Breast cancer: introduction

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The most frequent cancer type in females in the Western world is breast cancer, with a lifetime risk of the order of 1/10. Our understanding of the molecular events relating to breast cancer biology and pathogenesis has greatly increased over the last decade. The development of breast cancer involves several types of genes that need to be activated or inactivated in order to promote malignancy. The sequential steps in gene alterations with respect to tumour progression are not clear, and are far less well understood than what is currently the best example of tumour progression, that is, colo-rectal carcinoma. Still, the large number of alterations that have been identified in breast tumours at the genetic level fit the model of multistep carcinogenesis. Breast cancer is sometimes associated with predisposing mutations in the germline but is essentially a somatic cell genetic disease. In the present issue of *Seminars in Cancer Biology* selected topics on breast cancer biology and genetics are reviewed.

Putative precursor stages to invasive breast tumour growth are hyperplasias and cancer *in situ*. Breast cancer can also progress further to a metastatic disease, where axillary nodes are the most common sites, but distant metastases can also occur, where bone and bone marrow are among the major locations. Micrometastases in breast cancer patients have been studied recently, as reviewed by Klaus Pantel and Marcus Otte. One way to detect the micrometastases is to collect a bone marrow sample and score for epithelial characteristics of the cells, namely expression of cytokeratins by immunocytochemistry. Such micrometastases are relatively frequent in breast cancer (25–43%). Since metastatic cells are targets for therapeutics, it is optimal to detect them at a single-cell level and to detect their phenotype and viability. Recent developments in immunocytochemical and molecular methods

allow this identification but some of these methods still need to be standardized for routine clinical diagnosis. Detection of micrometastases in breast cancer patients may be an important adjunct for staging and therapy. Several reports have shown the prognostic relevance of detection of micrometastatic cells in bone marrow, and in breast cancer it is an independent prognostic factor in the node-negative disease. Identification of tumour markers, chromosome alterations, gene amplifications, gene mutations and expression profile in cytokeratin positive cells from bone marrow confirms their malignant nature and can explain their biological behaviour or phenotype. Both *in vitro* and *in vivo* models are of importance for better understanding of the biology of the disseminated tumour cells.

The role of oestrogen in growth, development and function of the mammary gland is well established, and detection of oestrogen receptors (ERs) in breast tumours has for some time been beneficial, together with progesterone receptors, for prognosis and selection of cancer therapy. The review by Stephanie Sommer and Suzanne A. W. Fuqua focuses on the recent understanding of ER action in relation to breast cancer. Some of the new discoveries of ER are: a new receptor (ER β), new ER α sequence variants (only in a minority of tumours), co-regulatory proteins modulating ER activity, the importance of conformational changes of ER, alternative downstream signalling pathways, and cross-talks of signal transduction pathways. Patients with ER positive tumours have better prognosis and these tumours are considered to be hormone-dependent and can be treated with tamoxifen or by other alternatives that affect oestrogen action. Nonetheless, some of the breast tumours are, or can become, resistant to hormonal therapy, even in cases where the ER is expressed. The anti-oestrogen resistance mechanism is not fully understood but could be explained by some of the following mechanisms: reduced expression of ER in the tumours (methylation of the promoter

may be involved in some cases), the existence of sequence variants (higher frequency has been reported in metastatic compared to primary tumour growth), up-regulation of co-repressors, down-regulation of co-activators or rapid inactivation of the hormone. Mutations in ER can modulate its activity by affecting residues that play a major role, including phosphorylation of a co-factor binding site. The model of oestrogen action is not as simple as formerly believed, partly by the definition of the different protein interacting partners, co-activators and co-repressors. In some of the non-classical models of ER activation, the AF1 or AF2 mediated ER regulated expression is dependent on cell types, and ER can enhance the activity of the Sp1 transcriptional factor through protein-protein interaction. In addition, ligand independent transcriptional activation by ER can be controlled by serine or tyrosine phosphorylation on AF1 or AF2. The ER action is further complicated by cross-talks with other signal transduction pathways. Some of the anti-oestrogens (e.g. tamoxifen) function as antagonists, that have oestrogen function in some tissues and anti-oestrogen in others, i.e. can act as SERMs (selective ER modulators). The gene for one of the co-activators of ER, the SRC3, is located on a chromosome region 20q found to be amplified and with increased protein expression in a fraction of breast tumours. This overexpression might result in enhanced oestrogen-induced growth stimulation. Further research on these recent findings on the ER will undoubtedly cast more light on some of the biological behaviour of breast cancer growth in the future.

The most frequent point mutations in breast tumours are in the TP53 tumour suppressor gene. But mutations in the TP53 gene occur only in about 30% of breast tumours, that is considerably less than the average 50% documented for all tumour types. Magali Olivier and Pierre Hainaut review the 1392 TP53 mutations in breast cancer so far described. Several reports show that TP53 mutations are associated with poorer prognosis but it is not as clear whether these tumours have reduced response to therapy. The p53 protein accumulates in over 50% of breast cancer, partly due to the more stable nature of some of the mutated forms and also because the p53 pathway can be activated in tumour cells, by stabilization of the w.t. p53 protein. The negative regulator of p53, the Mdm2 protein, which can promote p53 degradation, is found overexpressed in a fraction of breast cancers, and is presumably an additional mechanism that can turn off the p53 function in the

tumour cell. The TP53 differs from several other tumour suppressor genes by the high prevalence of missense mutations. Presumably this gives rise to mutated forms of p53 proteins that can act in a dominant negative manner. As this transcription factor acts as a tetramer, one mutated subunit could be sufficient to affect the function of the protein complex. In breast cancer 30% of TP53 mutations cluster at eight hotspot codons and a similar profile of TP53 mutations is detected as in other cancer types, except that there is a slightly lower frequency of transversions and slightly higher frequency of transitions. The transversions are generally more common in cancers in which carcinogens play an important role. The mutation pattern in the TP53 gene is different in Western countries compared with Japan, particularly in individuals diagnosed with breast cancer under 45 years of age, and there are also reports on higher prevalence of small deletions in specific regions of the USA. These differences in the TP53 mutations spectrum support the hypothesis that a fraction of breast cancer mutations may be induced in response to exposure to environmental carcinogens. In all, 196 germline mutations have been detected in the TP53 gene and 164 of them exist in cancer families. Breast cancer is the most frequent type of cancer in patients with inherited TP53 mutation, and mutation of one allele can predispose to early disease. The pattern of germline mutations in comparison with sporadic mutations show increased frequency of transitions at CpG sites and reduced frequency of transversions at non-CpG sites. This could possibly be explained by a specific endogenous mutagenic process that acts in the germline. Patients with germline mutation in BRCA1 or BRCA2 genes have a higher mutation rate in the TP53 gene and codon 163 is a hotspot. In these tumours there is a higher frequency of mutations at AT and a lower frequency of GC mutations at non-CpG sites in tumours with germline mutation in BRCA1 or BRCA2 genes, compared with sporadic tumours. Presumably this reflects the lack of a certain type of DNA repair in these tumours. Also, there could be a growth advantage of BRCA1 and BRCA2 mutated tumours upon deactivation of the p53 pathway, including escape from the apoptosis programme, normally turned on by a lack of DNA repair.

The only gene that is expressed at a constitutive fragile site, and so far analysed with respect to tumour pathogenesis, is the FHIT gene. Recent findings on the FHIT alterations in breast cancer are reviewed by Sigurdur Ingvarsson. Even though

classical point mutations of the FHIT gene are not found in breast cancer, relatively high levels of abnormalities are detected, including deletions, methylation and altered or reduced expression. The relevance of these alterations of FHIT for breast cancer pathogenesis is not clear. This high frequency of alterations could merely reflect the unstable nature of the fragile site in the breast tumour cell, but it is also possible that FHIT plays a tumour suppressor role. The evidence from cell and mouse models for the role of FHIT as a tumour suppressor has recently been increasing dramatically.

John L. Hopper writes a critical review on hereditary breast cancer, mainly focussing on BRCA1 and BRCA2, but BRCAX, ATM and TP53 are also addressed. In general it is widely accepted that germline mutations in BRCA1 or BRCA2 considerably increase the lifetime risk of developing breast cancer. It was a major breakthrough in the breast cancer field when the BRCA1 and BRCA2 were cloned. Until then, it was known that breast cancer was partly familial and some of the families showed linkage to chromosomes 17q and 13q. After the discovery of the BRCA1 and BRCA2, the first material to be screened for mutations was from high-risk multicancer families. This work led to the conclusion that there was relatively high penetrance of breast cancer when one of these genes was mutated in germline. Today, several population-based studies show that the penetrance may be much lower than originally estimated. There can be a great variation in risk, and not all mutations appear to confer a high risk in all settings. This variance in penetrance is detected for different genes and different mutations within the particular gene. Therefore it is difficult to generalize, and there is uncertainty about the cancer risk in mutation carriers. The risk estimate associated with a mutation depends not only on its type and location, but also on the sampling frame of the study from which that estimate was derived. In Hopper's review the putative modifications (genetic, epigenetic and environmental) of cancer risks in mutation carriers are discussed. Since breast cancer is a common disease, small familial clusters could occur by chance alone. This means that cases believed to be hereditary may be sporadic. A large number of BRCA1 or BRCA2 carriers do not appear to have a family history of breast cancer, nor are they members in a multiple-case family. This proportion is presumably higher in families with a low number of individuals in each generation, and where modifying factors are favourable for suppression of tumour growth. One

possible explanation is that there are factors shared by mutation carriers within a family which modify their risk. These familial modifiers could be different genetic variants of BRCA1 or BRCA2, or other genes, or environmental or lifestyle factors.

Information on the Brcal and Brca2 proteins has been rapidly increasing over recent years, mainly with respect to protein-protein interaction and their role in transcription, DNA repair and genome integrity. Furthermore, expression of Brcal and Brca2 and phosphorylation of the Brcal is regulated during the cell cycle. Despite several similarities affecting these cell functions, the Brcal and Brca2 proteins are unrelated with respect to amino acid sequence. As Åzke Borg reviews, the Brca proteins are part of multiprotein complexes. The Brcal protein is part of the BASC (Brcal associated genome surveillance complex) that includes other DNA repair proteins and some of the complexes detected also include proteins involved in transcription, protein degradation and mitotic checkpoints. All this interaction suggests that the Brcal protein (and Brca2) has an essential function in multiple biological pathways. Borg also reviews the histological and chromosomal characteristics of breast tumours in individuals carrying BRCA1 or BRCA2 mutations. Tumours arising in carriers of BRCA1 mutation differ morphologically and immunohistochemically from those caused by BRCA2 mutations and also from sporadic cases. Compared with sporadic tumours, the BRCA1 and BRCA2 tumours have higher overall grade, higher mitotic activity, lower frequency of lobular breast cancer, higher frequency of non-infiltrative and smooth edges (continuous pushing margins), and lower frequency of tubule formation/tubular tumours. In addition BRCA1 tumours have lower frequency of cribriform cancers and ductal carcinoma *in situ*. Atypical lymphocyte infiltration is also detected in BRCA1 tumours, and may be due to the presence of specific antigens. In general tumours with BRCA2 mutations are more heterogeneous with respect to phenotype than BRCA1 mutated tumours. Chromosome abnormalities are also of higher frequency, as demonstrated by pleomorphism and aneuploidy of BRCA1 tumours, and several chromosome abnormalities detected in BRCA1 and BRCA2 tumours. These chromosome abnormalities are of higher frequency in hereditary than in sporadic tumours, and a particular pattern is detected that distinguishes BRCA1 from BRCA2 tumours, as well as both of these tumour types from sporadic

tumours. This phenotype of the genome in BRCA1 and BRCA2 tumors is compatible with the normal role of these genes in the maintenance of genomic stability. The general aggressive appearance could be related to intrinsic chromosomal instability, defective DNA repair and centrosome regulation. Particular expression profiling is detected in BRCA1 and BRCA2 tumours (see also the last review in this issue). Thus histology, chromosome alterations and expression profiling can distinguish between sporadic, BRCA1 and BRCA2 breast tumours, and this information could be relevant for the management of breast cancer.

Important information on the function of the BRCA1 and BRCA2 genes and proteins has been obtained from mouse knockouts, as reviewed by Chu-Xia Deng and Steven G. Brodie. APC, ATM, PTEN and TP53 knockout mice are also reviewed, including the recent studies showing that APC mutation may be more relevant to mammary tumorigenesis than previously believed. Also, a certain mouse strain with heterozygous p53 knockout may serve as a unique model to study breast cancer in the Li-Fraumeni syndrome. One of the advantages of these knockout mice in studying tumour pathogenesis is that mice with different mutations can be interbred to access potential interaction between genes. Some of the findings based on the knockout mice include activation of the p53 pathway in response to genome alterations due to BRCA1 or BRCA2 mutations. Several functional aspects of the Brcal and Brca2 proteins have also been clarified in these studies; including their role in DNA repair, transcription, apoptosis and cell cycle progression; in general showing their importance for genome integrity. Some of these findings are based on mouse embryo fibroblasts, since the double knockouts are lethal at the embryonic stage. But hypomorphic mutations of Brca2, where the mice survive embryogenesis and develop tumours, and conditional knockouts, where one copy of the Brcal gene is inactive in germline and the other copy is conditional (spatially and temporally) for the mammary tissue, using the Cre-loxP system, have also been described. These conditional knockout experiments have provided direct genetic evidence that p53 loss accelerates mammary tumour formation in association with Brcal mutation. Loss of Brcal or Brca2 in the knockout mouse models has shown that this results in genome instability and tumour growth, where higher prevalence of mammary tumours is detected in the conditional knockouts. These studies have added

much to our understanding of the roles of these genes in mammary tumour initiation, promotion and progression. Loss of Brcal function does not directly initiate tumorigenesis, but promotes genetic instability, triggering further alterations that are critical for tumour formation, such as in tumour suppressor genes (TP53) and oncogenes, ultimately leading to tumour formation (see, also, the review by Oliver and Hainaut on the TP53 gene). This process of mutation accumulation requires time and may account for the latency of cancer incidence in germline mutation carriers and the rare incidence of somatic BRCA1 or BRCA2 mutations. This tumour progression may start with increased apoptosis that is defeated at later stages by further gene alterations.

Microarray (cDNA, CGH and tissue) studies in breast cancer are reviewed by Outi Monni, Elizabeth Hyman, Spyro Mousses, Anne Kallioniemi and Olli-P. Kallioniemi. The combined use of these methods allows integration of the cytogenetic and functional views of the cancer genome, which facilitates the understanding of the molecular basis of breast cancer development. Data are now available on the expression of a large number of genes from the overall genome or particular chromosome regions of interest, i.e. for identification of targets of chromosomal changes in breast tumours. Based on gene expression profiles, sporadic breast cancer can be divided into two major groups of basal and luminal types. These studies include the demonstration that ER negative clusters have a subset of basal-like rather than luminal-like tumours, suggesting different tumour behaviour and prognosis. Comparison of breast cancer in BRCA1 and BRCA2 mutant carriers, with each other and with sporadic breast tumours, shows that gene expression profiles are distinct in these three subgroups. The difference in expression includes down-regulation of ER and certain cytokeratins in BRCA1 tumours and up-regulation of cyclinD in BRCA2 tumours. Gene expression profiles of the individual tumour samples could possibly be used to accurately predict which genetic mutation they carried. These recent methodological developments have the potential to have a large impact on breast cancer research, where they could contribute to improving the specificity of tumour classification, that will eventually be developed into a useful tool for oncologists and lead to the identification of new therapeutic agents. However, some significant questions concerning the design, data interpretation and clinical utility of gene-expression profile studies remain to be answered.