# Assessment of genomic instability in blood and tissues from prostate cancer patients by random amplified polymorphic DNA analysis

# تقييم عدم الثبات المجيني لعينات الدم والنسيج في مرضى سرطان البروستات بواسطة تحليل الـ RAPD

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**Abstract:**

**All genetic alterations in the genome that predispose to the development of cancer are utmost importance in gaining a complete understanding of the exact molecular events involved in the development of tumorigenesis. In this study, the random amplified polymorphic DNA (RAPD) technique was used for assessing genomic instability in prostate cancer patients. DNAs were obtained from blood and tumor tissues of five prostate cancer patients, then amplified individually by RAPD with five different 10-mer arbitrary primers. The ability to detect genomic instability in five cancer tissues by each primer ranged from 20 to 100 percent. Change in the genome that revealed by RAPD technique included deletion or insertion and allelic losses or gains. The most important finding is that deletions were also observed in blood DNAs, while the corresponding fragments were present in the tumor DNA. Our results display an insertion of a 1093 bp fragment in 2 of 5 tumor samples using primer OPA-03. A deletion of the same 1093 bp fragment was observed in a blood sample using the primer OPA-03. These results confirm other reports that RAPD technique is useful for assessing genomic damage in cancer.**

**المستخلص**

**ان معرفة التغيرات الوراثية في الجينوم ( المادة الوراثية ) والتي تهيء الى حدوث السرطان يساعد في امكانية فهم للاحداث الجزيئية الدقيقة التي تتضمنها عملية تطور السرطان . في هذه الدراسة تم التحري عن عدم الثبات المجيني في دنا خمس عينات مأخوذة من نسيج دم لمرضى سرطان البروستات وخمس عينات نسيج الورم من المرضى انفسهم ، استعملت تقنية التضاعف التضاعف العشوائي المتعدد الاشكال لسلسلة الدنا (RAPD) المعتمدة على التفاعل التضاعفي لسلسلة الدنا (PCR) من اجل التحري عن عدم الثبات المجيني بأستعمال خمس بادئات عشرية القواعد اعتباطية التعاقب . تضمنت التغيرات في المجين والتي اظهرتها نواتج التضاعف العشوائي المتعدد الاشكال حذف او اضافة او فقدان او اكتساب الاليلات . فضلاً عن وجودها في دنا النسيج لوحظ الحذف في بعض عينات الدنا المأخوذة من الدم ، في حين كانت نظيراتها موجودة في دنا الورم المأخوذ من المريض نفسه . أظهرت النتائج إضافة لقطعة دنا ذات وزن جزيئي 1093 زوج قاعدة في عينتي نسيج من أصل خمس عينات باستعمال البادئ OPA-03 تؤكد نتائج هذه الدراسة في كون تقنية الـ RAPD مفيدة في تقييم الضرر المجيني في السرطان .**

## Introduction

Genomic instability is a hallmark of neoplastic transformation and a herald of genomic damage. Progress in molecular cancer genetics has facilitated the detetion of mutations related to cancer [1]. Several polymerase chain reaction (PCR) based methods have been developed to detect mutations, including random amplified polymorphic DNA (RAPD); multiplex PCR; single strand confirmation polymorphism (SSCP); and short tandem repeats (STR). All of these methods, except for RAPD, are based on the use of known gene sequences as primers for amplifications; hence, they exclude the possibility of detecting mutation of unknown and novel gene(s) that may be involved in tumorigenesis [2]. RAPD is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence [3]. This technique has been used extensively for DNA-fingerprinting, as well as for species classification and strain determination [4]. It has also been demonstrated that the instability within the genome can be detected with this fingerprinting method [5]. In present study, the assessment of genomic instability in human prostate cancer was investigated using RAPD analysis.

## Materials and Methods

### DNA Samples

Prostate cancer tissues and corresponding acid citrate dextrose (ACD) peripheral blood were obtained from five prostate cancer patients who underwent surgery at AL-Saadon Private Hospital (Baghdad, Iraq), DNAs were extracted from cancer tissues and corresponding blood samples using the standard phenol / chloroform extraction and ethanol precipitation method [6]. 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 ?C 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 ?C 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 these primers were; (i) OPA-0.3: 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).

Thermal Cycler was programmed according to Noil *et al.*, 1997[7]. The reaction program included one cycle at 94 ?C for 2 min. for initial denaturation of template DNA. Then, 40 cycles of amplification were done; each cycle included 1 min at 94 ?C for template denaturation, 1 min at 36?C for annealing of primers, and 2 min at 72 ?C for final extension. Amplification products were analyzed by electrophoresis in 1.2% agarose gel and detected by staining with ethidium bromide.

**Results:**

The primers used in RAPD analysis revealed 62 amplified DNA fragments which exhibited presence or absence or reductions and enhancements in the intensity which indicate genomic instability. The ability to detect genomic instability by each primer was different, ranging from 20 to 100 %. Sample 4 of the prostate cancer tissues showed the highest genomic instability, since it was detected by all primers used in this study, whereas, sample 1 displayed the lowest genomic instability because it could be detected by three out of five primers used Table (1).

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

| **Prostate cancer tissues** | | | | | | |
|---|---|---|---|---|---|---|
| Primer number | 1 | 2 | 3 | 4 | 5 | Primer detectibility (%) |
| OPA-03 | + | + | + | + | + | 100 |
| OPA-13 | - | - | - | + | + | 20 |
| OPA-13 | + | + | + | + | - | 80 |
| OPD-20 | - | + | + | + | + | 80 |
| PE-07 | + | + | + | + | + | 100 |

**+, 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-03 revealed 7

amplified DNA fragments. There was a missing of 805 bp amplified DNA fragment in tumor DNA sample compared to blood DNA sample. Furthermore, another missing of 468 bp amplified DNA fragment, but this was absent in blood DNA sample compared to tumor DNA sample Figure (1). 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 tissue sample compared to blood sample. Primer OPE-07 displayed two amplified DNA fragments in blood and tumor DNAs with changes in band intensity in the tumor DNA Figure (5). The other three primers did not reveal reliable DNA amplified products Figures (2, 3, 4).

**Table (2): Number of bands detected by RAPD with primers OPA-03 and OPE-07 in the Blood and tumor DNAs for sample 1**

| Primer number | OPE-07 | | OPA-03 | |
|---|---|---|---|---|
| 1. | B | T | T | B |
| 2. | + | + | + | + |
| 3. | + | + | + | + |
| 4. | + | + | | |
| 5. | + | + | | |
| 6. | + | – | | |
| 7. | + | + | | |

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

Sample 2, primer OPA-03 led to amplified 7 DNA fragments. Three were absent in the tumor DNA compared to blood DNA. These missing amplified DNA fragments were of 1225 pb, 1159 bp and 1093 pb. The heightened intensity of the amplified DNA fragments was observed in blood sample Figure (1). Primer OPD-20 displayed five amplified DNA fragments, the fifth band was absent from blood DNA Table (3) Figure (4). Four bands were observed with primer OPE-07 in blood DNA corresponding to a smear in the tumor DNA Figure (5). Primer OPA-10 and OPA-13 failed to reveal explainable RAPD products Figures (2, 3).

**Table (3): Number of bands detected by RAPD with primers OPA-03 and OPD-20 in the blood and tumor DNAs for sample 2**

| Primer number | OPA-03 | | OPD-20 | |
|---|---|---|---|---|
| Band number | B | T | B | T |
| 1 | + | – | + | + |
| 2 | + | – | + | + |
| 3 | + | – | + | + |
| 4 | + | + | + | + |
| 5 | + | + | – | + |
| 6 | + | + | | |
| 7 | + | + | | |

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

Sample 3, one band (1093 bp) among five bands was absent in blood DNA when using primer OPA-03 with changes in band intensity in the tumor DNA compared to blood DNA Table (4) Figure (1). Primer OPE-07 revealed three amplified DNA fragments with enhancements in the intensity of the amplified DNA fragments belonging to the tumor DNA Figure (5). Other primers did not succeed in achieving RAPD analysis for this sample Figures (2, 3, 4).

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

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

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

All primers used resulted in reliable RAPD products when used with sample 4. Primer OPA-03 revealed 5 amplified DNA fragments. A missing of a 1093bp amplified fragment was observed in the tumor DNA compared to blood DNA Table (5) Figure (1). There were differences in the intensity of these DNA fragments that revealed by this

primer in the tumor DNA. Four similar amplified DNA fragments were observed in DNAs of tumor and blood samples with primer OPA-10 Figure (2). Primer OPD-20 displayed 6 amplified DNA fragments with three missing fragments in blood DNA compared to the tumor DNA. These missing fragments were with 739 bp, 673 bp, and 581 bp. There were also changes in band intensity in the tumor DNA Figure (4). Primers OPA-13 and OPE-07 resulted in two and three amplified DNA fragments in blood DNA respectively, and smears in the tumor DNA Figures (3, 5).

**Table (5): Number of bands detected by RAPD with primers OPA-03, OPA-10 and OPD-20 in the blood and tumor DNAs for sample 4**

| Primer number | OPA-03 | | OPA-10 | | OPD-20 | |
|---|---|---|---|---|---|---|
| Band number | B | T | B | T | B | T |
| 1 | + | + | + | + | + | + |
| 2 | + | – | + | + | + | + |
| 3 | + | + | + | + | + | + |
| 4 | + | + | + | + | – | + |
| 5 | + | + | | | – | + |
| 6 | | | | | – | + |

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

Sample 5 revealed four amplified DNA fragments with primer OPE07; the first three were missing (1920 bp, 1634 bp and 1225 pb) in blood DNA compared to the tumor DNA Table (6) Figure (5). Single amplified fragment of DNA was observed with primer OPA-13 which revealed a smear in the tumor DNA Figure (3). There were no reliable RAPD results with primers OPA-03, OPA-10 and OPD-20 Figures (1, 2, 4).

**Table (6): Number of bands detected by RAPD with primer OPE-07 in the blood and tumor DNAs for sample 5**

| Primer number | OPE-07 | |
|---|---|---|
| Band number | B | T |
| 1 | – | + |
| 2 | – | + |
| 3 | – | + |
| 4 | + | + |

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

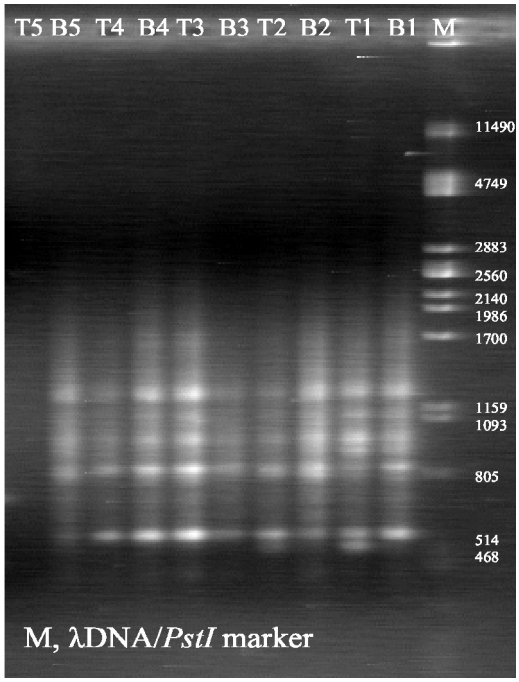



**Figure (1): DNA banding patterns of 5 prostate cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPA-03**

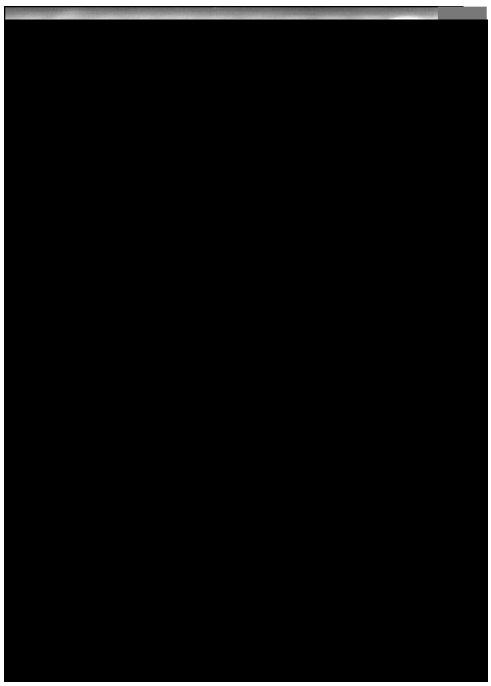
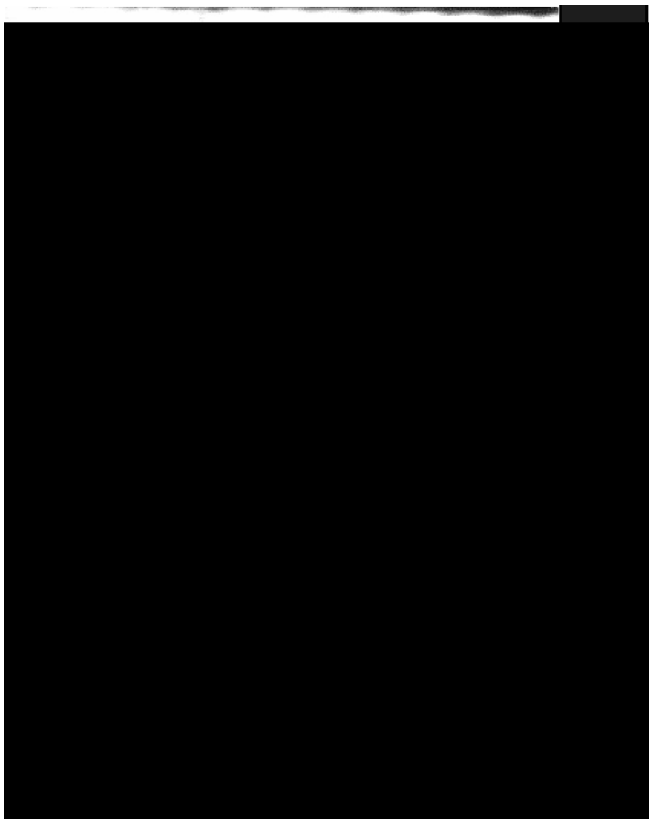


**Figure (2): DNA banding patterns of 5 prostate cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPA-10**

**Figure (3): DNA banding patterns of 5 prostate cancer tissues (T) and corresponding blood DNA samples (B) obtained with aprimer OPA-13**

**Figure (4): DNA banding patterns of 5 prostate cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPD-20**



**Figure(5): DNA banding patterns of 5 prostate cancer tissues (T) and corresponding blood DNA samples (B) obtained with primer OPE-07**

## Discussion

In the present study, we used RAPD fingerprinting to evaluate the genomic instability in prostate cancer. RAPD priming approach provides several advantages; it is only method that permits the cloning, in a single step, of DNA sequences that have undergone the two most common alterations in the cancer cells genome: loss of heterozygosity (LOH) and gain of extra gene sequence [8]. The difference in RAPD fingerprints arise from nucleotide substitutions that create or abolish primer sites and form deletion, insertion or inversion of a priming site or of fragment between priming sites [9]. The arbitrary nature of the priming events during RAPD allows for the detection of the large number of sequences amplified, generates a complex DNA fingerprint can be used to detect qualitative and quantitative differences in the entire genome of normal and tumor cells (2).

In the current study, the ability to detect genetic instability by each primer was different; this indicates that some loci in the DNA or chromosome underwent changes of nucleotide sequences like the sequence in the primers OPA-03 and OPE-07. On the other hand, the highest genomic instability represented by sample 4 of tumor tissues which reflects a lot of DNA damages or changes had happened during the evolution of cancer [10].

Changes in the genomic that were observed in this study included deletions or insertions, indicated by the absence or presence of amplified DNA fragments, and allelic losses or gains indicated by differences in the intensity of amplified DNA fragments in tumor or blood samples. The disappearance (deletion) or appearance (insertion) of an amplified DNA fragment might be associated with genetic rearrangements or unequal mitotic recombination (2). The most important finding that emerged from this study is that deletions were also observed in blood DNAs which revealed by the absence of amplified DNA fragments from blood DNA, while the corresponding fragments were present in the tumor DNA, this can be explained by that there are different genetic alterations and mutations which participate in the cancer development. Other significant result is that an insertion of a 1093 bp amplified fragment was observed in 2 of 5 tumor samples using primers OPA-03. Moreover, a deletion of the same fragment was found in a blood sample using the same primer OPA-03. it appears, therefore, that the insertion of this fragment in the genome of prostate tumor is most likely the result of mutation or a genetic alteration commonly associated with prostate tumor, and presumably, is specific for the prostate tumor occurring at the OPA-03 primer binding sites. The appearance of more than one amplified DNA fragment in the genome may refer to accumulated genetic alterations that may lead to 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 the intensity could be a result of aneuploidy. Any of these events could play an important role in the development of cancer or they could occur during the clonal expression of the genetically unstable tumor cells (2). Moreover, data suggest the molecular nature of the flanking region of the target site determines the relative intensity of the RAPD bands [11]. Our result demonstrates that RAPD can measure generalized genomic damage in prostate cancer cells.

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