

Summary

Breast cancer remains one of the leading causes of cancer death in women worldwide, despite significant improvements in survival over the past 25 years. One of the greatest challenges faced by researchers and clinicians in this field is that breast cancer is not a single entity, but rather a heterogeneous group of tumours consisting of several subtypes displaying distinct differences in biological and clinical behaviour. A primary aim in breast cancer management is to tailor clinical decisions to the individual, based on a detailed understanding of breast cancer tumourigenesis. This thesis describes a combined approach of two fundamental concepts in studying breast cancer development; the genetic predisposition to breast cancer and the molecular classification of breast cancer.

In Chapters 2 and 3, we performed a combined analysis of copy number, loss of heterozygosity (LOH) and gene expression profiling to investigate whether *BRCA1*-mutated and *CHEK2**1100delC-mutated breast tumours harbour characteristic genomic and/or transcriptional aberrations as compared to non-*BRCA1/2-CHEK2*-mutated familial breast tumours (BRCAX tumours). The identification of such characteristic genomic aberrations could prove valuable in clinical testing for *BRCA1* and perhaps *CHEK2* involvement in patients and could lead to a better understanding of the underlying process of oncogenesis.

During the analyses, we found that large numbers of tumour infiltrating lymphocytes (TILs) have a detrimental impact on genomic profiling of breast carcinomas. For this reason we developed a gene expression immune infiltrate signature to select for samples with relative low levels of TILs. Also, all supervised analyses for *BRCA1*-mutated and *CHEK2*-mutated tumours were restricted to the Basal-like and Luminal/HER2+ subtypes respectively.

A number of *BRCA1*-associated copy number aberrations (CNAs) were found, and these proved capable of discriminating *BRCA1*-mutated from BRCAX/sporadic Basal-like tumours in multiple cohorts and across array-platforms. In contrast to the *BRCA1*-mutated breast tumours, no apparent specific somatic CNA profile for *CHEK2**1100delC-mutated breast cancers was found. This difference in CNAs profiles might be explained by the need for *BRCA1*-deficient tumour cells to acquire survival factors, by for example specific CNAs, to expand. Such factors may not be needed for breast tumours with a defect in a non-essential gene such as *CHEK2*.

To demonstrate the impact of TILs on genomic profiling of tumour material, we performed DNA flow cytometry on paraffin-embedded, formalin-

fixed, material of *BRCA1*-mutated breast carcinomas selected for large numbers of TILs (> 40% of nuclei). Copy number profiles obtained by shallow whole genome sequencing analysis of sorted tumour cell derived DNA clearly showed profiles that were not detected in the matching unsorted tumour samples.

Chapter 4 describes how the mRNA immune infiltrate signature is applied as a standardized assessment of general tumour inflammatory cell infiltrate to investigate its association with patient survival and molecular subtypes in large cohorts of lymph node negative primary breast cancer patients who did not receive any adjuvant therapy. From the analyses we can conclude that in lymph node negative primary breast cancer, high levels of tumour inflammatory cell infiltrates are associated with better metastasis-free survival, especially in HER2+ breast cancer patients.

During the analyses described in chapter 2, we identified a small number of BRCAX Basal-like breast tumours with shared genomic features. These features include recurrent characteristic copy number aberrations and most notably, similar to the *BRCA1*-related tumours in this study, copy number neutral LOH of large overlapping genomic regions on chromosome 17. In chapter 5 we performed whole exome sequencing analysis of germline DNA from these five Basal-like BRCAX cases in an effort to identify putative novel high or moderate-risk breast cancer genes. A novel pathogenic *RAD51C* germline mutation was identified in one of the BRCAX cases. We did not find conclusive evidence for causal mutations in unknown breast cancer risk genes in the other four patients.