Polymerase the chain reaction mediated p53 polymorphisms biology essay

Literature

p53 is the good known tumor suppresser cistron because of its protective map on the mutant of the cistrons. But there are several polymorphisms in p53 cistron and it is associated with malignant neoplastic disease formation. Therefore, research workers are extremely interested to observe the p53 polymorphism in many malignant neoplastic diseases.

In our experiment, we besides tried to measure the p53 polymorphism from MCF-7 chest malignant neoplastic disease cell line by utilizing PCR with known 9 sets of primers. Our informations revealed that codon 47 and 360 part is found to be polymorphism in this chest malignant neoplastic disease cell line. And so, we can reason that these polymorphisms itself increase the chest malignant neoplastic disease hazard or by combination with other mutant in chest malignant neoplastic disease specific cistron like BRCA1, BRCA2.

Introduction

Breast malignant neoplastic disease is distributing out all over the universe and it is the most common malignance in adult females population. There are different types of chest malignant neoplastic disease such as ductal, lobular and invasive, non-invasive. The incidence is varied depend on the geographic and cultural group.

The incidence is four crease higher in northern American than the Asians (Dale, 2003) . Breast malignant neoplastic disease is related with different familial changes such as mutants in transforming genes and tumour suppresser cistrons. Mutants in the p53 cistron are considered to stand for

the most common familial change in human malignant neoplastic disease including chest malignant neoplastic disease (Runnebaum et al. , 1991) .

Furthermore, p53 polymorphisms and their associated malignances could be differing from one population to another because of their familial background (Goldman and Shields, 1998) . p53 is a tumour suppresser cistron and besides known as defender of genome. Similarly, it governs the major defences against the tumour growing. If it is damaged either omission or mutant can take to bring on tumour formation because it regulates normal cell growing and division, written text of the cistron, DNA fix and genomic stableness (Haffner and Oren, 1995) . In the province of nerve-racking conditions such as hypoxia, heat daze, ionising radiation, ultraviolet irradiation and chemotherapeutic agents that are related to activation of p53 cistron. These nerve-racking stimulations give rise to adaptative responses to apoptosis (Agarwal et al.

, 1998) . The protein merchandise of p53 is phosphor-protein and non usually found because of their short life (Bond et al. , 2005) . The p53 protein is a powerful transcriptional factor and in normal wild type phase, it is responsible for cell rhythm apprehension, programmed cell death, suppression of angiogenesis and cellular aging by moving on CDKN1A, BBC3, BAX, PERP and THBS1 cistrons. Interaction of p53 DNA adhering sphere with genomic Deoxyribonucleic acid is the critical in the bulk of p53 mutants that often occurs in the human malignant neoplastic disease (eL-Deiry et al.

, 1992) . Therefore, p53 protein can advance or quash the look of mark cistrons in response to DNA harm (Vogelstein et al. , 2000 and Vousden &

A ; Lane, 2007) . There are several polymorphisms in p53 cistron and polymorphous allelomorphs at codon 72 of the p53 cistron plays a function in increased susceptibleness of chest malignant neoplastic disease (Costa et al.

, 2008) . Most of these fluctuations are intronic and no malignant neoplastic disease related biological effects. Merely few of the polymorphism can change the biochemical map on malignant neoplastic disease hazard population. Therefore, it is an on-going challenge to choose the discrepancies in research lab based surveies (Boldrini et al. , 2008) . In this experiment, we tried to turn up the polymorphisms of p53 protein from MCF-7 chest malignant neoplastic disease cell line at codons 47, 72, 1414, 217, 267, 278, 290, 360 utilizing the polymerase concatenation reaction method with specific 9 sets of forward and backward primers.

Materials and methods

1.

Deoxyribonucleic acid extractionWe use the Deoxyribonucleic acid from commercial MCF-7 chest malignant neoplastic disease cell line. And so, trypsinise to take the cells form civilization flask and extracted the Deoxyribonucleic acid by utilizing Wizard Genomic DNA purification kit (Promega, catalogue figure A 1120) . 2. Deoxyribonucleic acid quantificationExtracted Deoxyribonucleic acid was read in spectrometer at wavelength 260nm and 280nm by doing dilution of genomic Deoxyribonucleic acid with H_2O 1 in 100 times. 3. Restriction enzyme

digestion for makingAfter quantified the Deoxyribonucleic acid, we check the quality of DNA once more before PCR tally.

The extracted DNA was digested with EcoRI limitation enzyme from Fermentas. And so, electrophosed on 1 % agrose gel, stained with ethidium bromide and look into the Deoxyribonucleic acid by utilizing UV visible radiation gel physician system. 4. Fix 9 sets of primers for PCRWe selected these 9 sets of primers to observe the sites of polymorphisms in p53 cistron47 F – 47R72 F – 72 Roentgen72 F – 72_1414_R217 F – 217 Roentgen267 F – 267 Roentgen278 F -278 R290 F – 290 R1290 F – 290 R2360 F – 360 Roentgen5. Polymerase concatenation reaction (Promega PCR maestro mix cat: M7502)Polymerase concatenation reaction (PCR) was done with genomic DNA and 9 sets of primers by utilizing PCR maestro mix catalog M7502 (Promega, Madison USA) . Each reaction was carried out with 9. 5ul of H2O, 12.

5ul of PCR Master Mix (which consists of 50units/ml of Taq DNA polymerase, 400uM of dNTPs and 3mM of MgCl2) , 1ul of forward primer, 1ul of backward primer and eventually adds 1ul of genomic DNA. The entire mixture will be 25ul. We prepared two sets of PCR machines with different tempering temperature: 55 ‘ C for one machine and 60’C for another one. All reactions were ab initio denatured with 94A°C at 4 proceedingss.

Subsequent denaturations were done at 94A°C for 45 seconds followed by 60A° or 55 A°C for 45 seconds and so, 72A°C for 1 minute. Last, concluding extension at 72A°C for 10 proceedingss was recommended. These rhythms were repeated 35 times and hive away the samples at 4’C for nightlong. 6.

Runing the PCR merchandises on gel cataphoresisAll these PCR merchandises were run on 1 % agarose gel after staining with ethidium bromide that intercalates with DNA. We used the DNA ladder to corroborate the figure of basal braces of PCR merchandises. Run the gel at 120V until the lowest set base on balls through half of the gel and so visualized under UV visible radiation with gel physician system.

Findingss and consequences

The extracted genomic Deoxyribonucleic acid was quantified by spectrophotometer.

Under 260nm, the optical denseness (OD) was 0. 253 and under 280nm, OD was 0. 175.

Therefore, the ratio of optical density under 260 and 280nm was 1. 44. In 1 optical denseness, the sum of DNA is 500I? g/ml at Absorbance 260nm.

So, the extracted genomic Deoxyribonucleic acid has sum of 1. 26 I? g/ml. Figure 1: exposure of agarose gel under gel physician system demoing the consequences of EcoRI limitation enzyme digested symbolsAs you seen in the image, there was no set in the lane 2 where it contained undigested genomic Deoxyribonucleic acid.

In contrast, diluted genomic Deoxyribonucleic acid in lane 3 was banded. And besides EcoRI restricted DNA in lane 4 appeared as a set. Two Deoxyribonucleic acid ladders were added in lane 1 and lane 5. Figure 2: exposure of agarose gel under physician system demoing the sets of PCR merchandisesMerchandises of PCR were stained with ethidium bromide and

separated on the 1 % agarose gel by cataphoresis. Then, when they were visualized with UV visible radiation under gel physician system, amplified fragments were seen as sets in their several lanes. Furthermore, two Deoxyribonucleic acid ladders were clearly seen in lane 1 and 11. But, there were no sets in lane 2 and 10.

This meant that there was no amplified PCR fragment in those lanes. Hence, codon 47 part and codon 360 parts could non be amplified. Lane 3 incorporating 72 show the set about 328 base brace (bp) whereas lane 4 incorporating codon 72 with fragment 1414 show the set but it does non make their appropriate size. Codon 217 in lane 5 besides appear the set of about 300 bp.

Lane 6 with codon 267 show the existent size of 778 bp set. Another amplified fragment in lane 7 (codon 278) contain about 479 bp while lane 8 (codon 290) besides show the set. Second last lane (lane 9) merely appears the set, approximately 569 bp.

Discussion

The parts of polymorphism in p53 cistron from MCF-7 chest malignant neoplastic disease cell line can be identified by comparing the consequences with normal wild type p53 proteins consequences. Harmonizing to our experiment, sum of extracted Deoxyribonucleic acid that we quantified in first measure earlier PCR tally is 3795ng. After that, we checked the purification of Deoxyribonucleic acid by ration of A 260: A 280 and the consequence is about 1. 44. It besides reveals that the sample is purified plenty harmonizing to the criterion protocol that is 1.

5. In the figure 1, there are multiple sets in the lane 4, it means EcoRI digestion was achieved and EcoRI restricted the genomic Deoxyribonucleic acid at the multiple sites. We added pure genomic Deoxyribonucleic acid in lane 2 and diluted genomic Deoxyribonucleic acid in the lane 3 as a control. There is a set in lane 3 and lane 2 shows no set.

It is due to non-diluted DNA (pure genomic Deoxyribonucleic acid) is somewhat tough to run during cataphoresis. Positive sets in lane 3 mean that there is no mistake in gel readying and cataphoresis. In figure 2, there are no seeable sets in lane 2 and 10 on the Polaroid exposure after PCR running. It means that the part of codon 47 and codon 360 may non be amplified.

The consequence may be due to proficient mistake or mutant and altering the base brace in this part. Some of the surveies show that the mutant in the codon 47 from proline to serine (CCG to TCG) is associated with reduced phosphorylation in p38 MAPK and besides lessening in programmed cell death that leads to malignant neoplastic disease formation (Kruse and Gu, 2008) . Similarly, primers of codon 360 did non temper in the lane 10 because this part may be mutated. Mutant in codon 360 is associated with permutation of glycine to alanine, this is the linker part to domain of p53. In bend, consequences in decrease transactivation of pro-apoptotic cistrons (eg. BAX, MDM2) .

The other lanes such as lane 3, 4, 5, 6, 7, 8, 9 show a set severally. These consequences are the same with wild type p53 consequence significance there is no polymorphism in these codon parts. To reason that p53 cistron

play a function in normal cell division and DNA fix and are critical for observing inappropriate growing signals in cells. If these cistrons, as a consequence of familial or acquired mutants, become unable to work, familial mutants in other cistrons can predate unbridled, taking to neoplastic transmutation.

Therefore, p53 polymorphisms can be associated with non merely breast malignant neoplastic disease but besides other types of tumour such as lung, colorectal and ovarian malignant neoplastic disease (Sjalnder et al., 1995)

Recognitions

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