

# UNRAVELLING THE MOLECULAR GENETIC TRAITS OF BREAST CANCER

Breast cancer is a heterogeneous disease with a variety of distinct biological and clinical behaviours. We are applying high-throughput pathology and molecular genetic methods to identify molecular traits of the disease that could be used ultimately to advance breast cancer diagnosis and treatment.



**Jorge Reis-Filho**  
MD PhD MRCPATH

Jorge Reis-Filho is Team Leader of the Molecular Pathology Team in the Breakthrough Toby Robins Breast Cancer Research Centre at The Institute of Cancer Research.

## Pathological features and molecular genetic aspects of breast cancer

Historically, breast cancer development was perceived as a multistep model in which normal breast cells acquired a cumulative series of genetic mutations leading them to transform and evolve through a series of precursors, eventually giving rise to invasive cancer. It was believed that, once malignant, breast cancer cells would acquire additional changes, and become increasingly more undifferentiated and aggressive, leading to the development of metastases. In the process, breast cancer cells acquired distinct microscopic morphological features which were used for diagnostic and prognostic determination by histopathologists. In other words, it was believed that breast cancer was a single disease and that the different microscopic patterns observed in breast cancers simply represented different stages of evolution.

## Breast cancer: More than one disease

This concept has dramatically changed in the last decade. Combined histopathological and molecular genetic analysis of breast cancers has revealed that there are at least two main groups of breast cancers: well-differentiated cancers (ie, low histological grade) which are characterised by very simple genomes with recurrent loss of genetic material in the long arm of chromosome 16 (16q); and poorly-differentiated cancers which have much more complex genomes. Interestingly, although poorly-differentiated cancers have genomes that are much more complex than those seen in well-differentiated cancers, loss of 16q is rarely found suggesting that progression from well-to poorly-differentiated breast cancers is unlikely.

This concept is also underpinned by clinical observation studies that demonstrate that primary breast tumours and their recurrences usually show remarkably similar histopathological characteristics.

Although current classification systems for breast cancer are solely based on their morphological features, it is now accepted that breast cancer is a heterogeneous disease even within morphologically-defined groups. Whilst it is understandable that tumours with distinct morphological features may be characterised by distinct patterns of genetic changes, it has also become clear that tumours with strikingly similar morphological features can also behave differently and that these differences may be underpinned by distinct genetic abnormalities. For example, it is known that whilst the majority of breast cancers are morphologically-defined as infiltrating ductal carcinomas (IDC), a subgroup of IDCs which display amplification and overexpression of the HER2 receptor (ie, HER2 positive tumours) carry a much worse prognosis but are more likely to respond to treatment with trastuzumab (a humanised antibody anti-HER2; Herceptin) when compared to HER2 negative IDCs.

### Breast cancer molecular pathology: One size does not fit all

It is unlikely that a reductionist approach to the study of breast cancer will be successful following the realisation that:

- There are molecular subgroups of breast cancers with similar morphological characteristics;
- Distinct genetic features of breast cancer can have a direct impact on clinical outcome;
- The same molecular genetic change may have distinct effects depending on the histological/molecular type of breast cancer.

The heterogeneity of breast cancer now poses a significant challenge to its effective diagnosis and if we are to treat breast cancer patients effectively, a paradigm shift is required.

▣ The time for us to move from a purely morphologically-based descriptive and prognostic classification system has come. Unravelling the significance of distinct molecular genetic changes in different types of breast cancer holds the key for the development of a functional and predictive molecular pathological classification of breast cancer. ▣

▣ By investigating molecularly-defined groups separately (ie, more homogeneous groups), it may become possible to identify their actual biological drivers and, therefore, develop more effective tailored therapies for breast cancer patients. ▣

### Our approach

Our research programme is devoted to the study of the distinct molecular genetic changes in breast cancer precursors and specific types of breast cancers using a combination of traditional pathology methods with high-throughput technologies developed at The Institute. Our aim is to develop a more biologically and clinically meaningful classification system for breast cancer precursors and specific types of breast cancer, based on a thorough analysis of their pathological features and molecular genetics. By characterising more homogeneous genetic subtypes of breast cancers, we aim to identify more novel therapeutic targets specific to each entity which could be used to target breast cancer in the precursor stages, thus preventing the development of invasive disease, and to tailor therapy for specific subgroups of breast cancer patients.

### FGFR1 as a therapeutic target: A proof-of-principle

We have started our analysis of specific types of breast cancer by studying classic invasive lobular carcinomas, which are the second most frequent histological type of the disease and account for approximately 10% of all breast cancer. The mainstay of therapy for patients with lobular cancers remains surgery followed by endocrine therapy, as these tumours are positive for hormone receptors, lack HER2 expression and are reported not to respond to chemotherapy. However, distant recurrences are observed in up to 30% of patients. By studying invasive lobular cancers using a combination of traditional pathology methods and molecular genetic analysis, we have identified a subgroup characterised by recurrent amplifications of a cell-surface tyrosine kinase receptor gene, the fibroblast growth factor receptor 1 (*FGFR1*) gene (see Figure 1). Detailed molecular genetic expression profiling and immunohistochemical analysis of breast cancer cell lines identified one (MDA-MB-134) that has all the characteristics of a molecular subgroup of lobular cancers with *FGFR1* gene amplification. Using methods to silence *FGFR1* signalling, we have demonstrated that MDA-MB-134 cells depend on

FGFR1 signalling for their survival (see Figure 2). On the other hand, cell lines with similar molecular genetic patterns but lacking *FGFR1* gene amplification did not show any dependence on this signalling pathway. In collaboration with Professor Ian Ellis from the University of Nottingham, we have recently completed the analysis of *FGFR1* gene status in 880 patients with breast cancer. Results showed that the *FGFR1* gene is amplified in approximately 9% of all breast cancers. Most importantly, *FGFR1* gene amplification has proved to be an independent predictor of outcome in patients with oestrogen receptor-positive disease. Taken together, our findings suggest that a subgroup of patients with *FGFR1*-amplified invasive lobular cancers and oestrogen receptor-positive cancers may benefit from therapeutic strategies targeting *FGFR1* gene signalling. Drugs targeting this receptor are currently being tested for the management of some haematological malignancies and inflammatory diseases.

▣ If *in vivo* tests with FGFR1 tyrosine kinase inhibitors prove effective, we aim to set up a clinical trial to test these drugs for the management of patients with *FGFR1*-amplified breast cancers within the next two years. ▣

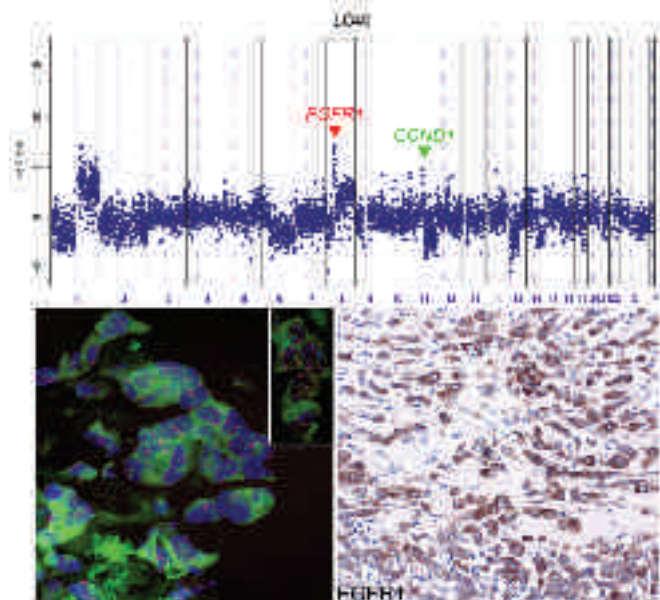


Figure 1: Microarray-based comparative genomic hybridisation (CGH) analysis of classic lobular carcinomas of the breast (top) revealing a subgroup that harbours co-amplification of *FGFR1* and *cyclin D1* gene (*CCND1*) (bottom left) and expresses *FGFR1* (bottom right) at high levels.

## Novel therapeutic targets for triple negative tumours

Patients with triple negative tumours (ie, oestrogen receptor, progesterone receptor and HER2 negative cancers) at present cannot be targeted with tailored therapies. These tumours account for up to 15% of all breast cancers and have a rather aggressive clinical behaviour. We have recently demonstrated that up to 50-60% of these tumours express the epidermal growth factor receptor (EGFR). EGFR tyrosine kinase inhibitors and humanised antibodies against EGFR have been used for the management of patients with some types of colon, lung and head and neck cancer. However, it has been demonstrated that EGFR expression is a poor predictor of response to these agents. Interestingly, tumours where EGFR expression is driven by an underlying genetic aberration on the *EGFR* gene (ie, activating gene mutation or gene amplification) show higher response rates to EGFR tyrosine kinase inhibitors than those with EGFR expression alone. Whilst analysing the molecular genetics of a rare specific histological type of triple negative cancer called metaplastic breast cancer, we have identified a subgroup harbouring *EGFR* gene amplifications (see Figure 3). Currently, we are testing *in vitro* whether triple negative breast cancer cells are sensitive to EGFR inhibitors.

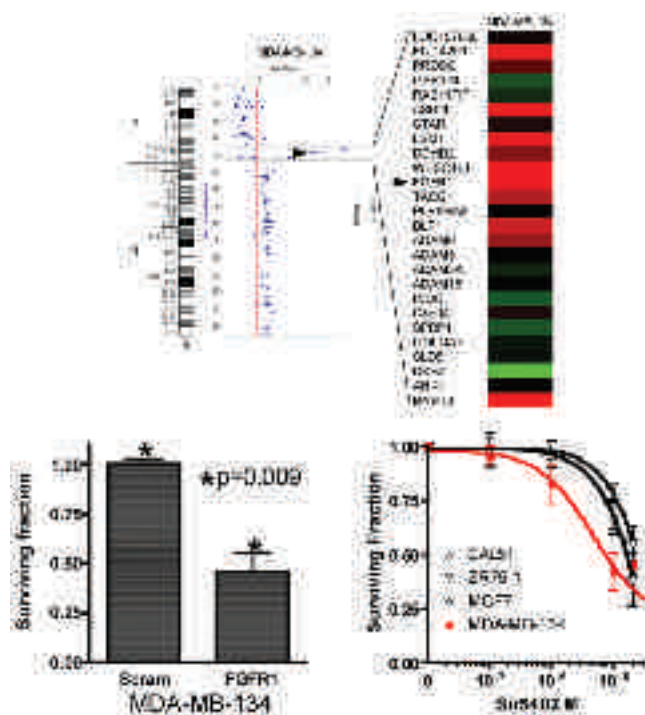


Figure 2: The cell line MDA-MB-134, which has all the hallmark molecular features of classic lobular carcinoma (ie, positivity for hormone receptors, lack of HER2 and E-cadherin expression, 16q-), was shown to harbour *FGFR1* and *CCND1* co-amplification and express high levels of *FGFR1* mRNA (top, Red: high expression; Green: low expression). When transfected with siRNA against *FGFR1* (bottom left) or treated with SU5402 (bottom right), a *FGFR1* tyrosine kinase inhibitor, the growth of MDA-MB-134 cells was significantly inhibited.

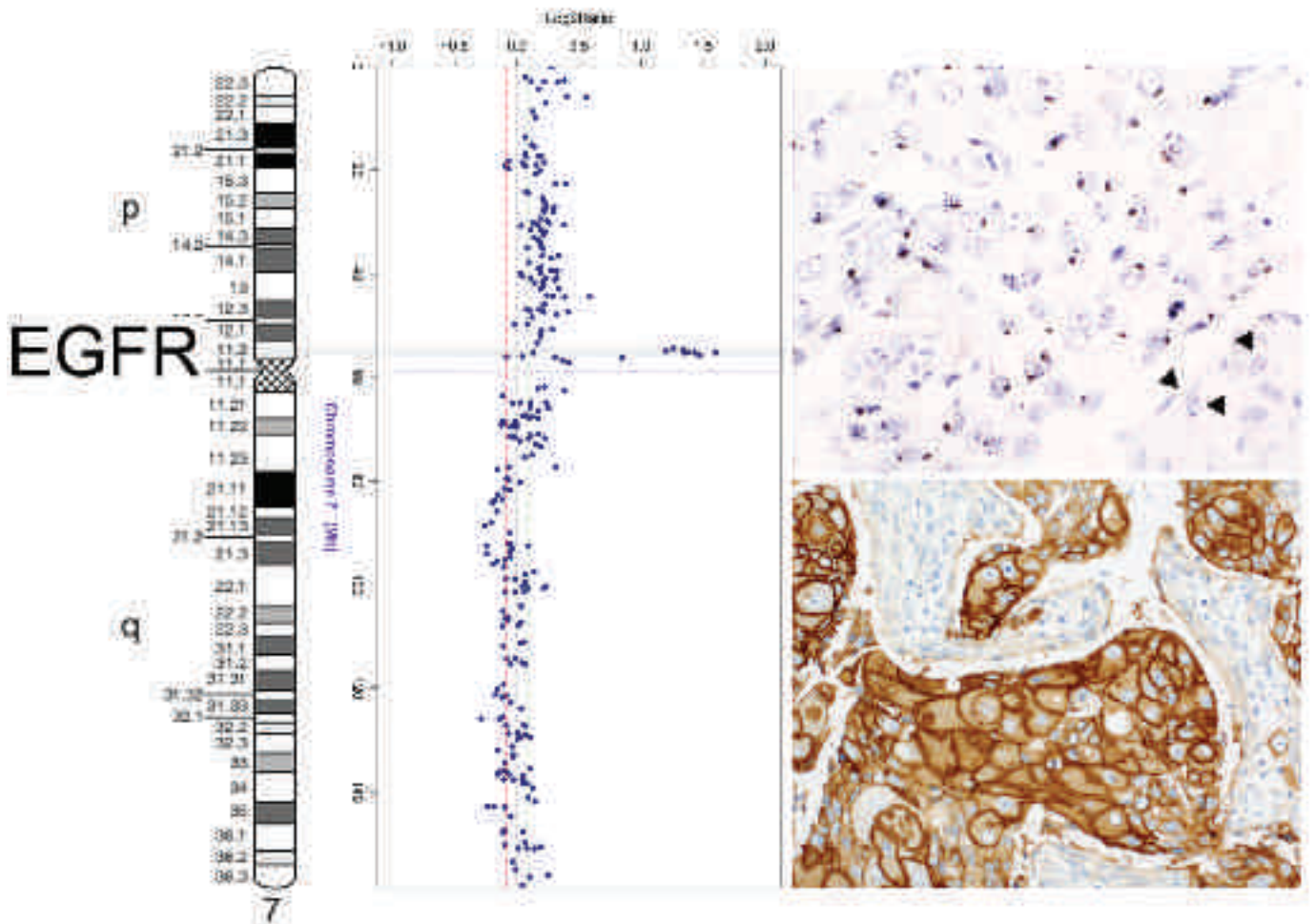


Figure 3: Triple negative metaplastic breast cancer with *EGFR* gene amplification. Left – microarray CGH analysis revealing an amplification of 7p11.2, mapping to the *EGFR* gene locus. Top right: Chromogenic *in situ* hybridisation showing clusters of signals in neoplastic cells and only two signals per nucleus in normal cells (arrowheads). Bottom right: Tumour cells strongly expressing *EGFR*.

▣ Our findings may provide an effective alternative for the management of patients with a subgroup of triple negative/basal-like breast cancers. ▣

### Future developments

We believe that by combining pathology and genetics we can unravel the complexity of breast cancers and refine the current classification system. However, molecular pathology is not merely an exercise in 'tumour philately'! With the identification of more homogeneous subgroups of patients, we will expedite the identification of the molecular drivers of tumour growth and progression and, therefore, of therapeutic targets in cancer.