O-29. IS THE MCF-7 BREAST CANCER CELL LINE OF VALUE IN RESEARCH?

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The established cell line MCF-7 is a widely used model in breast cancer research. Since it was first derived 30 years ago, several studies have produced conflicting results. We believe that significant in-vitro mutation, clonal evolution, and divergence of culture techniques have produced marked variability between sources. We have used multi-coloured fluorescent in-situ hybridization (M-FISH) to assess the genetic variability of MCF-7 from different sources for the first time at the single cell level.

MCF-7 cells from our laboratory were analysed using M-FISH at two time points, (#1 and #2), 18 months apart, and compared with MCF-7 lines from two other sources (#3) and (#4). Seven to 10 metaphase spreads, each containing 44 to 74 chromosomes, were examined for structural variability.

Translocations found in at least 50% of metaphases from all sources included 2;3, 10;7, 8;16, 8;11;16, and 15;16. Aberrations, common to two, or three of the four samples, were also present. However, a number of abnormalities were unique to each MCF-7 specimen, such as the marker chromosome t(8;19;12) found in MCF-7(#1), and t(6;8;19;12) identified 18 months later in MCF-7(#2). Unique marker chromosomes detected in MCF-7(#3) and (#4) included der(8)t(8;1;8) and t(16;1;20;9) respectively.

We have shown for the first time using M-FISH, that MCF-7 has sustained a vast number of mutations, which will render its properties inconsistent between labs, and in the same lab over a period of time. Such cell lines are already known to have limited relevance to primary tumours. Because of the current results we propose that future work performed using cell lines be preceded by a full karyotype analysis to permit accurate inter-study comparison.

O-30. CerbB2 ONCOPROTEIN EXPRESSION IS INCREASED IN BREAST CANCER BONE METASTASES

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Aim: Type 1 Tyrosine Kinase receptors such as Epidermal Growth factor receptor (EGFR) and CerbB2 oncoprotein have been shown to predict for a poor prognostic outcome in early breast cancer (EBC), but the incidence of such receptors in breast cancer at metastatic sites is unknown. We determined the incidence of these receptors in breast cancer bone and visceral metastases compared to primary EBC.

Method: Using standard immunohistochemical techniques, the incidence of EGFR and CerbB2 expression was determined on sections of formalin fixed primary and metastatic breast tissue. Disclosure of the primary antibodies was performed with a swine anti-rabbit antibody, streptavidin and diaminobenzidine-hydrogen peroxide. Positive staining was classed as greater than 5% of epithelia expressing the target antigen. A positive control was used in each run and a control slide used for each test sample. Scoring was performed by a single observer, and verified by an experienced pathologist. Results were analysed using the Chi squared test.

Results:

	CerbB2	EGFR
Bone metastases	*11/21 (52%)**	6/21 (29%)
EBC	21/76 (28%)	25/70 (36%)
Visceral Metastases	9/22 (40%)	10/18 (55%)

Expression of CerbB2 oncoprotein was significantly increased in bone metastases, compared to EBC (*$p = 0.029$). The frequency of EGFR expression was highest in visceral metastases.

Conclusion: The pattern of metastases is related to the type of Tyrosine Kinase Receptor expressed. The increased expression of CerbB2 oncogene in breast cancer bone metastases may explain the low response rate to hormonal therapy in bone and supports the rationale for expressing receptor status and the use of therapy directed against Type I Tyrosine Kinase receptors in breast cancer.

O-31. HER-2 STATUS IN LOCALLY ADVANCED BREAST CANCER

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Her-2 neu amplification is increasingly regarded as the best indicator of response to anti-her-2 therapies. Using FISH, we have studied 57 locally advanced breast cancers, and found 22 (39%) to have gene amplification. Amongst the non-inflammatory cancers, 17/32 (53%) were amplified, significantly ($p = 0.01$) more than in inflammatory 5/24 (21%), a rate not different to operable breast cancers (42/223).

There was no difference in survival between non-inflammatory and inflammatory cancers, and those tumours with Her-2 amplification appeared to do worse ($p = 0.08$). In the ER+ve subgroup, Her-2 amplification did predict for a poorer outcome ($p = 0.001$), although numbers were small. Conversely, in the ER−ve group, all of whom received tamoxifen, Her-2 amplification had no prognostic value ($p > 0.9$). Her-2 overexpression is thought to sensitize ER+ve cancers to low levels of oestrogen. It is therefore possible that in cancers with low levels of ER and normal oestrogen a similar effect could be seen.

These data suggest two hypotheses. Firstly, that Her-2 drives non-inflammatory cancers, so that they present at an advanced stage, but cannot explain the clinical behaviour of inflammatory cancers. Secondly, that Her-2 modulates the effect of tamoxifen in ER+ve cancers, but not in tamoxifen-treated ER−ve tumours.

Further studies of patients with ER +ve and ER −ve tumours should determine whether Her-2 amplification sensitizes ER+ve cancers to the agonistic effects of tamoxifen.