polymorphism of breast tumors using polymerase chain reaction based technique. Ph.D. Thesis, College of Science, Baghdad University.

12. Venugopal, G., Mohapatra, S., and Salo, D. (1993): Multiple mismatch annealing: Basis for random amplified polymorphic DNA fingerprinting. Biochem. Biophys. Res. Commun., 197: 1382-1387.