GENERAL INTRODUCTION
General Introduction

Cancer is a disease of impaired genome stability. That the genetic instability is underlying cause of abnormal growth in cancer was first proposed by Boveri (1914). Genomic instability is the dynamic process of genomic changes in a tumor cell over time. It is widely accepted that cancer results from the accumulation of mutations in the genes that directly control cell birth and death. It has been argued that an underlying genetic instability is absolutely required for generating multiple mutations that underlie cancer (Loeb et al., 1991; Hartwell et al., 1992). The genome of cancer cells displays elevated mutation rates during tumor progression, resulting in alterations in the base sequences and/or large changes in chromosome architecture. There is, however, a controversy on the origin of genomic defects in cancer. One group maintains that genomic instability is needed early on, to set cells on the path to cancer; whereas another group asserts that cancer cells start out with no more proneness to genomic instability than normal cells. In the latter situation, it has been suggested that normal rates of mutation coupled with waves of clonal expansion are sufficient for the process to occur in humans (Tomlinson et al., 1996; Wang et al., 2002).

Two main forms of genomic instability are associated with tumor cells (Lengauer et al., 1998). The mutational instability (MIN) phenotype is characterized by point mutations or small deletions. This type of instability usually arises due to mismatch repair (MMR) gene defects, which also is associated with microsatellite length variation, referred to as microsatellite instability (MSI). The chromosomal instability (CIN) phenotype is characterized by the gross rearrangement of chromosomes. The common chromosomal alterations include the loss or gain of whole chromosomes or chromosome fragments, and the amplification of chromosome segments. Chromosomal instability (CIN) is associated with the defects in the double-strand-break repair pathways (Van Gent et al., 2001, Lo et al., 2002, Rassool et al., 2003) or even with the stalled replication forks at template lesions (Cox et al., 2000, Weitao et al., 2003, Thompson et al., 2003).

A form of chromosome instability (CIN) commonly observed as loss of heterozygosity (LOH) has very often been implicated in some cancers, leading to allelic imbalance and haploinsufficiency. LOH is a hallmark of the presence of a tumor suppressor gene (TSG). All cells contain two copies of autosomal genes-one copy inherited from each parent; and
if a cell develops a mutation in one allele of a tumor-suppressor gene (TSG), loss of the remaining wild type allele is characterized as loss of heterozygosity (LOH). According to Knudson’s ‘two-hit’ hypothesis (Knudson 1971), inactivation of both alleles of a TSG is required for cancer formation. The mechanism of somatic hyper-mutability provides sufficient justification for identifying predisposition genes by studying somatic mutation in a candidate gene region. In sporadic cancers, a somatic mutation targeting each allele is required to completely inactivate a TSG. Inactivating mutations include point mutations, loss of chromosomal material, gene conversion, or mitotic recombination or deletion (Knudson 1978, Cavenee et al., 1983). Therefore, chromosomal regions that frequently exhibit allelic losses are expected to harbor putative TSGs (Johnson-Pais et al., 2003).

Loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) analysis are currently the two methods most frequently used for detection of chromosomal losses and for mapping the genes (Konishi et al., 2003, Melendez et al., 2003, Cesari et al., 2003, Narayan et al., 2003, Wessendorf et al., 2003). Mapping of sub-chromosomal regions, which exhibit loss or deletion in tumors are key to localize one or more putative TSGs in a critical region (Hahn et al., 1996; Li et al., 1997). In one such chromosome, the chromosome 17, chosen in this study, alterations are being reported in various cancers (van Dartel et al., 2002; Dunn et al., 2003; Santos et al., 2003; Uppal et al., 2003; Suspiro et al., 2003; Konishi et al., 2003; van Dartel et al., 2003; Zhao et al., 2003; Doneda et al., 2003) and in breast cancer, too (Watters et al., 2003), suggesting its role and importance in cancer pathogenesis. This chromosome harbors two important TSGs, TP53 (17p13) and BRCA1 (17q21). Both susceptibility genes are infrequent targets for somatic inactivation in sporadic breast tumors (Sullivan et al., 2002; Esteller et al., 2000). In addition to the presence of germline mutations in BRCA1, located on 17q21, another related gene, the BRCA2, located on 13q12-13 is known to cause genetic susceptibility in approximately 10% of the breast cancer cases which cluster in families. The majorities (approx. 90%) of cases, however, are sporadic and their defined genetic components are yet not known.

The pace of gene finding in cancers by deletion mapping using sequence tag site (STS) markers has been aided by the information available in the human genome sequence map (Lander et al., 2001; Venter et al., 2001) and its exploration with in-silico tools. In this study we have adopted the approach of using widely spaced (low resolution) STS markers on both short and long arm of chromosome 17, around the known critical regions involved in breast cancer or other cancers. The study which is the focus of the first chapter
(CHAPTER 1) narrows down the region of interest by low resolution mapping of STS markers and by scoring the frequency of LOH and MSI for these markers. The purpose of the study has not been to add more number of patients of sporadic breast cancer (which incidentally were available in limited numbers) but to proceed with further studies. Once the region of interest with the highest LOH is defined, the work progresses to find out the putative TSGs in the region through *in-silico* mapping and analysis of markers on the most recent Build-33 sequence map (http://www.ncbi.nlm.nih.gov/mapview/) released by National Center for Biotechnology Information (NCBI), for *ab-initio* gene prediction in the second chapter (CHAPTER 2). An attempt is also made to authenticate the presence of a new gene (partially) in the region used for ‘unknown’ gene prediction. Since the loss of genomic integrity is associated with cancer and the candidate genes which play the role of “genome guardians” could be the target for damage, the role of some of the randomly selected genes which could or are known to play a role in genomic stability, are also studied as a subject of the third chapter (CHAPTER 3). Incidentally, it has been observed that the tumors of BRCA2 mutation carriers appear to have more similarities with the sporadic cases than those of BRCA1 carriers (Lakhani *et al.*, 1998) which has prompted us to concentrate on BRCA2 gene survey in this chapter. The role of BRCA2 in maintaining genome stability is also another reason for its inclusion in this study. Since the genetic components are yet not defined for sporadic breast cancers and it is said that sporadic breast cancers could be the result of mutations in the multiple known and unknown low penetrance genes (DNA damage repairs, growth related genes, immuno-regulatory genes/molecules) (reviewed in Nathanson *et al.*, 2001) and the susceptibility to cancer could be greatly influenced by gene–gene interaction between low penetrance genes (de Jong *et al.*, 2002), a set of other candidate genes both relevant (IFN-γ, IL-6, TGF-β1) and innocuous (hair specific KRT6H6) (for comparison purposes) have been studied in this chapter too (CHAPTER 3). The purpose of choosing to study specific regions of these genes has been to assess the proneness to somatic mutations in the tumor samples in sporadic breast cancer patients.
References

Boveri, T. In Zur Frage der Entstehung Maligner Tumoren 1-64 (Gustav Fischer, Jena, 1914)


Moskovitz AH, Linford NJ, Brentnall TA, Bronner MP, Storer BE, Potter JD, Bell RH Jr, Rabinovitch PS. Chromosomal instability in pancreatic ductal cells from patients with


Aims and Objectives

The study was designed to carry out in three parts, distributed as three chapters in this presentation. The aim of the work was to look for a candidate region on chromosome 17 which could be explored for the presence of a previously unknown relevant sequence or a gene and authenticate the involvement of such a sequence or its association with sporadic form of breast cancer. Since role of more than one gene is attributed in the causation of any sporadic form of tumors, some of the relevant candidate genes and an innocuous gene for comparison purposes, were included in the study to understand if their studied region could be a target for somatic mutation in sporadic breast tumors. The study was carried out with the following objectives:

i) to carry out low resolution mapping of chromosome 17p13.3 and 17q21-23 regions by studying loss of heterozygosity (LOH) and microsatellite instability (MSI); to define the minimal lost region (MLR) using thinly placed, syntenic, PCR amplifiable, STS markers in the blood and tumor samples of sporadic breast cancer cases and to draw a correlation, if any, with available clinical parameters.

ii) to fine map the informative region by in-silico analysis on build 33 sequence map, carry out gene prediction with ab-initio approaches and validate the presence of a sequence or a gene partially or fully (if possible) which shows either allelic instability (LOH/ MSI) or germline/ somatic mutation(s).

iii) to study the somatic variation in tumor samples in selected region(s) of the following candidate genes i.e. BRCA2 (known to depict germline mutations in familial breast cancers with a role in repair and genome stability), IFN-gamma, IL-6, TGF-beta-1 (known to play a role in immune surveillance, cellular response and genome stability) and KRTHB6 (an innocuous hair specific keratin gene - a candidate for control comparison).