General Discussion
Breast cancer could be classified in different ways based on histology, stage and grade, laterality, genetic susceptibility etc. These tumors can also be classified depending on the age of the patient at onset of tumor. It is evident from studies that BC diagnosed at a young age is associated with an aggressive tumor phenotype and poor prognosis. These findings have raised the hypothesis that BC affecting younger women may have a biologically different origin, possibly caused by diverse pathways of genetic and epigenetic alterations. In-depth analysis of molecular alterations might provide some insights into the biological and behavioral features of BC in the two age groups. Moreover, molecular alterations in other breast lesions (except carcinomas) are not well understood. Thus, attempts have been taken in this study to delineate the molecular alterations (deletion/mutation/methylation/expression) of some candidate TSGs located in chromosomes 3 and 9 in the two age groups of BC and other breast lesions of Indian patients.

Compilation of data from Chapters 1, 2 and 3 revealed some interesting observations. The alterations of the candidate TSGs showed differential association in both early-onset (group-A) and late-onset (group-B) BC indicating differences in the molecular pathways in the two age groups (Figure 1). Significant association was seen between alterations of (i) PTCH1 and XPA, (ii) p16, p14 and p15 in both age groups of BC suggesting some synergistic association of these candidate TSGs in development of the disease. It seems that deregulation of these candidate TSGs might provide some selective growth advantage to the malignant clones.

**Figure 1.** Schematic representation of the interrelation among different genes. A: in early-onset BC and B: in late-onset BC. → → : significant interrelation between the genes, ← → : probable interrelation between the genes not observed in this study.
On the other hand, in group-A; the alterations of hMLH1 showed significant association with that of HYA22, FANCC and SH3GL2. No such association was observed in group-B. Similarly, the association as observed between FANCC and HYA22 in group-A was absent in group-B. Thus, the association of mismatch repair and double strand break repair genes in group-A suggests of the possibility of higher chromosomal abnormalities (aneuploidy, high S-phase fraction etc.) and greater aggressiveness of the disease. The significant associations of alterations of SH3GL2 with p16 and p14 in group-A only also supported this phenomenon.

Alteration in FANCC was common to all 14 other breast lesions. However, hMLH1 alterations was seen in 50% (7/14) of these samples similar to what was observed in BC samples (Table 1). This suggests that both mismatch repair and double strand break repair genes has important roles in the development of these tumors as well. Deregulation of any one of the cell-cycle regulatory genes (p16, p14, p15 and HYA22) was seen in 43% (6/14) of these tumors indicating their importance in the development of the disease. The candidate genes SH3GL2 and PHF2 were also seen to be altered in 21% (3/14) and 36% (5/14) other breast lesions respectively. Thus, it seems that alterations in DNA repair genes, cell cycle regulatory genes and some signal transduction genes might be involved in the development of other breast lesions also.

Table 1: Compilation of alterations (deletion/methylation/microsatellite size alteration) in bilateral tumors and multiple lesions. Symbols are same as in Table 5 of Chapter 1.
Analysis of chromosomal alterations in bilateral tumors has opened some new insights into understanding the clonality of breast tumors. The chromosomal alterations are compiled in bilateral tumors/multiple lesions (Table 2; Figure 2). It was seen that concordant alterations in different genes were present in corresponding bilateral tumors. Interestingly, FANCC gene was concordantly altered in 3 bilateral tumors while 2 showed concordant hMHL 1 alterations indicating the importance of these repair genes in initiation of these lesions. Except case #848, all the other bilateral cases have previously reported to show concordant alterations in other chromosomal loci [Chunder et al. 2004c]. Thus, it can be suggested that multiple tumors could arise from a common clone and subsequently diverge following different pathways and acquire late stage progressional damage and is expressed as different somatic phenotypes. However, reports have suggested concordant relationship between genetic abnormalities and menopausal status at time of onset of tumors as discordant pattern of alterations were seen in women experiencing cessation of menses between the first and second tumor [Imyanitov et al. 2002].

Table 2. Schematic representation of the pattern of alterations of different genes observed in bilateral breast tumors. Symbols are same as in Table 5 of Chapter 1.
Thus it may be concluded from the present study that molecular pathways associated with tumorigenesis of early- and late-onset BC might be different. Understanding these molecular pathways requires detailed functional analyses of the involved candidate genes. Analysis of few bilateral and multiple tumors in an individual suggests their common clonal origin. Further study considering a greater number of these tumors needs to be done.