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CHAPTER 1: Defining minimal lost region (MLR) on 17p13 and 17q21-23 by Low resolution LOH mapping in sporadic breast tumors and their correlation with clinical parameters.

1.1 Introduction 11-24

i. Breast cancer incidence
ii. Breast cancer biology
iii. Chromosome 17 and Breast cancer Genetics: Questions unanswered
iv. LOH mapping to study Minimal lost region (MLR)
v. Loss of heterozygosity LOH at 17p & 17q region in breast tumors
vi. Simple tandem repeat alterations (Microsatellite instability: MSI) in breast cancer

1.2 Materials & Methods 25-28

i. Chemicals and biochemicals
ii. Blood and tumor tissue samples
iii. High Molecular Weight Genomic DNA Isolation from Peripheral Blood Samples
iv. High Molecular Weight DNA Isolation from Tumour Tissue
v. Agarose Gel Electrophoresis
vi. PCR amplification of STS markers
vii. Loss of heterozygosity analysis
viii. Statistical analysis.

1.3 Results
1.4 Discussion
1.5 References

CHAPTER 2: In-silico analyses of marker regions on build 33 sequence maps and an ab-initio gene prediction from a selected marker region.

2.1 Introduction

i. In-silico cloning

ii. ab-initio gene finding methods

iii. Recent advances in ab-initio gene findings

2.2 Material & Methods

i. Markers and their sequences

ii. Chemical and biochemicals

iii. In-silico analysis of studied STS marker regions on Build 33 sequence map

iv. Ab-initio gene prediction analysis of D17S934 marker region

v. Isolation of total cellular RNA from blood lymphocyte and breast tissues.

vi. Single strand cDNA synthesis using oligo dT

vii. Transcript analysis by cDNA amplification

viii. Positive transcript (cDNA) sequencing

ix. Mutation analysis of the amplified predicted gene region in Sporadic breast cancer patients
2.3 Results

i. In-silico analysis of 17p13.3 region covered by D17S5-D17S379 Marker

ii. In-silico analysis of 17q21-23 region covered by D17S855-D17S934-D17S787-D17S948 Markers

iii. Ab-initio gene prediction in the D17S934 Marker region

iv. PCR & RT-PCR analysis of the coding regions of predicted gene (BK 000585)

v. Genomic and cDNA sequencing and BLAST analysis with human genome

vi. Mutation analysis of novel EST region in sporadic breast tumors by SSCP and SSLP.

2.4 Discussion

2.5 References

CHAPTER 3: Genomic instability and mutation analysis of candidate gene regions.

3.1 Introduction

i. BRCA2

ii. Interferon-γ (IFN-γ)

iii. Interleukin-6 (IL-6)

iv. Transforming growth factor –β 1 (TGF-β 1)

v. KRTHB6 (keratin gene)

3.2 Material and Methods

i. Chemicals and biochemicals

ii. Blood and tumor tissue

iii. PCR of the selected region of candidate genes

iv. Mutation analysis of selected region of candidate genes

v. DNA band elution from silver stained PAG
vi. Elution of DNA bands from agarose gels
vii. Cloning of PCR product in pGem vector
viii. Preparation of competent cells by calcium chloride treatment
ix. Transformation of competent cells
x. Single colony PCR
xi. Plasmid DNA isolation
xii. DNA sequencing
xiii. Statistical analysis

3.3 Results

i. Mutation analysis by PCR-SSCP and sequencing of the selected regions of BRCA2 gene
ii. Mutation analysis of IFN-γ gene in sporadic breast cancer cases
iii. Mutation analysis of IL-6 gene promoter region
iv. Mutation analysis of TGF-β promoter, 5’UTR and exon 1 gene region in sporadic breast tumors
v. Mutation analysis of promoter and helix termination motif (HTM) region of type II keratin gene KRT6B
vi. A case control study of TGF-β promoter, 5’UTR and exon-1 polymorphism

3.4 Discussion

3.5 References

Summary

Appendix