Chapter-5

General Discussion

The notion of cancer being a consequence of genetic alterations, is almost intuitive and the advances in molecular biology and genomics have given us many tools to understand and possibly to combat cancer. Since science has always existed on a continuum, the genetic alterations in cancer have to be understood in the context of cellular organization, differentiation, tissue organization host response and susceptibility angiogenesis etc. The properties that are taken to typify cancer cells are also present in normal cells. These include cell division, migration and even invasion. However what marks out cancer cells is dysregulation and inappropriate expression of these attributes. Typically the genetic alterations in cancer can be said to include three major types of genes, oncogenes, tumour suppressor genes and genes that preserve the integrity of the genome.

5.1 Significance of cytopathological screening in breast cancer

The combination of the precision of H&E and immunohistochemistry with the visualization provided by a sensitive detection system, is a science that has its origins in the work of Albert Coons, 60 years ago. Building upon the work of Coons, who initially sought to prove the presence of immunoglobulin on plasma cells, the past half century has seen tremendous refinements in the development of the antibody tools and detection systems. Immunohistochemistry started with a brilliant yet disarmingly simple idea; to have antibodies bind the specific antigens being sought and to make those antibodies visible by hooking them to a fluorescent compound. All subsequent modification\'s such as use of non-fluorescent chromogen, the amplification of reaction and unmasking of antigens are technical improvements.

The advancement of monoclonal antibody technology has been of great significance in assuring the place of immunohistochemistry in modern accurate microscopic diagnosis of human neoplasms as a method of choice in histopathology. The production of antibodies enabling the detection of genetic abnormalities including mutations, gene amplifications or specific

chromosomal translocations associated with novel chimeric proteins promises to yield further insights into the genesis and behaviour of tumours.

In gene mutations, it is used to show the loss of protein expression, reflecting the final common pathway that results from mutation, promoter methylation or other genetic mechanisms. The molecular signature of lobular carcinoma of breast is a genetic alteration which results in loss of E Cadherin protein, which can be determined by immunohistochemistry.

The future perspectives point for new discoveries that should make the immunostaining methods simpler. This involves an inert polymer in which many molecules from the primary antibody and peroxidase are chemically connected, consequently decreasing the number of incubation stages, and is currently commercially available. Despite its high cost, this type of technology could be fundamental to pathology laboratories in which the diagnostic routine is very extensive. In addition it could also be of great value in the standardization of the employed technique and the reproducibility of the results. Without doubt, the development of quantification methods for the immunohistochemistry technique, mainly those which are computer-assisted, have increased not only the accuracy in the detection of markers, but also the reliability of their results. However, with the recent spread, practicality, reproducibility and reliability of obtaining results along with falling costs of systems of computer assisted image analysis is changing this panorama. At present, immunohistochemistry quantification is widely employed in many areas, not only in pathology, but also in various medical areas with particular impact on the daily clinical practice. The scientific, diagnostic and prognostic applications of this methodology must be explored in a bid to benefit of the patient. In order to achieve this goal the collaboration and pooling of knowledge between these two valuable medical areas is vital. Pathologists have used many special techniques over the years to confirm, complement and refine the information they were able to obtain with their old faithful armamentarium i.e. formalin fixation, paraffin embedding and haematoxylin

eosin staining. These techniques include special staining, tissue culture, immunohistochemistry and molecular biology methods. It could be fair to say that today no special technique has influenced the way pathology is practiced as profoundly as immunohistochemistry or has even come close to it. It would not be an exaggeration to speak of a revolution, particularly in the field of tumour pathology.

Other important uses of immunohistochemistry include determination of unknown primary tumours diagnosis of central nervous system tumours, diagnosis of Paediatric small round blue cell tumours, carcinomas, Melanoma and sarcomas. Small round blue cell tumours and solid paediatric malignancies require immunohistochemistry for the diagnosis, evaluation of recurrent or metastatic disease and in some cases prognostic classification. It is only one of the very important tools available for the pathologists to categorize solid tumours of childhood and adolescence. Immunohistochemistry has been shown to have an impact on patient diagnosis and has been reported to be a cost effective ancillary test in patient diagnosis. The discovery of neoplasm associated antigens has not only made the most accurate diagnosis of human cancer feasible but has also shed the light on extensive immunophenotypic heterogenicity of even the most closely linked to human malignancies. Future antineoplastic therapeutical appearances should see the inclusion of a variety of immunotherapies, in the form of an individualized "Cocktail" specific for the particular immunophenotypical pattern associated with each individual patient's neoplastic disease.

5.2 Reputation of mutational screening in breast cancer

Naturally cancer is a multi-step process and several genetic alterations are required for a full blown cancer phenotype. In the last 10 years many studies have focused on screening of mutations in breast/ovarian cancer. Mutations in BRCA genes have been established to predispose women to breast and ovarian cancers, the endpoint of BRCA protein dysfunction.

Although previous studies have implicated both *BRCA1* and *BRCA2* in the cellular response to DNA damage, little is known about the mechanism by which BRCA proteins modulate this response. Extensive research has revealed that BRCA proteins bind and interact with a number of regulatory proteins. Accumulating evidence suggests that *BRCA1* participate in multiple functions, including DNA repair, transcription, and cell cycle control. In the near future, numerous other proteins that bind to BRCA proteins will probably be identified, leading to the discovery of new functions. It is unclear why a BRCA-related predisposition to cancer is apparently site-specific, affecting the breast and ovary, despite the fact that the known functions of BRCA proteins are essential to all cell types. A possible explanation is that breast or ovarian epithelia is particularly vulnerable to transformation when heterozygous for *BRCA* gene mutations. This increased vulnerability could be attributed to tissue-specific effects of the haploinsufficiency involved in the hormone-responsive proliferative changes unique to these cells. At present, however, the roles of BRCA proteins in epithelial cell biology and transformation remain uncertain. In this study majority of patients were at the fourth stage and a large percentage of the patients who come to the CCHRC seeking care owing to their nominal incomes. Usually, breast cancer can occur at any age but younger women are less susceptible to ward's breast cancer (Mathew et al., 2004). Our study comprises of a lower mean value of age that revealed the occurrence of this disease a decade earlier, as compared to western countries (Sandhu et al., 2010). The probable reason for the early onset of this dreaded disease in the younger women may be due to personal history with a breast cancer / ovary cancer (Liang et al., 2011), family history of breast cancer, particularly in a mother, sister and daughter (Metcalfe et al., 2010), history of radiation therapy to the chest before age 40 (Narod 2011).

This mutational research analysis explores the incidences and distribution of mutations in North-Eastern region of India concerning the other factors relating to breast cancer. For the diagnosis of breast carcinoma, FNAC

technique was used as it is a useful diagnostic tool because of its cost efficient and rapidness (Sandhu et al., 2010). Three deleterious nonsense mutations resulting in a premature termination codon were identified in *BRCA1*, 185DelAG in exon 2; 1014DelGT and 3889DelAG in exon 11, rather absent in the observed control group. Nonsense mutations of these three specific mutations are very detrimental to the protein; it can render the resulting protein non-functional due to formation of stop codon at the early stage. Unexpectedly, we have gathered 185DelAG in North-East Indian Hindu patient residing in Cachar district who claimed to have family history but not to Jewish ancestry. In India, 185DelAG has been reported in all populations studied (Saxena et al., 2005; Hedau et al., 2004; Kumar et al., 2002; Valarmathi et al., 2004). Similarly, Lakhotia et al., found the same mutation with the help of conformation sensitive gel electrophoresis in four Indian breast cancer families (Lakhotia et al., 2010). Worldwide population studies have revealed that the 185DelAG mutation predates the severance of Sephardi and Ashkenazi Jewish populations and is probably 2000 years old (Bar-Sade et al., 1998). *BRCA1* 1014DelGT was detected in two Muslim index cases of without any family history of Karimganj District, but both have the personal history of ovary cancer. Interestingly, the same mutation was reported in a heterogeneous Pakistani population of the Muslim religion (BIC-NHGRI). The specific position of 1014DelGT comes under the part of a DNA binding region where other tumor suppressor proteins could not bind and unable to form complex protein for the downstream act of protein. These observations suggest that 1014DelGT might be a common mutation in the Muslim community and might have migrated to the Indian population through a pool of Muslim immigrants (Liede et al., 2009). In the North-Eastern region, the mutation of 3889DelAG is higher than the rest of the mutation found. Out of nine, three have the family history and found scattered in studying population; it also found in various populations of the world (Thirthagiri et al., 2008; Farooq et al., 2011). The location of 3889AG is towards the C terminus of BRCA1, within the transcriptional activation domain, a region as well reported

to interact with the *BRCA2* protein, which plays an important role in the double stranded break (DSB) repair (Roy et al., 2012). Nevertheless, the number of mutations identified in the studied North-East Indian population is higher. It may be due to the selected candidate who comes under the three patterns of the study. This significant proportion of mutation from *BRCA1* suggests one of the several possibilities for genetic predisposition in the North-East Indian population.

The results of this primary study put forward that the mutational spectrum in exons 2 and exon 11 of the *BRCA1* gene in this population may be at variance from what has been observed in other Indian populations. Also the mutational screening of the whole *BRCA1* gene from different geographical regions of India will help to identify the mutations. It may provide the knowledge of biological properties of the protein corresponding to polymorphism. Through this aspect of proper counselling, patients and pre-symptomatic mutation carriers' studies would be able to make better decision about medical and surgical preventive options.

5.3 Low penetrance genes may contribute in breast cancer

Several studies have addressed the role of *GSTT1* and *GSTM1* gene deletions as risk factors in breast carcinoma, but the results are conflicting (Helzlsouer et al, 1998; Gumundsdottir et al, 2001; Krajinovic et al, 2001; Mitrunen et al, 2001). The findings, which showed that GSTs enzymes play crucial role in the detoxification of numerous products induced by cancer therapy, prompted us to evaluate the prognostic significance of GSTs deletions in breast carcinoma. The present case/controlled study showed a borderline significant increase in the risk of breast carcinoma in unselected subjects carrying the null-*GSTT1* genotype. This association becomes clearly significant for premenopausal women. Thus the *GSTT1* deletion seems to be associated specifically with the early onset of breast carcinoma. No direct correlation was found between polymorphism in the *GSTM1* gene and the breast carcinoma onset in

Tunisians. Our data provide evidence against a substantially increased risk of breast carcinoma associated with *GSTM1* homozygous gene deletion. The generation of ROS and their byproducts is a large part of the cytotoxic activity of chemotherapy agents. The *GSTT1* and *GSTM1* enzymes have been shown to have removal activity toward lipid hydroperoxides. The lack of these enzymes could conceivably be associated with better response to chemotherapy. In this study, we initiate the prognostic significance evaluation of the GSTs deletions by investigating the potential association between *GSTT1* and *GSTM1* gene deletion and the clinical response to chemotherapy induction. This evaluation indicated that only *GSTT1* gene deletion is associated with the clinical response to chemotherapy. This prognostic significance was particularly high for patients with axillary's lymph node-negative breast carcinoma. Although no significant association was found between *GSTM1* gene deletion and the response to chemotherapy, a combined effect of *GSTT1* and *GSTM1* gene deletions was seen in the response to chemotherapy induction. Indeed, none of the patients with lymph node-negative and carrying the double null-*GSTT1*-*GSTM1* genotype had poor response to chemotherapy. These findings support the hypothesis that patients with *GSTT1*-null genotypes have reduced detoxification of therapeutic agents and, in the case of high-dose therapy for acute myelocytic leukaemia, worse outcomes. In the present study of primary breast carcinoma patients, the better response to chemotherapy that was observed among *GSTT1*-null patients, who were not treated with high-dose therapy, can be explained by the increased efficacy of treatment.

There have been only a few studies of GST genetic polymorphism and survival after treatment of breast carcinoma (Lizard-Nacol et al, 1999; Ambrosone et al, 2001). For the most part, prior studies were undertaken on a small or heterogeneous population. Our results suggest that *GSTM1, GSTT1*, and *CYP1* genotypes do play a role in susceptibility to breast cancer, in agreement with most previous studies. However, inability to detect effects for

GSTs could result from failure to include relevant environmental exposures or genes that interact with GSTs. The selection of axillary's lymph node-negative breast carcinoma allowed the appearance of a significant association between *BRCA1* of breast carcinoma and the *GSTT1* gene deletion. Significant association was found with the *GSTM1* gene deletion. However, there was an increase in *BRCA1* mutations for patients carrying both gene deletions for *GSTT1* and *GSTM1*. ORs for *GSTM1* null and *GSTT1* null genotypes were elevated slightly among women with family history of breast cancer, and age at diagnosis was lower among women with a family history and *GSTM1* null genotype. Helzlsouer et al. reported 2-fold elevated ORs for all three GST genes among women with a family history of breast cancer, whereas Kelsey et al. reported no modification of ORs for *GSTM1* by family history. The positive associations for *GSTM1* and *GSTT1* genotypes among women with a family history could be attributable to unmeasured genetic or environmental factors that interact with GST genes to increase risk of breast cancer and/or age at onset. Family-based studies that incorporate genotyping and environmental exposure assessment are the ideal study design to test such a hypothesis (Zhao et al., 1997). We did estimate joint effects for GST genotypes and *BRCA1* status because of the large number of BRCA carriers in the studied population.

In conclusion, this study suggests that the *GSTT1* gene deletion may be an attractive susceptibility marker for the mutation of breast cancer gene 1. This genetic marker represents not only a predictor of chemotherapy response but also a prognostic variable for predicting relapse in patients with lymph node-negative breast carcinoma. We examined the relation of *GSTM1*, *GSTT1*, and *GSTP1* genotypes and breast cancer risk in hospital-based study, casecontrol study of women in Northeast Assam. *GSTM1*, *GSTT1*, and *GSTP1* genotypes were associated with breast cancer risk in women from Assam. Previous studies reported association between *GSTM1* genotype and breast cancer risk. Charrier et al. reported positive associations for the *GSTM1* null

genotype among postmenopausal but not premenopausal women, whereas Ambrosone et al. reported a positive association for *GSTM1* null genotype among younger postmenopausal women. In contrast, Garcia-Closas et al. reported no association for *GSTM1* null genotype in pre- or postmenopausal women. Helzlsouer et al. reported no association for *GSTT1* null genotype and breast cancer in pre- or postmenopausal women, whereas Garcia-Closas et al. observed an inverse association for *GSTT1* null genotype among premenopausal women. Here we reported a positive association for *GSTP1* Val/Val genotype in women.

The results of this primary study put forward that the mutational spectrum in *BRCA1* gene in the population may be at variance from what has been observed in other Indian populations. Also the mutational screening of the whole *BRCA1* gene from different geographical regions of India will help to identify the mutations. It may provide the knowledge of biological properties of the protein corresponding to polymorphism. Through this aspect of proper counselling, patients and pre-symptomatic mutations carriers' studies would be able to make better decision about medical and surgical preventive options.