Chapter -1

Introduction

1.1 An overview of cancer

The health scenario today is significantly different from last millennium with marked improvements in all the vital indicators of health development. However, much remains to be done in cancer treatment to overcome the high mortality rate from the disease in India. As we stand at the beginning of a new millennium, it is imperative to review the specific patterns and pace of cancer, its enormous burden and our preparedness to meet the challenges in the developing countries. Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Globally, the burden of new cases in 2000 was estimated to be 10.1 million representing a 20% incidence over the previous decade with 53% occurring in the developing world. Similarly 56% of the estimated deaths from cancer occur in the developing world. This is projected by the WHO to dramatically increase to 20 million by 2020 to 70% in the developing world which has access to only 5% of the global resources (Parkin et al., 2005). In India, it is estimated that there are approximately 2 - 2.5 million cases of cancer in the country at any given time. Nearly 800,000 cases were diagnosed in the year 2000 and several deaths due to cancer occurred in the Indian population. In fact, it is estimated that, around 555 000 people died of cancer in India in 2010 (Dikshit et al., 2012).

Miller and Miller in 1947 proposed that carcinogenesis resulted from a permanent alteration or loss of protein essential for control of growth described in deletion hypothesis proposed by (Ruddon, 2007). In 1958, Potter proposed the "feedback deletion hypothesis" which suggested that "repressors" crucial to the regulation of genes involved in cell proliferation are lost or inactivated by the action of oncogenic agents on the cell, either by interacting with DNA to block repressor-gene transcription, or by reacting directly with repressor proteins and inactivating them. This prediction anticipated the discovery of tumor-suppressor proteins, such as *TP53* and *BRCA1*, by about 25 years (Ruddon, 2003). Hanahan and Weinberg have

suggested that cancer is the result of six essential alterations in cell physiology namely self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These capabilities are acquired by and common to most human tumors, and have been referred to as the "Hallmarks of Cancer" (Hanahan and Weinberg, 2011). Lee and Muller provide a basic idea from their work to understand the function of tumor suppressor genes and its malfunction when it undergoes mutation (Lee and Muller, 2010). *BRCA1* gene plays a major role in breast cancer for that reason it is called as "a strong candidate for breast cancer" (Miki et al., 1994).

1.2 Breast cancer menace in the population of southern Assam

Among all cancers, breast cancer is the most frequently diagnosed cancer in women (Jemal et al., 2011). Gender is a crucial risk factor for breast cancer. Risk is also increased by inherited genetic mutations in the tumor suppressor genes, a personal or family history of breast cancer, high breast tissue density, biopsy-confirmed hyperplasia, and high-dose radiation to the chest, typically related to a medical procedure. Reproductive factors that increase risk include a long menstrual history, never having children, recent use of oral contraceptives, and having the first child after the age of 30. Some potentially modifiable factors that increase risk include being overweight or obese after menopause, use of postmenopausal hormone therapy (especially combined estrogen and progestin therapy), physical inactivity, and consumption of alcohol. Breast cancer in men is a rare disease, accounting for 1% of all breast cancer cases. Major genetic factors associated with an increased risk of breast cancer for men include *BRCA2* mutations, which are believed to account for the majority of inherited breast cancer in men. Epidemiologic risk factors for male breast cancer include disorders relating to hormonal imbalances such as obesity, testicular disorders and radiation exposure.

At least 5 percent of all breast cancer cases are thought to result from a hereditary predisposition to the disease. Women who inherit loss-of-function mutations in one allele of either the *BRCA1* or *BRCA2* gene have an up to 85 percent risk of breast cancer by the age of 70. Carriers of mutations in these genes are also at elevated risk of cancer in the ovary, pancreas and prostrate (Friedenson, 2005). Because *BRCA1* and *BRCA2* are expressed in a broad spectrum of tissues and cell types it is not clear how loss of their functions can lead to tissue and gender-specific cancers. The breast and ovary are estrogen-responsive tissues with estrogen metabolites thus acting as tissue-specific carcinogens. Moreover the breast and ovarian epithelia proliferate rapidly under the influence of estrogens, the progeny of this proliferative burst being retained in the breast lobules and ovarian inclusion cysts. A cancer-predisposing mutation, such as in *TP53* in a lobular precursor cell, accompanied by loss of *BRCA* mutations, could thus increase the risk of breast and ovarian cancer (Scully and Livingston, 2000; Sowter and Ashworth, 2005).

A very high incidence of cancers at all sites in general has been reported in Northeast region of India. However, the available data on tobacco usage and pesticide exposure alone is not sufficient to explain the high incidence. Northeast region of India has different customs, food habits, life-style, diverse ethnic groups, type and pattern of tobacco use in as compared to the rest of the country. It is very well known that the carcinogenicity of tobacco is attributed to nitrosamines, polycyclic aromatic hydrocarbons (PAHs), benzene, Benzo(a)pyrene etc. Moreover there is extensive use of pesticides in tea gardens in Northeast which can lead to widespread occupational and environmental exposures. Indian council of Medical Research (ICMR) has started Cancer Registries in the Northeast under the National Cancer Registry Program (NCRP). Multicentric studies have been initiated by ICMR to find out the genetic factors, in addition to common environmental exposures, tobacco smoking, alcohol consumption, pesticide

exposure and dietary habits which could possibly explain the high prevalence of breast head and neck cancers in Northeast India. These multi-centric studies is investigating the link between carcinogenic contents of tobacco and pesticides used in the Northeast and genetic variation including polymorphism/mutations associated with ethnic variation. India is facing a cancer epidemic. By 2020, breast cancer is set to overtake cervical cancer as the most common type of cancer among all women in India (Shetty, 2012). And she also disclosed Ravi Kannan words "Women sometimes come into Cachar Cancer Hospitals and Research Centre (CCHRC), Assam, Northeast India, with lumps in their breast that are 30-35 cm large. These heavy masses of cancerous cells protrude outside the body, ulcerating or teeming with maggots". Our study in collaboration with CCHRC have been screened the breast cancer patients of Southern Assam with three methodological ways such as cytopathological screening, mutational screening and low penetrance gene's involvement in breast cancer patients.

1.3 Cancer associated genes in breast cancer

Cancers occur when a buildup of genetic mutations in critical genes those that control cell growth and division or the repair of damaged DNA allow cells to grow and divide uncontrollably to form a tumor. In most cases, these genetic changes acquire during a person's lifetime and are present only in certain cells. These changes, which are called somatic mutations, are not inherited. Less commonly, gene mutations inherited from a parent increase the risk of developing cancer. In people with these inherited genetic changes, additional somatic mutations in other genes must occur for cancer to develop. Variations of the *BRCA1, BRCA2, CDH1, STK11,* and *TTP53* genes increase the risk of developing breast cancer. The *AR, ATM, BARD1, BRIP1, CHEK2, DIRAS3, ERBB2, NBN, PALB2, RAD50, and RAD51* genes are associated with breast cancer (Koboldt et al., 2012). In addition to specific genetic changes, researchers have identified many personal and environmental factors that may influence a person's risk of developing breast cancer. These factors include

gender, age, ethnic background, a history of previous breast cancer, certain changes in breast tissue, and hormonal factors. A history of breast cancer in closely related family members is also an important risk factor, particularly if the cancer occurred at an early age. Some breast cancers that cluster in families are associated with inherited mutations in particular genes, such as *BRCA1* or *BRCA2*.

Somatic mutations also have been identified in breast tumors. For example, somatic mutations in the ERBB2 (also called Her-2/neu), DIRAS3, and *TP53* genes have been associated with some cases of breast cancer. Tumor suppressor genes are genes whose loss-of-function releases the constraint on cell growth and is therefore tumorigenic. They thus have an effect opposite to oncogenes. Tumor suppressor genes are vulnerable sites for critical DNA damage because they normally function as physiologic barriers against clonal expansion or genomic mutability and are able to hinder the growth and metastasis of cells driven to uncontrolled proliferation by oncogenes (Nowak et al., 2006). Loss of tumor-suppressor function can occur through damage to the genome by mutation, chromosomal rearrangement and non-disjunction, gene conversion, imprinting or mitotic recombination. The tumor suppressor activity can also be neutralized by interaction with other cellular proteins or viral oncoproteins (Harris and Hollstein, 1993). Several tumor suppressor genes associate in breast cancer have been shown in Table 1.1.

Sl. No.	**Gene**	**Function**	**Chromosome Location**	**Tumor types observed**
1	***BRCA1***	Cell cycle control, controlling protein degradation, DNA damage repair, and transcriptional regulation; interacts with Rad51 in DNA repair	17q21	Breast ovarian
2	***BRCA2***	Transcriptional regulation of genes involved in DNA repair and homologous recombination	13q12.3	Breast ovarian
3	***TP53***	Cell cycle regulation, apoptosis	17p13.1	Breast,

				Brain sarcomas, leukemia
4	***CDH1***	Cell-cell adhesions, mobility and proliferation of epithelial cells	16q22.1	Breast, gastric
5	***STK11***	Cell metabolism, cell polarity, apoptosis and DNA damage response	19p13.3	Testicular laryngeal
6	***AR***	Regulates the activity of androgen-responsive genes	Xq12	Breast
7	***ATM***	Cell division and DNA repair	11q22-23	Breast, lung, ovarian
8	***NBN***	Response to DNA damage and the maintenance of chromosome integrity	8q21	Breast Cancer
9	***PALB2***	Interacts with the *BRCA2* protein and plays a role in homologous recombination and double-stranded DNA repair	16p12.2	Breast and pancreatic
10	***RAD50***	Double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis	5q31	Breast
11	***RAD51***	Maintaining the stability of a cell's genetic information	15q15.1	Breast
12	***ERBB2***	Cell adhesion, cell specialization, cell movement	17q12	Breast, Lung,Brain, Stomach

Table 1.1 Associated genes with breast cancer (Adapted from Fisher, 2001)

1.4 Associated tumor suppressor genes in breast cancer

As tumor suppressor genes function in the prevention of tumorigenesis, it is evident that they are required not only to control cellular proliferation, but also to main the stability of the genome. Kinzler and Vogelstein have accordingly classified tumor suppressor genes into "gatekeepers" and "caretakers" (Kinzler and Vogelstein, 1997). Here we consider two tumor suppressor genes to study *BRCA1* and *TP53* in breast cancer of southern Assam.

1.4.1 *BRCA1* as caretaker gene

The breast cancer-associated *BRCA1* gene is located in humans on chromosome 17 at position 17q21. The *BRCA1* gene was identified by positional cloning methods as the gene responsible for increasing susceptibility to breast and ovarian cancer by (Miki et al., 1994). The gene shown in Figure 1.1 encompasses 24 exons in approximately 81 kb of genomic DNA and codes for a protein of 1863 amino acid residues (Oesterrich and Fuqua, 1999). *BRCA1* mutations are thought to account for about 45% of families with high breast cancer risk, and greater than 80% of families with high risk of early onset breast and ovarian cancers (Nadeau et al., 2000). The *BRCA1* gene contains 42% *Alu* sequences and 5% non-*Alu* repeats. The presence of such repeats may be responsible for the large deletions in and around the *BRCA1* gene observed in both inherited and sporadic breast and ovarian cancers, due to homologous recombination between the repeat sequences (Welsch and King, 2001).

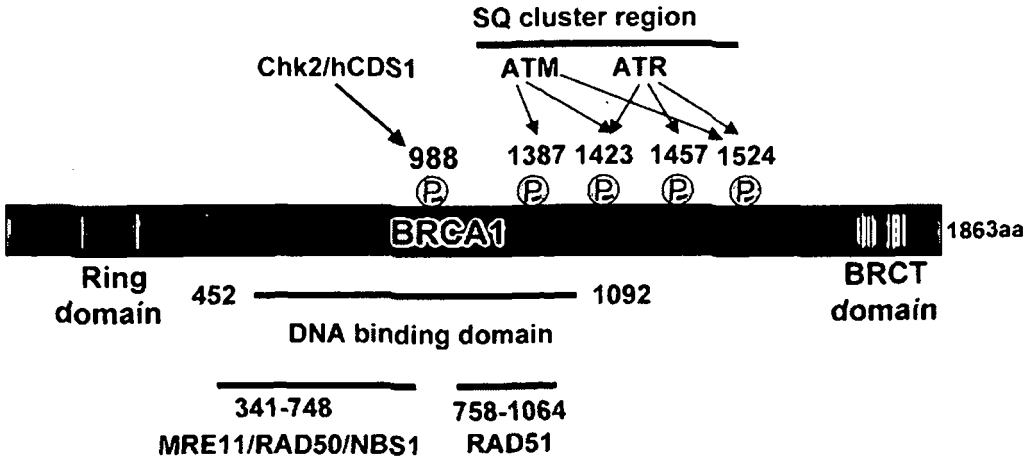

Figure 1.1 A schematic diagram of *BRCA1* protein structure, with its DNA binding domain, ring domain and BRCT domain (adapted from Zhang and Powell, 2005).

The N-terminal RING finger of *BRCA1* interacts with *BRCA1*-associated RING domain 1 (BARD1) protein, which recruits *BRCA1* to the nucleus, and with the deubiquitinating enzyme, *BRCA1*-associated protein-1 (BAP1). *BRCA1* also has two nuclear localization signals (NLS) and nearby regions which interact with *TP53*, c-myc, pRb, RAD50, MRE11 and NBS-1. A domain within *BRCA1* (amino acids 758-1064) interacts with RAD51. The *BRCA1* C-terminal (BRCT) repeats interact with *BRCA2*, histone deacetylase (HADC) 1 and 2, RNA helicase A (RHA), p300/CBP and the CtBp-interacting protein (CtIP) (Clark et al., 2012).

1.4.1.1 Role of *BRCA1* in Breast Cancer

Biochemical, genetic and cytological studies have revealed multiple functions for *BRCA1*. The *BRCA1* proteins have been found to be involved in the control of homologous recombination (HR) and double-strand break (DSB) repair in response to DNA damage (Welsch and King, 2001). *BRCA1* is involved in various protein-protein interactions relevant to DNA repair. It interacts with the MRE11/RAD50/Nbs1 complex, known to participate in DSB repair with MRE11 encoding a nuclease activity which resects flush DSB ends to generate ssDNA tracts, which are substrates for HR. *BRCA1* may also have local activities at DSB sites through its interaction with enzymes that alter chromatin structure. *BRCA1* also functions by regulating the expression of *GADD45*, a tumor suppressor gene that is also a downstream target of the *TP53* pathway. *GADD45* transcription is normally suppressed by a co-repressor complex in which *BRCA1* associates with the KRAB domain transcription factor ZBRK1. After ionizing irradiation, phosphorylation of *BRCA1* by ATM relieves *GADD45* repression. In this way, *BRCA1* processes signals through ATM to achieve transcriptional regulation of *GADD45* in response to DSB (Yoshida and Miki, 2004).

BRCA1 is rapidly phosophorylated after DNA damage in dividing cells by the *ATM*, *ATR* and *CHK2* kinases. As shown in Figure 1.2, phosphorylation

of *BRCA1* by each of these kinases is activated by distinct stimuli and targeted to distinct clusters of serine residues- ATM and CHK2 phosphorylate *BRCA1* after ionizing radiation, whereas ATR is more specifically activated after UV-irradiation or replication arrest (Zhang et al., 2013). *BRCA1* then induces *TP53*-independent G1/S- arrest by interacting with hypophosphorylated pRb or by p21-induction (Rastogi et al., 2010). *BRCA1* may also induce *TP53*-dependent G1/S arrest, and a study demonstrated that the BRCA1- BARD1 complex is required for ATM/ATR-mediated phosphorylation of *TP53* at Ser-15 following IR or UV radiation-induced DNA damage (Deng, 2006). Further, it was observed that phosphorylated BRCA1 is involved in the S-phase cell cycle checkpoint, and, BRCA1 mediates G2/M arrest via its interaction with CHK1 and CHK2 (Deng, 2006).

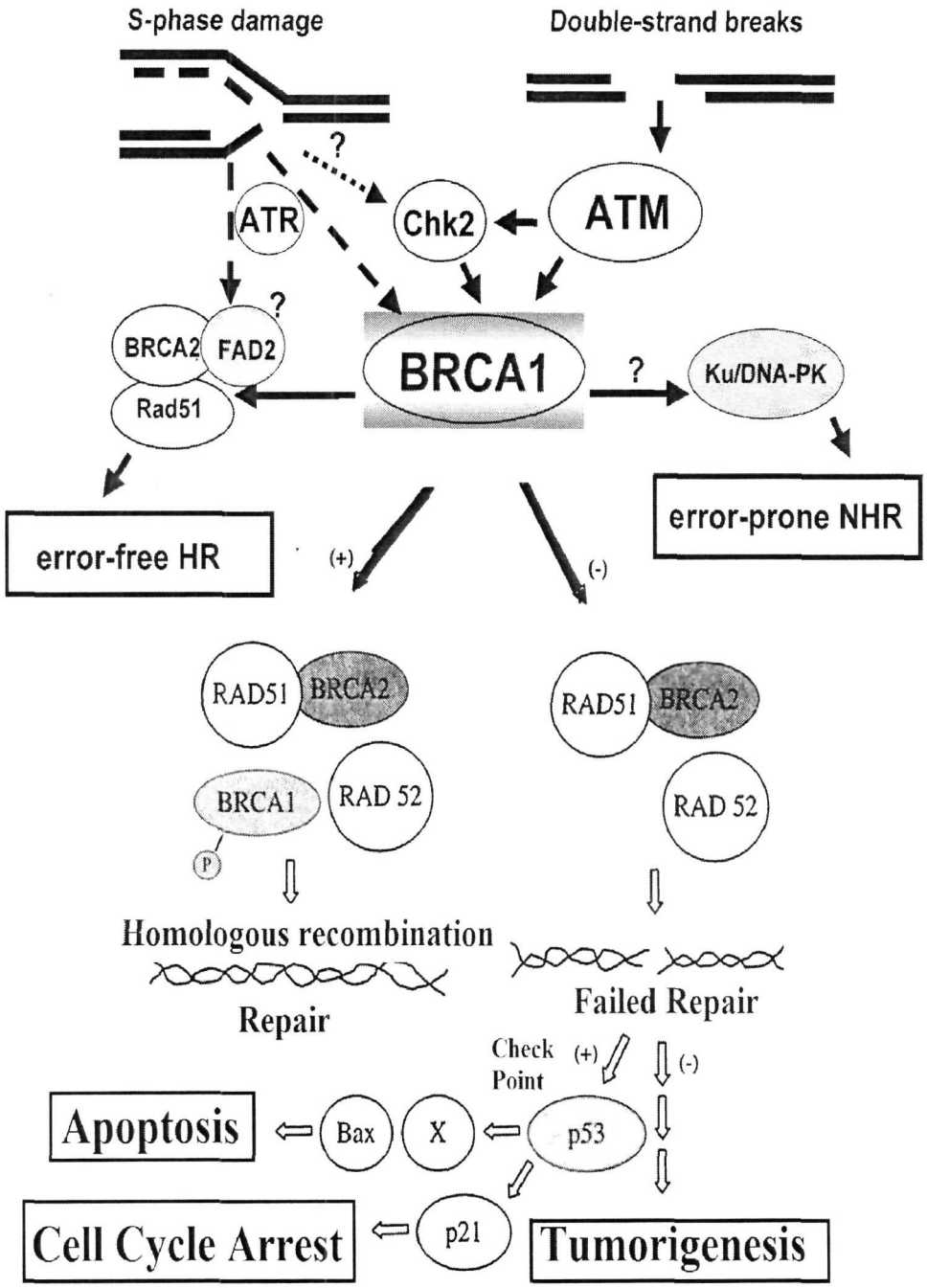

Figure 1.2 A schematic diagram of *BRCA1* protein functions, apoptosis, cell cycle arrest and tumorigenesis (adapted from Zhang and Powell, 2005).

1.4.2 *TP53* as a gatekeeper gene

The *TP53* gene was discovered in 1979 as a gene coding for a 53 kDa nuclear phosphoprotein bound to the large T antigen of the simian virus 40 (SV40) DNA virus (Lane and Crawford, 1979; Linzer and Levine, 1979). The *TP53* gene is located in humans on chromosome 17 at location 17p13.1. The gene is composed of 11 exons, the first of which is non-coding. Exons 5 through 9 of the *TP53* gene contain conserved sequence blocks (Figure 1.3).

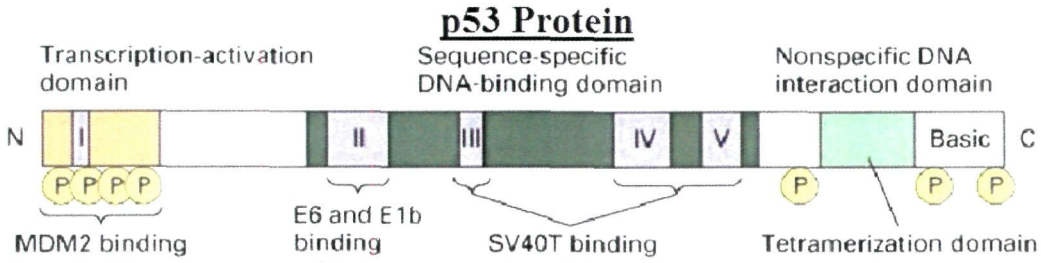

Figure 1.3 A schematic diagram of *TP53* protein with its transcription-activation domain, sequence-specific domain and nonspecific DNA interaction domain (adapted from Somasundaram and Ei-Deiry, 2000)

The wild-type *TP53* gene codes for a protein of 393 amino acids which is composed of three distinct structural and functional domains: (i) a N-terminus containing an amino-terminal transactivation domain (residues 1-42) which is required for transactivation activity and interacts with various transcription factors including acetyltransferases and MDM2 (murine double minute 2); and, a proline-rich region with multiple copies of the PXXP sequence (residues 61-94, where X is any amino acid). The proline-rich region plays a role in *TP53* stability regulated by MDM2, wherein *TP53* becomes more susceptible to degradation mediated by MDM2 if this region is deleted (ii) a central core domain (residues 102-292) constitutes the DNA-binding domain which is required for sequence-specific DNA binding. The consensus sequence contains two copies of the 10-bp motif 5'-PuPuPuC (A/T)-(T/A) GPyPyPy-3', separated by 0-13 bp (iii) a C-terminal region (residues 301-393) containing an

oligomerization domain (residues 324-355), a strongly basic carboxyl terminal regulatory domain (residues 363-393), a nuclear localization signal sequence (NLS) and nuclear export signal sequences (NES). The basic C-terminus of *TP53* functions as a negative regulatory domain and has also been implicated in induction of cell death. The central domain of *TP53* is its most highly conserved region, not only when *TP53* is compared with its homologs from *Drosophila* and *Caenorhabditis elegans*, but also as compared with its mammalian family members, p63 and p73. In fact, structural studies of *TP53* have revealed that majority of *TP53* mutations found in various cancers are missense mutations that are mostly located in this central DNA-binding domain (Figure 1.3) (Rivlin et al., 2011).

1.4.2.1 Role of *TP53* in breast cancer

It was observed that only mutant *TP53* could co-operate with *ras* during cellular transformation, and wild-type *TP53* could in fact inhibit transformation induced by the combined effect mutant *TP53* with E1A antigen or *ras*. It was also demonstrated that colorectal carcinomas in which one allele of the *TP53* gene is lost, also harbored mutations in the remaining allele, a characteristic hallmark of loss of tumor suppressor function according to Knudson's hypothesis. This pattern was found to be true for breast cancers (Velculescu and El-Deiry, 1996).

In normal unstressed cells, *TP53* is an unstable protein with a half-life ranging from 5 to 30 minutes, and is present at very low cellular levels (Levine, 1997). Following various intracellular and extracellular stimuli, such as DNA damage (induced by various factors including ionizing radiation, UV radiation, exposure to cytotoxic or chemotherapeutic agents and viruses), heat shock, hypoxia, and oncogene overexpression, wild-type *TP53* is activated and emerges as a pivotal regulatory protein which triggers diverse biological responses, both at the level of a single cell as well as in the whole organism.

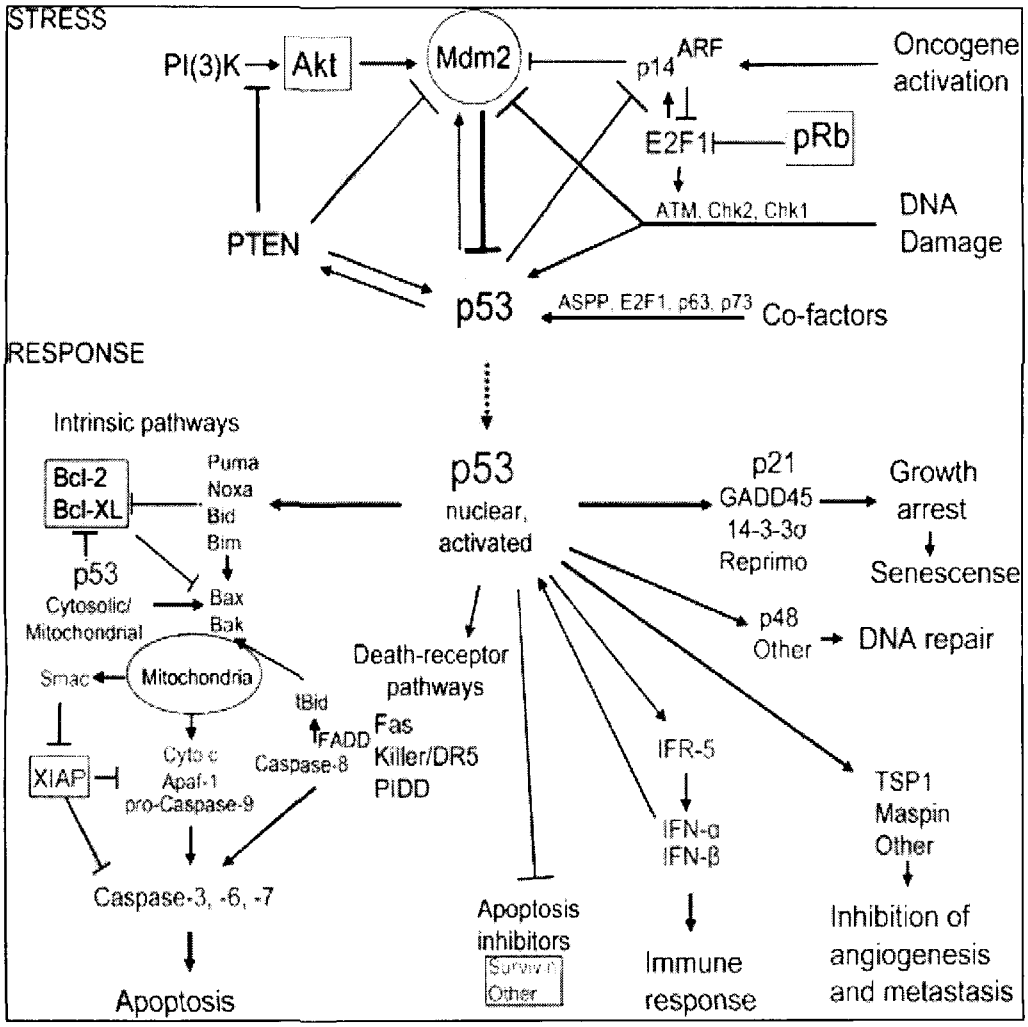

Figure 1.4 A schematic diagram demonstrating some of the known components of the *p53* functional circuit (adapted from Stewart and Pietenpol, 2001).

TP53 activation involves an increase in overall *TP53* protein level by inhibition of MDM2-mediated degradation, as well as qualitative changes in the protein through extensive post-translational modifications including phosphorylation and acetylation, thus resulting in activation of *TP53*-targeted genes (Wang and Sun, 2010).

TP53 plays a critical role during the DNA damage-induced G1/S cell cycle checkpoint. After exposure of cells containing wild-type *TP53* to

genotoxic agents, *TP53* is activated and transcriptionally up regulates the Cdk inhibitor, p21. p21 then binds to, and inactivates cyclin-Cdk complexes that mediate G1 phase progression, resulting in pRB hypophosphorylation, E2F sequestration, and cell cycle arrest at the G1/S transition. *TP53*-dependent induction of p21 also results in an S phase checkpoint response, as p21 binds to the proliferating cell nuclear antigen (PCNA) and prevents PCNA from mediating recognition of the DNA primer template complex, thus inhibiting the elongation step in DNA replication (Stewart and Pietenpol, 2001).

After genotoxic stress, *TP53* modulates DNA repair through multiple mechanisms, including sequence specific transactivation and direct interaction with components of the repair machinery. *TP53* sequence-specific transactivation-dependent DNA repair occurs in part by transactivation of p21. p21 specifically inhibits PCNA-mediated DNA replication while allowing PCNA-regulated DNA repair. *TP53* also directly interacts with proteins that function in DNA repair pathways. Most of these proteins are members of the TFIIH complex that initiates basal transcription of RNA polymerase II and couples transcription with nucleotide excision repair (NER). *TP53* binds to the Cockayne syndrome B repair helicase and replication protein A (RPA), a trimeric protein complex that functions in DNA replication, homologous recombination, and NER. Besides, *TP53* may also be involved in DNA repair through direct interaction with DNA. Association of the carboxy terminus of *TP53* with single-stranded DNA ends facilitates the binding of the *TP53* core domain to DNA and helps in recruiting different repair factors. *TP53* also possesses intrinsic 3' → 5' exonuclease activity that is associated with the core domain of the protein. This exonuclease activity may play an important role in *TP53*-mediated repair, as tumor-derived *TP53* mutants are exonuclease-deficient and cells expressing such mutant *TP53* are defective in global NER (Stewart and Pietenpol, 2001).

The *TP53* apoptotic target genes can be divided into two groups; the first group encodes proteins that act through receptor-mediated signaling, and

the second group encodes proteins that regulate apoptotic effector proteins. *TP53*-dependent transactivation of IGF-BP3 induces apoptosis by blocking IGF-1 survival signaling to the IGF-1 receptor (IGF-1R). When combined with *TP53*-dependent repression of the IGF-1R, *TP53* signaling results in a highly efficient block of this survival pathway. *TP53* also mediates apoptosis through activation of the Fas/APO1/CD95 (Fas) and KILLER/DR5 death receptors. Early cell changes that occur during apoptosis are associated with mitochondrial changes mediated by members of the Bcl-2 family of proteins, including antiapoptotic Bcl-2 and pro-apoptotic BAX proteins. BAX facilitates the release of the apoptosis-inducing factor (AIF) and cytochrome *c* from the mitochondria, thus activating the caspase cascade. *TP53* inhibits expression of the anti-apoptotic Bcl-2 protein, which normally blocks apoptosis by preventing the release of AIF and cytochrome *c* from the mitochondria.

1.5 Role of Low penetrance candidate genes in breast cancer

The etiology of breast cancer is still poorly understood with known high penetrance gene mutations accounting for only a small proportion of the cases. Low penetrance candidate genes are found to in a variety of pathways, ranging from the detoxification of environmental carcinogens to steroid hormone metabolism and DNA damage repair. Glutathione S-transferase (GSTs) constitute a super-family of ubiquitous, malfunctioned enzymes, which play a key role in phase II cellular detoxification of a wide variety of exogenous and endogenous chemicals with electrophilic functional groups, by conjugating them to the tripeptide glutathione (GSH) thereby neutralizing their electrophilic sites and rendering the products more water soluble. Additionally they act to protect DNA damage and adduct formation through conjugation.

Three members of the GST family, *GSTM1*, *GSTT1* and *GSTP1* has been widely analyzed for polymorphic variants to elucidate their role in carcinogenesis. Independent gene deletion has been identified at both *GSTM1*

and *GSTT1*, and these deletion variants biochemically fail to express an active protein. Associations between *GSTM1* and *GSTT1* null genotypes and breast cancer have been reported with inconsistent results. GST gene polymorphisms have also been studied with respect to various cancers and have been variously associated with cervical cancer (Zhang et al., 2012), esophageal cancer (Yi and Li, 2012), colorectal cancer (Wang et al., 2012), and Hodgkin and non-Hodgkin lymphoma (Bin and Luo, 2013).

As the GSTs code for biotransfermation enzymes, their variants cause inter-individual variability in the metabolism of anticancer drugs also and may have an impact on clinical outcome. But, few studies have investigated the association between polymorphisms in the GST genes and survival in patients with breast carcinoma. The results of such studies have been mixed. It will also be of interest to investigate the role of GSTs in the survival of patients with a family history. It is with this background that in this study we are also investigating the role of this polymorphism in the survival of sporadic as well as familial breast cancer patients.

1.6 Mutational hotspot screening of selected genes in the population of southern Assam

Cancer is now well defined as failure of genes by means of genetic and environmental exposure. A mutation can be defined as a sequence change in a test sample compared with the sequence of a reference standard. This definition implies nothing about the phenotypic consequences (e.g., pathogenicity) of a mutation. A polymorphism may be defined as a mutation that occurs in a substantial proportion (>1%) of a population and is tacitly assumed to be non-pathogenic, although the true pathogenicity may be unknown. A polymorphism has also been defined as a Mendelian trait that exists in the population, with the frequency of the more rare of the two alleles greater than 1-2%. If we accept that DNA sequence is a Mendelian trait, then the two definitions of polymorphism are the same. The detection of a single

base change in the human genome requires a signal background ratio of 1 : 6 × 10^9 a formidable task. To achieve such selectivity in the field of electronics would require amplification and noise reduction, and it is no surprise that analogous processes are found in molecular genetics for example, amplification by the polymerase chain reaction (PCR) and noise reduction by the stringent annealing of probes and primers. Mutation detection techniques can be divided into techniques that test for known mutations (genotyping) and those that scan for any mutation in a par-Most of the cancers are both preventable and curable provided they are detected at an early stage. In case of female this one is in very harsh condition due to unawareness and in sufficient technique to detect cancer early. The need for early detection of breast cancer in order to decrease mortality is well known to all. For this extensive work will require several parts of the country, hence there is a need for innovative research to suit our socioeconomic conditions.

Changes in genetic factor by means of mutations are main causes of all cancer. In the last decade of the 20th century all avenues of biomedical research led to the gene. The human genome contains all the information necessary from conception until death. The completion of the first draft sequence of the human genome in the Human Genome Project has resulted in sequencing of the entire human genome. This remarkable achievement will reveal the genetic instructions that specify the molecular components, the design and the operating software of the human body. Following this achievement mutational screening of hot spot region in different responsible gene for cancer is now under consideration of research. This knowledge will transform medicine, giving us the means to see and to understand human anatomy, physiology and pathophysiology in molecular detail. This development will dramatically accelerate the development of new strategies for the diagnosis, presentation and treatment of diseases. Especially for common complex diseases such as cancer, genetic differences contribute to the risk of contracting the disease, clinical course of disease and respond to

different treatments. Since the discovery of oncogenes, tumor suppressor genes and more recently genes of apoptosis cancer has become one of the most important diseases in the design of approaches based on genetics and genomic research. The explosion of information generated by large-scale genomic related technologies has resulted in an exponential increase in the number of genes and proteins available for pharmaceutical and diagnostic research development. The increasing understanding of complex molecular pathways involved in cancer will shift clinical practice from empirical treatment to treatment based on molecular taxonomy of disease (Dancy et al., 2012).

1.7 Objectives of this study are as follows:

1) Survey based on proforma / registry book in hospitals and medical institutes of Barak valley for prevalence of Breast cancer.
2) Immunohistochemistry analysis of tissues from breast cancer patients.
3) Design of primer for amplification of most responsible genes in Breast cancer.
4) Detection of hotspot sequence mutation from breast cancer associated gene(s).
5) Bioinformatical analysis of breast cancer associated genes and the global databases in breast cancer.
6) Characters of low penetrance genes in breast cancer patients.