Recent advances in breast cancer

Compiled and annotated by J Louise Jones, Centre for Tumour Biology, Barts Institute of Cancer, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ, UK

Molecular analysis of breast cancer subtypes

Over the past decade gene array studies have had major impact in the breast cancer field. Expression arrays, array CGH, and gene signatures derived from such approaches have been used to better understand the morphological diversity of breast cancer. The impact of these developments on breast cancer classification, generation of prognostic information, and prediction of response to specific therapies is covered in a detailed review by Weigelt et al. [1]. One approach that has been proved particularly effective has been to study the less common special types of breast cancer in order to dissect the relationship between genetic and morphological diversity. Geyer et al. [2] used morphologically diverse metaplastic carcinomas to address the issue of intra-tumour genetic heterogeneity, which may be implicated in the emergence of tumour subclones resistant to chemo-or targeted therapies. Distinct genetic alterations in different parts of a tumour or at different stages in tumour development has important implications for planning of therapy, particularly in the modern era of targeted therapies that are dependent on activation or inactivation of specific pathways.

Using a similar approach, the same group analysed the genetic alterations in micropapillary carcinomas (MPC), both in pure form and admixed with invasive ductal carcinoma of no special type (IDC-NST) [3]. They identified consistent genetic alterations in this tumour phenotype and demonstrated greater similarity between mixed MPC and pure MPC, possibly explaining the more aggressive nature of mixed MPC compared to IDC-NST.

Whilst previous studies have reported differences in gene expression profile between invasive lobular carcinoma (ILC) and IDC-NST, these generally have been confounded by comparison with very diverse IDC, where ILC are almost always ER positive, grade I or II and of a luminal phenotype. A more meaningful comparison may be achieved using grade- and molecular subtype-matched IDC tumours, an approach used in the study by Wiegett et al. [4]. Gene expression profiling clearly separated ILC from IDC, and the differentially expressed genes between the groups provide clues to the mechanisms that determine the distinct clinicopathological features of these tumours.

1. The contribution of gene expression profiling to breast cancer classification, prognostication and prediction: a retrospective of the last decade
   Britta Weigelt, Frederick L Baehner, Jorge S Reis-Filho
2. **Molecular analysis reveals a genetic basis for the phenotypic diversity of metaplastic breast carcinomas**
   Felipe C Geyer, Britta Weigelt, Rachael Natrajan, Maryou BK Lambros, Dario de Biase, Radost Vatcheva, Kay Savage, Alan Mackay, Alan Ashworth, Jorge S Reis-Filho
   *The Journal of Pathology* 2010; 220: 562-73. (Original Paper)

3. **Mixed micropapillary-ductal carcinomas of the breast: a genomic and immunohistochemical analysis of morphologically distinct components**
   Caterina Marchiò, Marjan Iravani, Rachael Natrajan, Maryou BK Lambros, Felipe C Geyer, Kay Savage, Suzanne Parry, Narinder Tamber, Kerry Fenwick, Alan Mackay, Fernando C Schmitt, Gianni Bussolati, Ian Ellis, Alan Ashworth, Anna Sapino, Jorge S Reis-Filho

4. **The molecular underpinning of lobular histological growth pattern: a genome-wide transcriptomic analysis of invasive lobular carcinomas and grade- and molecular subtype-matched invasive ductal carcinomas of no special type**
   Britta Weigelt, Felipe C Geyer, Rachael Natrajan, Maria A Lopez-Garcia, Amar S Ahmad, Kay Savage, Bas Kreike, Jorge S Reis-Filho
   *The Journal of Pathology* 2010; 220: 45-57. (Original Paper)

**In vivo and in vitro models of breast cancer**

To study the biology of breast cancer, appropriate model systems are required. Transgenic mouse models have advanced significantly our understanding of the role of selected genes in tumour development, and their utility is further enhanced by the use of targeted conditional gene mutations such as the Cre/Lox P site specific recombination system, which allows spatio-temporal control of genetic aberrations. However, an ideal model of mammary breast cancer would allow normal development of the mammary gland, stochastic occurrence of mutant cells in a background of wild-type cells, and the introduction of more than one gene mutation, to more accurately reflect human tumourigenesis.

Evers *et al.* [5] describe an elegant model that achieves these goals, combining the selective targeting of the Cre/Lox P system with the greater flexibility afforded by transplantation into cleared fat-pads. This allows the study of a range of factors contributing to tumourigenesis, such as changes in the micro-environment, without the labour-intensive and time-consuming process of intensive breeding.

Infiltrating lobular carcinomas exhibit distinct clinical, morphological and genetic features but biological studies have been limited owing to lack of well-characterised invasive lobular breast cancer cell lines. Christgen *et al.* [6] describe the isolation of metastatic lobular breast cancer cells (designated IPH-926) from malignant ascites followed by clonal isolation and serial culture. The authors draw attention to an important consideration in the use of such model systems; the need for rigorous characterisation of cell lines to avoid mis-identification and consequent use of inappropriate cell populations.
Controversy still remains over the identity, nature and even existence of cancer stem cells, however, several generally accepted techniques for enriching these ‘tumour-initiating cells’ have been established, including growth of cells as mammospheres. Kok et al. [7] used this approach to identify a ‘mammosphere-derived’ gene set in ER-positive breast cancer cells. Interestingly, mammosphere cells showed reduced ER protein expression and a significant enrichment for cell cycle related genes, which were predominantly down-regulated. This gene signature identified a more favourable outcome in patients with ER-positive tumours. That a gene profile based on a cancer stem-cell enriched population relates to an improved outcome seems counter-intuitive and it is suggested that this may reflect distinct characteristics of ER-positive compared to ER-negative stem cell populations.

5. A tissue reconstitution model to study cancer cell-intrinsic and -extrinsic factors in mammary tumourigenesis
   Bastiaan Evers, Ewoud N Speksnijder, Eva Schut, Metamia Ciampricotti, Matthew J Smalley, Patrick WB Derksen, Jos Jonkers, Karin E de Visser
   The Journal of Pathology 2010; 220: 34-44

   Matthias Christgen, Henriette Bruchhardt, Catarina Hadamitzky, Cornelia Rudolph, Doris Steinemann, Dorothea Gadzicki, Britta Hasemeier, Daniel Römermann, Tim Focken, Till Krech, Matthias Ballmaier, Brigitte Schlegelberger, Hans Kreipe, Ulrich Lehmann

7. Mammosphere-derived gene set predicts outcome in patients with ER-positive breast cancer
   Marleen Kok, Rutger H Koornstra, Tania C Margarido, Renske Fles, Nicola J Armstrong, Sabine C Linn, Laura J Van t Veer, Britta Weigelt

Biological determinants of therapeutic response

Understanding the mechanisms underlying tumour response or resistance to therapeutic agents is key to the design of more effective treatments, and a number of recent papers in the Journal have tackled this area.

Parkin is an E3 ubiquitin ligase that catalyses protein ubiquitination; reduced expression and inactivation are frequently observed in human cancers, including breast cancer. The study by Wang et al. [8] focuses on the ability of Parkin to interact with microtubules and enhance the efficacy of binding of microtubule-targeting drugs such as Paclitaxel. Importantly, these in vitro data were supported by observations in human tissues, where high Parkin expression levels correlated with pathological response to neoadjuvant paclitaxel-containing chemotherapy, and also reflected in vitro sensitivity to paclitaxel treatment. This study provides mechanistic insight into how response to chemotherapeutic agents may be modulated and provides not only a predictive marker but potential for manipulation of chemosensitivity in breast cancers.
Holm et al. [9] demonstrate the power of analysing well-defined patient tissue samples to translate experimental findings into the clinical setting. Using patients from a randomised controlled study with extended follow-up, Holm et al. showed serine phosphorylation status of ERα to be a significant predictor of tamoxifen resistance in pre-menopausal women. The same was not shown in a separate patient cohort with relapsed disease, which may reflect changes in the tumour between primary and recurrent disease, or differences between the disease in pre-menopausal versus post-menopausal women, both of which were included in the second cohort.

Further insight into the mechanisms of anti-hormone resistance is provided by Gee et al. [10] who demonstrate that acquisition of resistance is associated with elevated expression of the transcription factor AP2-gamma, and that AP2-gamma levels act as an independent predictor of outcome and response to tamoxifen, even in traditionally good prognosis ER-positive and Her2-negative subgroups. Further studies to identify the transcriptional targets of AP2-gamma may offer potential targets for therapeutic manipulation in anti-hormone-resistant tumours.

A key feature of invasive lobular breast cancer is down-regulation of E-cadherin, a feature also evident in atypical lobular hyperplasia and LCIS, and frequently mediated by promoter hypermethylation. Interestingly, Zou et al. [11] detected CDH1 promoter hypermethylation in adjacent non-neoplastic epithelium though not in breast tissue from women without cancer, suggesting this may be the first-hit in the silencing of CDH1 gene, predisposing to lobular neoplasia. Since methylation is potentially reversible, this possibly represents a novel chemopreventive target for lobular neoplasia.

Ashworth’s group [12] make the case for classifying tumours on the basis of their genetic defect, specifically focusing on different forms of genomic instability, that may then be exploited for the development of novel therapeutic approaches. In relation to breast cancer, the power of this approach has been most elegantly demonstrated in dissecting the role of BRCA1 and BRCA2 proteins in the repair of double stranded breaks (DSB) by homologous recombination. Exploiting the concept of synthetic lethality, the defective DSB repair exhibited in cells mutated for either BRCA1 or BRCA2 is made lethal by the concurrent inhibition of the base excision repair enzyme PARP1. As well as being highly effective against mutated cells, the approach gives a degree of selectivity towards the abnormal cells not normally achieved with conventional chemotherapies. This exciting discovery effectively demonstrates the importance of dissecting fundamental biological processes to inform the design of therapeutic agents.

Whereas BRCA1 mutation carriers generally develop ER-negative breast cancers, BRCA2 hereditary breast cancers are usually ER-positive. Malone et al. [13] suggest this may be a consequence of oestrogen-regulated activation and stabilization of BRCA2. They demonstrate that as a result of this interaction, oestrogen mediates enhanced radiation survival in breast cancer cells and suggest this molecular interaction may offer opportunities for combination therapies, for example hormone therapy with agents targeting BRCA2 DNA repair pathways.

8. **Parkin regulates paclitaxel sensitivity in breast cancer via a microtubule-dependent mechanism**
   Hongxia Wang, Bingbing Liu, Chao Zhang, Guoyuan Peng, Min Liu, Dengwen Li, Feng Gu, Quan Chen, Jin-Tang Dong, Li Fu, Jun Zhou
9. Phosphorylation of the oestrogen receptor α at serine 305 and prediction of tamoxifen resistance in breast cancer
C Holm, M Kok, R Michalides, R Fles, RHT Koornstra, J Wesseling, M Hauptmann, J Neefjes, JL Peterse, O Stål, G Landberg, SC Linn

10. Overexpression of *TFAP2C* in invasive breast cancer correlates with a poorer response to anti-hormone therapy and reduced patient survival
JMW Gee, JJ Eloranta, JC Ibbitt, JFR Robertson, IO Ellis, T Williams, RI Nicholson, HC Hurst

11. Epigenetic silencing in non-neoplastic epithelia identifies E-cadherin (*CDH1*) as a target for chemoprevention of lobular neoplasia
Donghui Zou, Han-Seung Yoon, David Perez, Robert J Weeks, Parry Guilford, Bostjan Humar

12. Genomic instability and the selection of treatments for cancer
Sarah A Martin, Madeleine Hewish, Christopher J Lord, Alan Ashworth

13. Oestrogen-mediated phosphorylation and stabilization of BRCA2 protein in breast
JL Malone, AC Nelson, R Lieberman, S Anderson, JT Holt

**Molecular pathology directs treatment decisions**

An accurate assessment of Her2 amplification status as distinct from chromosome 17 polysomy is essential for the selection of patients to be offered trastuzumab. Dual-colour FISH, using a centromeric probe in addition to a Her2 probe, is widely adopted to correct for Chr17 polysomy, however, the study by Marcio et al. [14] suggests that even in the presence of abnormal *CEP17* copy numbers, this is rarely due to polysomy but more frequently the result of complex genetic aberrations involving the centromeric/paracentromeric region of Chr17. These findings have important implications in terms of the potential mis-classification of a patient’s tumour as Her2 non-amplified, and this is reinforced in the commentary from Viale [15], who supports the suggestion that such misclassification may be the basis for reports indicating benefit for patients treated with trastuzumab despite allegedly Her2 negative tumours.

14. Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence *in situ* hybridization and microarray-based CGH analysis
Caterina Marchiò, Maryou B Lambros, Patrizia Gugliotta, Ludovica Verdun Di Cantogno, Cristina Botta, Barbara Pasini, David SP Tan, Alan Mackay, Kerry Fenwick, Narinder Tamber, Gianni Bussolati, Alan Ashworth, Jorge S Reis-Filho, Anna Sapi
15. Be precise! The need to consider the mechanisms for CEP17 copy number changes in breast cancer
   Giuseppe Viale

**Refining prognostic signatures**

The importance of p53 alterations in breast cancer is well recognized but is likely to have been under-estimated. Ellis’s group hypothesized that a more accurate assessment of p53 disregulation could be gained by the analysis of the functional state of p53-regulation proteins [16]. They showed that, regardless of p53 levels, patients with tumours displaying a completely inactive p53 pathway had poorer prognosis and poorer outcome even following adjuvant therapy. This once again demonstrates the importance of dissecting molecular interactions and translating this to well defined patient cohorts with accurate associated clinicopathological data. Such an approach may also be valuable in assessing the functional status of other complex molecules, such as BRCA1, which may help elucidate the causes and extent of ‘BRCAness’ in the absence of gene mutations. A further important point made by Thompson and Lane in an accompanying commentary [17] relates to the choice of antibody used to detect p53, some being specific for phosphorylation at serine residues, which may offer additional information on p53 functional status.

16. The biological, clinical and prognostic implications of p53 transcriptional pathways in breast cancers
   Tarek M Abdel-Fatah, Desmond G Powe, Johnson Agboola, Martyna Adamowicz-Brice, Roger W Blamey, Maria A Lopez-Garcia, Andrew R Green, Jorge S Reis-Filho, Ian O Ellis
   *The Journal of Pathology* 2010; 220: 419-34. (Original Paper)

17. p53 transcriptional pathways in breast cancer: the good, the bad and the complex
   Alastair M Thompson, David P Lane
   *The Journal of Pathology* 2010; 220: 401-3. (Invited Commentary)
Questions

The following questions can be answered by reading and reflecting upon the above annotation and the papers that are cited within it. Within the Royal College of Pathologists Continuing Professional Development (CPD) scheme, CPD points may be earned by writing reflective notes on the papers in this Virtual Issue and the questions are designed to act as a focus for this activity. To do this, you may wish to use the Royal College of Pathologists’ reflective notes form.

**Question 1**  What are metaplastic carcinomas and why are they useful to study intra tumoural heterogeneity?

**Question 2**  What genetic aberrations identified in micropapillary carcinomas may prove to be useful as therapeutic targets?

**Question 3**  In mouse models, what is meant by conditional knockout and what are the advantages over conventional transgenic mice?

**Question 4**  What are the key genetic alterations that have been identified in invasive lobular carcinomas and how may they contribute to disease progression?

**Question 5**  How may tumour initiating or cancer stem cells be identified in vitro?

**Question 6**  What is synthetic lethality and in what way can this be used to design novel therapies?

**Question 7**  What are the mechanisms by which CEP17 copy number may be increased?

**Question 8**  In the context of microarray data, what is meant by supervised and unsupervised hierarchical cluster analysis?

www.thejournalofpathology.com