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Review

Relevance of breast cancer cell lines as models for breast tumours: an update

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Summary

The number of available breast cancer cell (BCC) lines is small, and only a very few of them have been extensively studied. Whether they are representative of the tumours from which they originated remains a matter of debate. Whether their diversity mirrors the well-known inter-tumoural heterogeneity is another essential question. While numerous similarities have long been found between cell lines and tumours, recent technical advances, including the use of micro-arrays and comparative genetic analysis, have brought new data to the discussion. This paper presents most of the BCC lines that have been described in some detail to date. It evaluates the accuracy of the few of them widely used (MCF-7, T-47D, BT-474, SK-BR-3, MDA-MB-231, Hs578T) as tumour models. It is concluded that BCC lines are likely to reflect, to a large extent, the features of cancer cells *in vivo*. The importance of oestrogen receptor-alpha (gene *ESR1*) and Her-2/*neu* (*ERBB2*) as classifiers for cell lines and tumours is underlined. The recourse to a larger set of cell lines is suggested since the exact origin of some of the widely used lines remains ambiguous. Investigations on additional specific lines are expected to improve our knowledge of BCC and of the dialogue that these maintain with their surrounding normal cells *in vivo*.

Introduction

A considerable part of our knowledge on breast carcinomas is based on *in vivo* and *in vitro* studies performed with breast cancer cell (BCC) lines. These provide an unlimited source of homogenous self-replicating material, free of contaminating stromal cells, and often easily cultured in simple standard media. The first line described, BT-20, was established in 1958 [1]. Since then, and despite sustained work in this area, the number of permanent lines obtained has been strikingly low (about 100). Indeed, attempts to culture BCC from primary tumours have been largely unsuccessful. For instance, Gazdar et al. [2] obtained cell lines from only 18 of 177 primaries, while the percentage of success reported by Amadori et al. was as low as 0.7 (1/136) [3]. This poor efficiency was often due to technical difficulties associated with the extraction of viable tumour cells from their surrounding stroma. Most of the available BCC lines issued from metastatic tumours, mainly from pleural effusions. Effusions provided generally large numbers of dissociated, viable tumour cells with little or no contamination by fibroblasts and other tumour stroma cells. However, even with metastatic samples, success in long-term propagation has been limited. For instance, Cailleau et al. [4], Meltzer et al. [5], and Gazdar et al. [2] fruitfully propagated tumour cells in only 10, 2, and 25% of cases, respectively.

Many of the currently used BCC lines were established in the late 1970s. A very few of them, namely MCF-7, T-47D, and MDA-MB-231, account for more than two-thirds of all abstracts reporting studies on mentioned BCC lines, as concluded from a Medline (http://www.ncbi.nlm.nih.gov/PubMed/)-based survey. The transposability to tumours of results obtained with such limited numbers of cell lines is questionable. To discuss the problem of representativeness, we have

Table 1. A series of BCC lines for which a somewhat more detailed description has been given in the literature

Cell line	Type of cancer	Original tissue	Modal chromosome number(s)	ER status	PgR status	Reference
'21-series'						[6]
AU565	IDC	M (Pl)	?	−	−	[7]
BOT-2	IDC	P	63[a]	?	?	[8]
BRC-230	IDC	P	60–61[a]	−	−	[3]
BrCa-MZ-01	MC	P	66–70[a]	+	+	[9]
BrCa-MZ-02	IC	M (Pl)	46–50[a]	−	−	[9]
BSMZ	IDC	M (Pl)	80[a]	+	+	[10]
BT-20	IDC	P	49[b]	−[g]	−	[1, 11]
BT-474	IDC	P	55[a], 104[c], 103[d]	+	+	[11, 12]
BT-483	IDC	P	72[a]	+	+	[11, 12]
BT-549	PIDC	P	74–76[d], 74[e]	−	−	[h]
CAL-18A	C	P	71[a]	−	−	[13]
CAL-18B	C	P	65[a]	−	−	[13]
CAL-51	IDC	M (Pl)	46[c,f]	−	?	[14]
CAMA-1	C	M (Pl)	78[d], 80[f]	+	+	[11, 15]
DU4475	IDC	M (Sk)	87–90[a], 88–93[c], 91[d], 93[e]	−	−	[16]
EFM-19	IDC	M (Pl)	62[d]	+	+	[17, 18]
EP	IDC	M (Pl)	53[a]	+	?	[19]
EVSA-T	IDCS	M (As)	84[b], 62[c]	−	+	[11, 20, 21]
GI-101	IDC	R (L)	98–100[a]	−	−	[22]
GCS	IDC	M (As)	?	+	+	[23]
HBL-100	See text		63[a]	−	−	[24, 25]
'HCC-series'						[2]
HDQ-P1	IDC	P	55–59[a], 92–107[c]	−	−	[26]
HH315	C	M (O)	113[a]	−	−	[27]
HH375	C	M (LN)	64 and 67[a]	−	−	[27]
'HMT-series'	See text					[28]
Hs578T	CS	P	58[a], 59[e]	−	−	[11, 29]
Ia-270	IDC	M (Pl)	?	+	+	[30]
IBEP-1	IDC	M (Pl)	52[a]	−	+	[31]
IBEP-2	IDC	M (Pl)	74[a]	+	−	[31]
IBEP-3	IDC	M (Pl)	57[a]	−	+	[31]
IIB-BR-G	IDC	P	56[a]	−	−	[32]
JCK	IDC	M (Pl)	?	+	+	[23]
KPL-1	IDC	M (Pl)	77–78[a], 77[c]	+	−	[33]
KPL-3C	IDC	M (Pl)	64[a]	−	−	[34]
KPL-4	IDC	M (Pl)	53[a]	−	−	[35]
LCC15-MB	C	M (F)	?	−	−	[36]
MA11	ILC	M (BM)	64[a]	−	−	[37, 38]
MAST	IDC	M (As)	60[a]	+	+	[39]
MaTu	IDC	M (LN)	66–69[b]	−	−	[40]
MCF-7	IDC	M (Pl)	88[a], 86[c], 79[d], 65[f]	+	+	[11, 41]
MDA-MB-134 VI	IDC	M (Pl)	43[a], 42[d], 44 and 66[f]	+	−	[4, 11, 42]
MDA-MB-157	MC	M (Pl)	65[a], 64–66[b], 54 and 95[d], 62, 116[f]	−	−	[4, 43]
MDA-MB-175 VII	IDC	M (Pl)	49[a], 48[f]	+	−	[4, 11, 42]
MDA-MB-231	IDC	M (Pl)	64[a], 69–70[b]	−	−	[4, 11, 42]
MDA-MB-330	ILC	M (Pl)	64[a]	−	−	[4, 11]
MDA-MB-361	AC	M (Br)	52[a], 54–56[d], 51[f]	+	+	[4, 11]

Table 1. (*continued*)

Cell line	Type of cancer	Original tissue	Modal chromosome number(s)	ER status	PgR status	Reference
MDA-MB-415	AC	M (Pl)	72[a]	?	?	[4, 11]
MDA-MB-435S	IDC	M (Pl)	64[a], 56[e], 57[f]	−	−	[4]
MDA-MB-436	AC	M (Pl)	45 and 80[a], 39 and 80[d]	−	−	[4, 11]
MDA-MB-453	AC	M (Pl)	45[a], 89[c]	−	−	[4]
MDA-MB-468	AC	M (Pl)	35[a]	−	−	[4]
MFM-223	IDC	M (Pl)	46–47[a], 46[d]	−	−	[44]
MPE-600	C	?	46[d]	?	?	[i]
MT-1	C	P (Tr)	103[f]	−	−	[45]
MT-3	C	P (Tr)	47[c], 46[f]	−	−	[45]
MW	IDC	M (Pl)	67[a]	−	?	[19]
PMC42	C	M (Pl)	66[a], 57[f]	?	?	[46]
SK-BR-3	IDC	M (Pl)	84[a], 79[d,f]	−	−	[11]
'SUM-series'	See text					[47]
T-47D	IDC	M (Pl)	66[a], 63[d], 65[e], 62[f]	+	+	[11, 48]
UACC-812	IDC	P	58–64[a], 63[d]	−	−	[5]
UACC-893	IDC	P	62[a], 59[d]	−	−	[5]
UISO-BCA-1	IDC	M (Pl)	54[a]	−	−	[49]
UISO-BCA-2	IDC	M (Pl)	61[a]	−	−	[49]
VHB-1	IDC	P	70–74[a]	+	+	[50]
ZR-75-1	IDC	M (As)	71–72[a], 71[d], 72[f]	+	+	[11, 51]
ZR-75-30	IDC	M (As)	81[a], 79[f]	+	−	[11, 51]

[a] Original reference.
[b] Reference [11].
[c] Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) data.
[d] Reference [52].
[e] American Type Culture Collection (ATCC) data.
[f] Reference [53].
[g] ESR1 mRNA present.
[h] Coutinho W.G. and Lasfargues E.Y., 1978 (unpublished data).
[i] Developed by Vysis International Inc.
IDC: invasive ductal carcinoma; MC: medullary carcinoma; IC: inflammatory carcinoma; PIDC: papillary invasive ductal carcinoma; C: carcinoma; IDCS = IDC, mucin-producing, signet-ring type; CS: carcinosarcoma; ILC: invasive lobular carcinoma; AC: adenocarcinoma.
P: primary; M: metastasis; R: recurrence Pl: pleural effusion; Sk: skin; As: ascites; L: local; O: omenum; LN: lymph node; F: femur; BM: bone marrow; Br: brain; Tr: transplanted.

here brought together and compared various data obtained, mostly in the last decade, on both tumours and BCC lines.

Presentation of BCC lines – the question of representativeness

Multiplicity and variability of BCC lines

Most of the BCC lines that have in the past been the subject of a somewhat detailed description are listed in Table 1. Also provided, where available, are data on the type of primary tumour (ductal, lobular, . . .); the tissue from which the BCC lines originated (primary or metastasis); the steroid receptor status and the modal chromosome number(s) of the cell lines.

Distinctive features of BCC lines. An exhaustive description of all BCC lines contained in Table 1 is beyond the scope of the present paper. However, it is of interest to mention that some aspect regarding their biology or their origin has distinguished many of them. For instance, DU4475 cells may grow in suspension *in vitro*, a feature rarely observed with BCC [16]. KPL-3C cells may produce tumours associated with micro-calcifications in nude mice [34]. CAL51 cells exhibit a normal karyotype [14] and appear

perfectly diploid by molecular cytogenetic analysis [53]. IBEP lines differ from the widely used MCF-7 and MDA-MB-231 cells by their spectrum of proteolytic activities [54]; they are also characterised by a relatively rare steroid receptor status (two of them are estrogen receptor-negative (ER−)/progesterone receptor-positive (PgR+), the third is ER+/PgR−) [31]. MFM-223 cells have a large amount of androgen receptors [44]. The epidermal growth factor receptor gene (*EGFR*) is amplified in BT-20 and MDA-MB-468 lines [55]; it is over-expressed without amplification in SUM-102, SUM-149, and SUM-229 cells [56, 57]. The fibroblast growth factor receptor 1 gene (*FGFR1*, at 11q13) is amplified in SUM-44 and SUM-52 cells [58], and highly amplified in MDA-MB-134 cells [59]. The fibroblast growth factor receptor 2 gene (*FGFR2*, at 10q26) is amplified in SUM-52 [58, 60]. BT-474, SK-BR-3, MDA-MB-361, MDA-MB-453, ZR-75-30, UACC-812, UACC-893, BSMZ, HCC1419, HCC1954, SUM-190, and SUM-225 lines have an amplified *ERBB2* (encoding Her-2/*neu*) at 17q11.2-q12 [2, 10, 58, 61, 62]. Close to this region, UACC-812 cells have an amplified *TOP2A* gene, while one copy of this gene is deleted in the MDA-MB-361 line; as a consequence, the sensitivity of both cell lines to topoisomerase II alpha inhibitors is modified [61]. One of the most intriguing cell lines is PMC42 [46], which is apparently derived from the stem cell compartment in the breast. These cells are heterogeneous, with at least eight different morphological types identified by phase contrast and electron microscopy, expressing both secretory and myoepithelial markers. These cells can express milk-specific genes through hormone and extra-cellular matrix interactions [63]. Moreover, they can be induced to undergo epithelial–mesenchymal transition (EMT, see below) [64]. Both of these attributes are quite unusual among the cell lines.

AU565 and SK-BR-3 lines were obtained from the same patient [7], as were CAL18A and CAL18B [13]. MDA-MB-330 and MA-11 issued from a lobular carcinoma, a type representing only 5–10% of all breast carcinomas [4, 11, 37, 38]. Hs578T cells were derived from a carcinosarcoma, a very rare form of breast cancer [29], as is medullary carcinoma, from which BrCa-MZ-01 and MDA-MB-157 originated [9, 43].

Besides the number of cell lines that have been obtained from pleural effusions, others have a more infrequent origin: MDA-MB-361 issued from a brain metastasis [4, 11], LCC15-MB cells from a femoral metastasis [36] and HH315 and HH375 from abdominal and supraclavicular lymph node metastases, respectively [27]. MA11 have been obtained from a bone marrow aspirate [37]. MAST cells have been isolated from an ascitic fluid [39], as were ZR-75-1 cells [51]. HBL-100 cells were established from an early lactation sample obtained in an apparently healthy woman. They exhibited characteristics of transformation from the very beginning and evolved during *in vitro* maintenance, until becoming tumourigenic in nude mice. They have been shown to harbour SV40 genetic information [24, 25]. HBL-100 cells have also been shown to carry the Y chromosome (ATCC web site), which raises some doubt as to its origin.

Finally, some BCC lines are characterised by their tropism to specific metastatic sites. This is the case for MT-1 cells, which tend to specifically give bone/bone marrow metastases in mice, while MA-11 preferentially establish in the brain, following injection into the left-ventricle of the heart [65].

BCC lines series. Some BCC lines are related, as they have been obtained from the same patient, or isolated in the same laboratory (and thus often established and maintained in the same culture conditions). Other lines have been derived by serial subculture from the same initial population. These cell lines constitute series that are well suited for comparative studies. 'HCC', 'SUM', 'HMT', and '21' cell lines are examples of such series.

'HCC (Hamon Cancer Centre) series'. In an attempt to obtain paired tumour and non-tumour cell lines from patients with breast cancer, 18 BCC lines were isolated from 177 primary tumours (and 3 from 12 metastatic lesions) [2]. This series allowed an extensive comparison of various features (morphology, ploidy, and marker expression) in paired BCC lines/original tissue [66]. The high number of original tissue samples also allowed the identification of criteria leading to the most successful isolation of BCC lines, thus pointing out that cell line establishment is a biased process (see below).

'SUM series'. A series of 11 tumour cell lines that were isolated and grown in media improved for the culture of normal breast epithelial cells. They were obtained from primary tumours, pleural effusions, a chest wall recurrence, or from a highly invasive cancer specimen grown for two transplant generations in immuno-deficient mice. Molecular cytogenetic analysis of these BCC lines was performed

[47, 58]. A detailed description of phenotypic/genotypic features of each SUM line is accessible (http://www.cancer.med.umich.edu/breast_cell/clines/clines.html).

'HMT series'. The HMT-3522 cell line was derived from a fibrocystic lesion of the breast. Issuing from breast tissue cultured on type IV collagen, these cells were first propagated as a near-diploid, non-tumourigenic sub-line in enriched but serum-free medium. These cells progressively developed, by subcultures in various conditions: p53 mutation; *MYC* amplification; EGF-independence accompanied by tumourigenicity in nude mice; EGFR, TGF-alpha, and c-erbB-2 over-expression. They have also been genetically engineered to study inter-relations between EGF and 17-beta oestradiol (E_2) action (for a review, see [28]).

'21-series'. Two primary and two metastatic cell lines with distinct phenotypes and genotypes established from the same patient diagnosed as having infiltrating and intra-ductal mammary carcinoma [6].

BCC variant lines. A number of variant sub-lines have been obtained, starting from the most widely used BCC lines. Some resulted from culture of the same unstable cell line by different groups and/or in different culture mediums. For instance, it is well-known that MCF-7 cell stocks from different laboratories may differ in their sensitivity to E_2, evaluated through cell proliferation rate and *TFF1* (encoding pS2) and *CCND1* (cyclin D1) gene induction. These effects have been associated with variations in ER protein and mRNA levels [67].

Other variants were obtained by selection of cell sub-populations resistant to a given agent, for instance anti-estrogen, vitamin D, doxorubicin, thymidylate synthase inhibitor... [68–71]. A good example of variant use to explore a specific resistance is provided by the MCF-7 BCC, which have been often considered as 'prototypes' for ER+ cells. Different levels of MCF-7 resistance to (anti)-estrogens have been found, illustrated by different variants. So are MCF-7/LCC1 hormone-independent but hormone-responsive, MCF-7/LCC2 (selected from LCC1) 4-hydroxytamoxifen (4-OH-TAM, a partial anti-oestrogen)-resistant but ICI 182,780 (a pure anti-oestrogen)-sensitive, and MCF-7/LCC9 (selected from LCC1) 4-OH-TAM- and ICI 182,780-resistant [72, 73].

Specific *in vivo* properties have been associated to a third category of variants. For instance, MDA-MB-435/LCC6 cells constitute an ascites model derived from MDA-MB-435S [74]. Distinct sub-populations of MDA-MB-231 BCC have been obtained on the basis of their preferential metastatic site (bone or brain) [75].

BCC lines from breast cancer patients with germline mutations. A particular subset of tumours is composed of those arising in women with germ-line mutations. Attempts to isolate corresponding cell lines appear to have been mostly unsuccessful.

To the best of our knowledge, only one *BRCA1* mutant cell line, HCC1937, has been described to date [76]. It was established from a primary non-metastatic IDC originating from a 24-year-old patient with a germ-line mutation. The cell line is homozygous for the *BRCA1* 5382insC mutation, whereas the patient's lymphocyte DNA was heterozygous for the same mutation, as were at least two other family members' lymphocyte DNA. HCC1937 BCC also have an acquired mutation of *TP53* with wild-allele loss, and an acquired homozygous deletion of the *PTEN* gene. No significant levels of progesterone or oestrogen binding were observed in either the primary tumour or the HCC1937 cultured cells. Only very low levels of Her-2/*neu* were expressed. HCC1937 cells have been extensively used to study *BRCA1* function, notably after ionising radiation-induced damage [77–86]. Considering the number of distinct mutations that may affect the *BRCA1* gene [87], there is clearly a need for additional cell lines. To be complete about *BRCA1*, it must be mentioned that the establishment of an immortalised breast cell strain containing the heterozygous form of a *BRCA1* 185delAG mutation has been described. These mutant cells appear to abundantly express the 220-kDa full-size *BRCA1* protein and to have growth and stress response characteristics similar to those of normal human breast cells, which is consistent with the hypothesis that loss of heterozygosity must occur to impair putative *BRCA1* function [88].

It seems that there is no available *BRCA2* mutant BCC line at the present time, suggesting that establishment of such line from *BRCA2* tumours could be especially difficult.

One cell line, HCC1569 [2], was found to have a mutated *FHIT* gene (G → T at nucleotide 651, changing valine to phenylalanine) that proved to be heritable, in that the patient's daughter also carried the same alteration. The tumour arose in an older patient (age 70) without a family history of breast cancer [89]. It is unclear whether the germ-line alteration that

occurred in this breast tumour (which otherwise appeared to be sporadic) was a causative factor in the development of her cancer.

Li-Fraumeni syndrome (LFS) is a rare, familial, dominantly inherited cancer syndrome characterised by a wide spectrum of neoplasms occurring in children and young adults. While LFS has been associated to germ-line mutations in *TP53*, no cancer cell line has been as yet isolated from LFS-associated breast tumours. However, the spontaneous *in vitro* immortalisation of normal breast epithelial cells obtained from a patient with LFS (with a mutation at codon 133 of *TP53*) has been described [90].

Other germ-line mutations that have been suggested to be associated with breast cancer susceptibility may be found in the *PTEN*, *ATM*, and *NBS1* genes [91]. To our knowledge, no BCC line has been obtained from carriers of these types of mutations.

The specificity of inflammatory breast cancer. Inflammatory breast cancer (IBC) is an advanced and accelerated form of breast cancer usually not detected by techniques such as mammograms or ultrasounds. It requires immediate aggressive treatment with chemotherapy prior to surgery and is treated differently than more common types of cancer. While no more than 5% of all patients with breast cancer have IBC in Western countries, this percentage may be higher than 10% in African countries such as Tunisia or Nigeria [92]. The majority of IBC tumours are ER-negative (ER−), PgR-negative (PgR−), Her-2/*neu*-positive (Her-2/*neu*+), and EGFR-positive (EGFR+) [93, 94]. They are also characterised by intense angiogenesis and a strong E-cadherin expression [95]. In view of their ER, PgR, and EGFR status, the presence of a high E-cadherin level in IBC is intriguing and contrasts with observations in most other breast cancers (see notably the sections Phenotype and invasiveness-based BCC line classification and Analysis of breast tumours – markers and grade – comparison with cell lines in this paper). IBC deserves a specific discussion here, as basic research on this form of cancer has recently greatly benefited from the introduction of human cell line and xenograft models.

The SUM-149 (ER−, PgR−, Her-2/*neu*−, and EGFR+) and SUM-190 (ER−, PgR−, Her-2/*neu*+ (*ERBB2* amplified), and EGFR+) cell lines are derived from primary IBC tumours. The SUM-149 BCC have been used to identify genetic determinants of the IBC phenotype. Among the genes found, *ARHC* (coding for the small GTPase RhoC) appears over-expressed in IBC. RhoC is involved in cytoskeletal re-organisation; specifically, it is involved in the formation of actin stress fibers and focal adhesion contacts. Its over-expression modulates induction of angiogenic factors in BCC. Treatment of cells with a farnesyl transferase inhibitor may lead to the reversion of RhoC GTPase-induced inflammatory phenotype. [93, 96–101]. Contrasting with *ARHC*, the expression of *LIBC* was found to be frequently lost in IBC. *LIBC* is now renamed *WISP3* (Wnt-1 inducible signalling pathway protein 3). It was shown that its loss of expression may contribute to the phenotype of IBC by regulating tumour cell growth, invasion and angiogenesis [96, 102]. *WISP3* is a member of a gene family ('CCN family'), also including *CTGF* (connective tissue growth factor), *CYR61* (cysteine-rich angiogenic inducer 61), and *NOV* (nephroblastoma over-expressed gene), which encode cysteine-rich secreted proteins with roles in cell growth and differentiation.

Although this article is mainly focused on cell lines, we need to mention the existence of two xenograft models of IBC. The first, MARY-X, grows as tight multi-cellular spheroids *in vitro* and as lymphovascular emboli *in vivo* in SCID/nude mice (animal models for tumourigenicity studies are discussed in the section Tumourigenicity of BCC lines in animal models of this paper). It is ER−, PgR−, Her-2/*neu*− and EGFR+. The primary tumour of origin of MARY-X exhibited identical markers, except that about 50% of its cells showed Her-2/*neu* amplification. Comparative studies of MARY-X with non-inflammatory xenografts indicated 10–20-fold over-expression of E-cadherin and MUC1, findings that were reflected in most cases of human IBC. The formation of spheroids and the lack of binding of the tumour emboli to the surrounding endothelium have been associated to: (a) an over-expressed E-cadherin/alpha, beta-catenin axis, determining strong homotypic cell interactions; (b) a decreased alpha-3 4-fucosyltransferase activity, which leads to reduced sialyl-Lewis X/A (sLe(X/A)) carbohydrate ligand-binding epitopes on the over-expressed MUC1 and other surface molecules that bind endothelial E-selectin. Moreover, the decreased sLe(X/A) fail to confer electrostatic repulsions between tumour cells, which further contributes to the compactness of the MARY-X spheroids by allowing the E-cadherin homodimeric interactions to go unopposed [103–105].

WIBC-9 is another human xenograft transplantable in SCID/nude mice. It is frequently accompanied by lung metastasis and exhibited erythema of the

overlying skin, reflecting its human counterpart. *In vitro*, WIBC-9 forms tube-like structures and loops, in concordance with its *in vivo* feature. Consistent with IBC, WIBC-9 is ER−, PgR−, and exhibits *ERBB2* gene amplification. Comparative studies of WIBC-9 and three established non-IBC xenografts, by reverse transcription-PCR, ELISA, and immunohistochemistry, indicated the over-expression of a series of angiogenesis-related genes in IBC [106].

The problem of representativeness in BCC lines

Permanent BCC lines have been isolated in order to understand the mechanisms underlying tumour initiation and evolution. Yet despite the considerable role that they continue to play in most aspects of cancer biology, they are still often viewed as non-representative models of the tumours from which they are derived.

At first, the metastatic origin of most BCC lines has raised questions as to their relationship to primary tumours. More generally, the pure and clonal population composing any single BCC line, regardless of its primary or metastatic origin, is *a priori* expected to poorly reflect the assumed heterogeneity of breast tumours. This heterogeneity is in line with the common view that breast cancer involves a sequential progression through clinical and pathologic stages, starting with atypical hyperproliferation, progressing into *in situ*, then invasive carcinomas and culminating in metastatic disease. These changes are believed to be associated with the sequential acquisition of various genetic and phenotypic changes in a single cell followed by clonal selection and expansion, thus leading to intra-tumoural diversity.

It has also been argued that most culture conditions relevant to the establishment of BCC lines will eliminate some types of tumour cells initially present in the cancer samples. For instance, BCC unable to grow well on plastic, or having an absolute requirement for factors only provided by their specific tumour environment, are unlikely to be represented in the panel of currently available BCC lines. Also, all else being equal, only the most proliferative cells extracted from the tumour samples to be cultured *in vitro* should be finally selected.

Cancer cells are genetically unstable. An additional criticism addressed to BCC lines is that, as compared to cells *in vivo*, they could undergo specific genotype/phenotype alterations resulting from long-term culture in simplified conditions. In addition to this divergence, lots of the same BCC lines distributed in different laboratories and/or cultured under various conditions could differently evolve and give rise to distinct sub-populations. This would prevent inter-laboratory comparisons of data obtained with the same line.

Finally, that the few widely used BCC lines could accurately reflect the inter-tumoural heterogeneity has been debated.

A response to these questions and criticisms will be given throughout the present paper.

Phenotype and invasiveness-based studies of BCC lines and tumours

Phenotype and invasiveness-based BCC line classification

As exemplified by the steroid receptor status, discordances have been pointed out between tumours and the panel of available BCC lines. Various explanations have been proposed. One of them, based on the epithelial–mesenchymal transition hypothesis, was suggested by initial observations of distinct cell line phenotypes. Subsequent studies confirmed the existence of two major 'portraits' for BCC lines.

Steroid receptor status and the bias in BCC line isolation. In the classification and comparison of cell lines (and breast tumours), no single criterion appears *a priori* more pertinent than the ER. As a mediator of (anti)-estrogen action, it plays a central role in breast cancer biology and treatment. One of the major proteins induced by estrogens is the progesterone receptor (PgR). ER and PgR amounts have been evaluated in tumours and cell lines for more than 30 years [107]. ER was long believed to be unique; however, an isoform named ER-beta and encoded by a specific gene, *ESR2*, was identified in the late nineties. The 'older' ER isoform (renamed 'alpha', and encoded by the *ESR1* gene) seems to be functionally the most important in breast tumours, as no clear picture has emerged to date about the ER-beta role in this pathology [108]. We have shown that the level of ER protein evaluated in breast tumours by ligand-binding assay, which measures both ER-alpha and -beta isoforms, was linearly correlated to the level of mRNA specific for *ESR1*, while the *ESR2* mRNA was undetectable in the samples [109]. In the present paper, we will consider that ER-beta, although not negligible (see notably [110]), is of secondary importance in breast tumours and cell lines, and the term ER will refer to the alpha isoform, unless otherwise indicated.

Cumulative data from a number of studies have revealed that steroid receptors are distributed in breast tumours as follows: 50–60% ER+/PgR+; 10–20% ER+/PgR−; 5–15% ER−/PgR+; 15–25% ER−/PgR−. In contrast, BCC lines listed in Table 1 (and added with the HCC and SUM series) are characterised by a clearly different distribution: 20% are ER+/PgR+; 7% ER+/PgR−; 5% ER−/PgR+; 68% ER−/PgR−. One explanation for these discrepancies supposes that the phenotype of BCC could change under *in vitro* culture, notably leading to the loss of steroid receptor expression. However, data presented in various parts of this paper suggest that this is unlikely.

On the other hand, it appears that steroid receptor-negative BCC lines are easier to establish *in vitro* than the receptor-positive ones. The 18 cell lines of the HCC series that were obtained from 177 primary tumours [2] exemplify this. Only a subset of carcinomas that had several features indicative of tumours with poor prognosis, absence of steroid receptors, hyperploidy or aneuploidy, Her-2/*neu* over-expression, positive immuno-staining detection of p53 protein expression, could be successfully cultured. Among the 18 HCC lines, 15 (83%) are ER−. It has been suggested that the secretion of various extra-cellular proteins, such as collagens, could provide a selective advantage to ER−/PgR− cells, by increasing their adherence to plastic. It also appears that ER−/PgR− cells may, more frequently than ER+ and/or PgR+ BCC, express both a series of growth factors (i.e., EGF, TGF-alpha, amphiregulin, heregulin, FGFs, IGFs) and their corresponding receptors, thus sustaining growth independently of exogenous growth factor supply (autocrine loop) [111].

Distinct phenotypes – the 'epithelial–mesenchymal transition' (EMT) hypothesis. To account for the under-representation of steroid receptor positivity in BCC lines, another explanation has been advanced. During the sequential *in vivo* progression of cancer from atypical hyperproliferation to metastatic disease, BCC might undergo phenotype alterations, subtended or not by genetic changes. These alterations would notably include the loss, to a variable extent, of epithelial-like features, and the gain of more aggressive and invasive mesenchymal-like traits. If steroid receptor-positive cells may occasionally lose their receptors (along with other epithelial markers) during tumour progression, at least a fraction of ER+ tumours could evolve to produce ER− metastases. As many cell lines have originated from metastatic cells, this could explain the discrepancies between these lines and the primary tumours. The concept of phenotype change in BCC, which is opposed to the idea that the 'portrait' of tumour cells remains essentially the same during cancer progression, received a more precise formulation with the 'epithelial–mesenchymal transition' (EMT) hypothesis.

The EMT hypothesis was mainly based on studies involving a relatively high number (up to 18) of BCC lines. These were found to distribute along a spectrum of differentiation from epithelial to mesenchymal appearances [112, 113]. Based on their phenotype and invasiveness (chemo-invasion through the reconstituted basement membrane, Matrigel, in a modified Boyden chamber), the cell lines could, however, be summarily classified into three groups:

— The first group expressed high amounts of markers typical of the luminal epithelial phenotype of breast cells: ER, E-cadherin (gene *CDH1*), zonula occludens-1 (*TJP1*), and desmoplakin I/II (*DSP*), the three latter being involved in adherens, tight, and desmosomal junctions, respectively. These 'luminal epithelial-like' cells grew as interconnected colonies of polygonal cells on plastic and as fused colonies in Matrigel. They were weakly invasive. BCC lines in this group included BT-483, MCF-7, T-47D, and ZR-75.
— The second group of cell lines, closely related to the first, was characterised by a 'weakly luminal epithelial-like' phenotype, with the expression, to a reduced extent, of at least some of the epithelioid markers found in the first group, and a weak *in vitro* invasiveness. Most of these cell lines grew as non-fused spheres in Matrigel. On plastic, they accumulated in clusters of loosely attached cells, reaching full confluency only rarely (personal observations). In this group were included the BT-474, CAMA-1, MDA-MB-134, MDA-MB-361, MDA-MB-453, MDA-MB-468 and SK-BR-3 cell lines.
— The third group of cell lines was clearly distinct from the two others. It did not express the epithelioid markers found in the 'luminal epithelial-like' and 'weakly luminal epithelial-like' groups, but in contrast exhibited a high level of vimentin (gene *VIM*), a marker also found in mesenchymal cells. Most of these lines had a fibroblastoid phenotype on plastic and grew as colonies with large stellate projections in Matrigel. They were highly invasive *in vitro*. BCC lines in this 'mesenchymal-like'

or 'stromal-like' group included MDA-MB-435S, MDA-MB-231, Hs578T, and BT-549.

Based on this classification, and since: (a) a hallmark of invasive (and metastatic) tumour cells is the ability to invade and traverse basement membranes; (b) strong migratory and invasive abilities are also characteristic of cells of mesenchymal origin, it was later postulated that BCC with epithelioid features might acquire a mesenchymal-like phenotype during tumour progression. This process would be reminiscent of the so-called 'epithelial–mesenchymal transition' that occurs during embryonic development at precise times and locations [114]. EMT in BCC would consist of the turning-off of genes encoding epithelial markers (estrogen receptor-alpha, E-cadherin, tight junction proteins,. . .) and the increase of markers such as vimentin, accompanied by morphological changes and increased invasiveness. In short, cells from the luminal epithelial-like group could evolve to resemble cells from the mesenchymal-like group, maybe by expressing a transitory, weakly luminal, epithelial-like phenotype.

There are data from *in vitro* studies that support the EMT hypothesis. For instance, in some MCF-7 and ZR-75 BCC selected for their resistance to doxorubicin, vimentin expression appeared to be turned on [115]. Moreover, the MCF-7/Adr cell line, obtained by exposure of the luminal epithelial-like MCF-7 to doxorubicin [116], was shown during the course of years to express a number of features mainly or exclusively found in mesenchymal-like lines such as MDA-MB-231 or Hs578T. Experimental expression of vimentin in MCF-7 BCC led to increased motility and invasiveness, suggesting that it was needed to allow successful invasion [117]. A sub-population, T-47Dco, was derived from the luminal epithelial-like T-47D cells. It had unstable vimentin expression and its most invasive cells were of fibroblastic/mesenchymal (VIM-positive) type [113]. Thus, phenotype instability or change, resulting in the acquisition of mesenchymal features, has been observed in BCC lines, and seems to confer to these cells increased mobility and aggressiveness. It has, however, rarely been described *in vitro*. Only a few ER+ cell lines have converted to an ER− phenotype and most efforts to obtain ER− sub-lines from ER+ MCF-7 and T-47D by selection or transformation have failed. Even in the cases where a hormone-independence was obtained, the ER was retained [72, 118]. EMT and more generally, important phenotype changes in cultured BCC, are likely to be more sporadic than systematic. In fact, the possibility of an EMT, even transient, has been demonstrated in two different cell systems: PMC42 and MCF10A. A PMC42 sub-line (PMC42-LA) displays an epithelial phenotype: the cells congregate into pavement epithelial sheets in which E-cadherin and beta-catenin are localised at cell–cell borders. They abundantly express cytokeratins, although 5–10% of the cells also express vimentin. Stimulation of PMC42-LA cells with epidermal growth factor (EGF) leads to EMT-like changes, including up-regulation of vimentin and down-regulation of E-cadherin. Vimentin expression is seen in virtually all cells, and this increase is abrogated by treatment of cells with an EGF receptor antagonist. Although E-cadherin staining at cell–cell junctions disappeared in response to EGF, beta-catenin persisted at the cell periphery. Further analysis revealed that N-cadherin was present at the cell–cell junctions of untreated cells and that expression was increased after EGF treatment. N- and E-cadherin are not usually co-expressed in human carcinoma cell lines but can be co-expressed in embryonic tissues, and this may signify an epithelial cell population prone to epithelio-mesenchymal-like responses [64]. MCF10A are not cancer cells, but immortalised normal breast epithelial cells. It is, however, of interest to mention that transient expression of vimentin may be induced in these cells. In an *in vitro* wound-healing model, analysis of the trajectories of the cells and their migratory speeds by time-lapse video microscopy revealed that vimentin mRNA and protein expression were exclusively induced in cells at the wound's edge, which were actively migrating towards the center of the lesion. Moreover, the vimentin protein disappeared when the cells became stationary after wound closure [119].

The data from BCC classification [112, 113] are in agreement with an EMT hypothesis according to which the weakly luminal epithelial-like phenotype could constitute a transitory step in tumour cell progression from the luminal epithelial-like portrait to the mesenchymal-like one. However, almost all of the weakly luminal epithelial-like cell lines in their study have later been found to exhibit specific gene amplifications underlying the over-expression of specific protein tyrosine kinases involved in growth factor signalling. BT-474, MDA-MB-361, MDA-MB-453, and SK-BR-3 cells over-express Her-2/*neu* and have an amplified *ERBB2* locus. This is also observed in about 30% of tumours. Her-2/*neu* over-expression has been associated to down-regulation of ER and breakdown of

Table 2. A list of genes differentially expressed in BCC lines (at least four cell lines examined) and tumours

Gene name	Gene product name(s)	Higher expression in			References
		Luminal epithelial-like and/or ER+ BCC lines	ER+ tumours	Low-grade tumours	
Panel A[a]					
ARHB	Ras homolog gene family, member B	Yes			[122]
C1orf34	Chromosome 1 open reading frame 34 (DEME-6)	Yes			[123]
CBX5	Chromobox homolog 5 (HP1 alpha homolog)	Yes			[124]
CDH1	Cadherin type 1, epithelial cadherin (E-cadherin)	Yes	Yes	Yes	[112, 113, 125]
CLDN1	Claudin 1	Yes			[126]
CLDN7	Claudin 7	Yes		Yes	[127]
DSP	Desmoplakin (DPI, DPII)	Yes		Yes	[112, 113, 128]
ESR1	Estrogen receptor, alpha	Yes	Yes	Yes	[129–131]
GATA3	GATA sequence binding protein 3	Yes	Yes		[132]
GPC3	Glypican 3	Yes			[133]
GRB14	Growth factor receptor-bound protein 14	Yes			[134]
GREB1	Greb1 protein	Yes	Yes		[135]
IGFBP2	Insulin-like growth factor binding protein 2	Yes			[136]
IGFBP5	Insulin-like growth factor binding protein 5	Yes			[137]
JUP	Junction plakoglobin	Yes			[113]
KLF4	Kruppel-like factor 4 (GKLF)	Yes			[138]
KRT18	Keratin 18	Yes			[139]
MDM2	Mdm2, p53 binding protein	Yes	Yes	Yes	[140, 141]
NME1	Protein expressed in non-metastatic cells (nm23A)	Yes		Yes	[142, 143]
PDZK1	PDZ domain containing 1	Yes	Yes		[135]
PGR	Progesterone receptor	Yes	Yes	Yes	[129, 130, 144, 145]
PRDM2	PR domain containing 2, RIZ (transcript 1)	Yes			[146]
PRLR	Prolactin receptor	Yes	Yes		[147, 148]
PTPN6	Protein tyrosine phosphatase, non-receptor type 6	Yes			[149]
RERG	Ras-like, estrogen-regulated, growth-inhibitor	Yes	Yes		[150]
SLC9A3R1	Solute carrier family 9, isoform 3 regulatory factor 1	Yes	Yes		[151]
SPINT1	Serine protease inhibitor, Kunitz type 1 (HAI-1)	Yes			[152]
ST14	Suppression of tumorigenicity 14 (matriptase, epithin)	Yes			[152]
STC2	Stanniocalcin 2	Yes			[153]
SYK	Spleen tyrosine kinase	Yes			[154]
TFAP2C	Transcription factor activator protein 2 gamma	Yes	Yes		[123]
TFF1	Trefoil factor 1 (pS2, BCEI)	Yes	Yes	Yes	[130, 145]
TFF3	Trefoil factor 3	Yes	Yes		[155]
TJP1	Tight junction protein 1 (ZO-1)	Yes		Yes	[112, 113, 156]
TPD52	Tumor protein D52	Yes			[157]

Table 2. (*continued*)

Gene name	Gene product name(s)	Higher expression in			References
		Mesenchymal-like ER-BCC lines	ER− tumours	High-grade tumours	
Panel B[b]					
AKT3	V-akt murine thymoma viral oncogene homolog 3	Yes	Yes		[158]
ANGPT1	Angiopoietin-1	Yes			[159]
BZRP	Benzodiazepine receptor (peripheral)	Yes			[160]
CDH3	Cadherin 3, placental cadherin (P-cadherin)		Yes	Yes	[161]
CDH11	Cadherin 11, osteoblast cadherin (OB-cadherin)	Yes			[162]
CDKN2A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Yes	Yes	Yes	[163]
CSF1	Colony stimulating factor 1 (M-CSF)	Yes			[164]
DFNA5	Deafness, autosomal dominant 5 (ICERE-1)	Yes	Yes		[165]
EGFR	Epidermal growth factor receptor	Yes	Yes	Yes	[55, 144, 166, 167]
ERBB2	c-erb-B2, Her-2/*neu*		Yes	Yes	[168–173]
ETS1	V-ets erythroblastosis virus E26 oncogene homolog 1	Yes			[174]
GPX1	Glutathione peroxidase 1	Yes	Yes		[175, 176]
GSTP1	Glutathione S-transferase pi	Yes	Yes		[177]
HMGIY	High-mobility group protein isoforms I and Y	Yes			[178]
HXB	Hexabrachion (tenascin-C)	Yes	Yes	Yes	[179]
IGFBP1	Insulin-like growth factor binding protein 1	Yes			[136]
IL6	Interleukin-6	Yes			[180]
IL8	Interleukin-8	Yes			[181]
IL11	Interleukin-11	Yes			[180]
LOX	Lysyl oxidase	Yes			[182]
LOXL2	Lysyl oxidase-like 2	Yes			[182]
MET	Met proto-oncogene (HGF receptor)	Yes			[183]
MMP14	Matrix metalloproteinase 14 (membrane-inserted)	Yes			[184, 185]
MSN	Moesin	Yes	Yes		[186]
MT1E	Metallothionein 1E	Yes	Yes		[187, 188]
NR3C1	Glucocorticoid receptor	Yes			[144]
NRG1	Neuregulin 1 (heregulin)	Yes			[55, 189]
PLAU	Plasminogen activator, urokinase	Yes	Yes	Yes	[164, 190–192]
PTN	Pleiotrophin (heparin binding growth factor 8)	Yes			[193]
RARB	Retinoic acid receptor, beta	Yes	Yes	Yes	[194]
S100A4	S100 calcium binding protein A4 (metastasin)	Yes	Yes		[195, 196]
SERPINE1	Plasminogen activator inhibitor type 1 (nexin)	Yes	Yes	Yes	[145, 190, 192]
SNAI1	Snail homolog 1		Yes	Yes	[197]
SNAI2	Snail homolog 2 (slug)	Yes			[198]
STMN1	Stathmin 1 (oncoprotein 18)	Yes	Yes	Yes	[199]
TIMP1	Tissue inhibitor of metalloproteinase 1	Yes	Yes		[200–202]
VIM	Vimentin	Yes	Yes		[112, 113, 179]

[a] Panel A: genes expressed at higher levels in luminal epithelial-like and/or ER+ BCC; in ER+ tumours; in low-grade tumours.
[b] Panel B: genes expressed at higher levels in mesenchymal-like/ER− BCC; in ER− tumours; in high-grade tumours.

cell–cell junctions [120, 121], and could indeed participate in the phenotype attenuation seen in weakly luminal, epithelial-like cells. For their part, MDA-MB-468 cells over-express EGFR and have an amplified *EGFR*. This is also seen in another weakly luminal epithelial-like cell line not studied by Sommers et al., BT-20 [55], but has been observed in less than 2% of breast tumours. Finally, MDA-MB-134 cells are characterised by the over-expression of the FGFR1 due to *FGFR1* amplification [59]. This event affects 5–10%

of tumours. Among the weakly luminal epithelial-like cell lines reported by Sommers et al., CAMA-1 were scarcely investigated afterwards, and are the sole lines for which the *EGFR*, *ERBB2*, and *FGFR1* amplification status is unknown. Considering the importance of growth factor signalling in cancer cell properties, it is speculated that abnormally increased tyrosine kinase activity of Her-2/*neu*, EGFR, or FGFR1 in BCC could significantly alter their phenotype and behaviour. In clear contrast to weakly luminal epithelial-like cells, none of the luminal epithelial-like or mesenchymal-like cell lines described by Sommers et al. has been found to exhibit an *ERBB2*, *EGFR*, or *FGFR1* amplification. As it is unlikely that BCC could progress by undergoing an amplification of one of these genes, followed by a return to a normal gene dosage, this may appear to be an argument against the EMT hypothesis. However, we cannot exclude the possibility that cancer cells might evolve from the luminal epithelial-like to the mesenchymal-like phenotype by supporting a transitory increase in tyrosine kinase activity not subtended by gene amplification. On the other hand, an EMT could not necessarily include a transition by the weakly luminal, epithelial-like phenotype.

Extended marker analysis. In the course of several years, the expression of many genes has been evaluated in BCC lines. Most of these studies, however, involved no more than two or three cell lines. When at least four cell lines were examined, they often comprised the two luminal epithelial-like MCF-7 and T-47D, and the two mesenchymal-like MDA-MB-231 and Hs578T lines. Such investigations revealed that the expression of the majority of studied genes was clearly associated to either one or the other phenotype, and positively or negatively correlated to ER expression. A series of these genes is mentioned in Table 2(A and B). In all cases where it was also measured, the protein amount reflected well the mRNA level.

Among other genes, luminal epithelial-like/ER+ cells preferentially expressed the epithelium-tied, serine protease *ST14* (matriptase) and *SPINT1* (matriptase inhibitor), *PRLR* (prolactin receptor), *SYK* (spleen tyrosine kinase), *IGFBP2* (insulin-like growth factor-binding protein 2), *IGFBP5*, *KRT18* (keratin 18) and, unsurprisingly, genes up-regulated by activated ER, such as *PGR* (progesterone receptor), *TFF1* (trefoil factor 1/pS2), *TFF3* (trefoil factor 3), *TPD52* (tumour protein D52), *RERG* (Ras-like, estrogen-regulated, growth inhibitor). Moreover, these cells had a higher expression of several transcription factor-encoding genes: *GATA3* (GATA binding protein 3, or GATA-3), *KLF4* (Kruppel-like factor 4), *TFAP2C* (AP-2, gamma isoform).

Among the genes preferentially expressed in mesenchymal-like/ER− lines were: *SERPINE1* (encoding plasminogen activator inhibitor-1), *PLAU* (urokinase-type plasminogen activator), and *MMP14* (membrane-type metalloproteinase-1), all implied in proteolysis; *ANGPT1* (angiopoietin-1), *IL8* (interleukin 8), and *MET* (hepatocyte growth factor receptor), all related to angiogenesis; the inflammation-related genes *IL6* (interleukin-6) and *IL11* (interleukin-11); genes associated to collagen processing, such as *LOX* (lysyl oxidase) and *LOXL2* (LOX-like 2); *IGFBP1*, *HXB* (hexabrachion, tenascin-C). All these genes are also frequently expressed in various mesenchymal cells such as fibroblasts or osteoblasts. The mesenchymal-like/ER− BCC lines were also characterised by higher amounts of several transcription factors, such as those encoded by *HMGIY*, *ETS1*, and *SNAI2*.

Thus, from expression analysis of an increasing number of genes, it appeared that the two phenotypes that had been previously summarily described [112, 113], the luminal epithelial- and the mesenchymal-like, were indeed highly different. This means that an eventual EMT would imply the turning-off of an extended set of genes, accompanied or followed by the gain in expression of another wide gene set. Research has identified a few genes that could play a key role in regulating numerous other phenotype-associated genes. They include *CEBPB* (encoding the transcription factor 'CCAAT/enhancer binding protein (C/EBP)beta'), *HMGA1* (architectural transcription factors 'high mobility group protein isoforms I and Y' – HMGI(Y)), *ID1* ('inhibitor of DNA binding 1, dominant negative helix-loop-helix protein'), *MTA3* ('metastasis associated 3', a sub-unit of the Mi-2/NuRD repressor complex), or *SNAI1* ('snail', a transcription factor) [203–207]. We cannot exclude the possibility that the expression of these genes could occasionally be more or less deeply altered in BCC, either spontaneously or in response to changes in cell environment, possibly leading to an at least partial EMT. This remains, however, to be clearly established.

Analysis of breast tumours – markers and grade – comparison with cell lines

A number of data have shown that the same major phenotypic markers distinguishing BCC lines – and clearly associated with the ER status – may also

discriminate between tumours. Moreover, their expression patterns largely overlap histological grade classification.

Molecular markers. Part of the genes associated to either the luminal epithelial-like or the mesenchymal-like phenotypes in BCC have also been examined in breast tumours. For all of them, their expression was found in at least a fraction of carcinomas. These genes are mentioned in Table 2(A) (genes positively correlated to ER in tumours) and B (genes negatively correlated to ER in tumours). In most cases, the genes correlated to the luminal epithelial-like/ER+ phenotype in BCC were positively correlated to the ER expression in tumours, while genes correlated to the mesenchymal-like/ER− phenotype in BCC were negatively correlated to the ER in tumours. None of the genes positively correlated to ER in BCC appeared negatively correlated to the receptor in tumours. None of the genes negatively correlated to ER in BCC was positively correlated to the receptor in tumours. Thus, discriminative phenotypic traits observed in BCC lines were also frequently discriminative features in tumours. Regarding ER, it is of interest to note that *ESR1* mRNA variants containing precise truncations in various exons have been identified in tumours; the same specific variants were also found in BCC lines [208].

Although infrequent, the co-existence in the same tumour of markers related to both luminal epithelial-like/ER+ and mesenchymal-like/ER− phenotypes has been observed. For instance, ER and EGFR levels are inversely correlated in BCC and in most tumours, as shown by numerous studies. Both receptors are, however, occasionally co-expressed in carcinomas, but are then, in the vast majority of cases, localised in distinct tumour cells, or in interspersed groups of cells ('mosaic expression', see for instance [209]). Whether ER-poor/EGFR-rich cells were derived from ER-rich/EGFR-poor BCC, for instance through EMT, in these tumours is unknown. If this was the case, the observations suggest that these events occur sporadically among cancer cells and do not seem to be related to any significant advantage for progression. Rare co-expressions have also been observed with other pairs of markers related to distinct BCC phenotypes (not discussed here).

Grade. One of the most widely accepted classification systems for breast carcinomas is grading. The majority of grading systems, such as those based on the Scarff, Bloom, and Richardson (SBR) method, combine histological assessment of nuclear pleomorphism, mitotic activity, and tubule formation [210]. According to such systems, tumours classified as 'grade I' or 'low-grade' have well-differentiated attributes, while 'grade III' or 'high-grade' tumours have poorly-differentiated attributes. Grade II tumours fall into an intermediate category. High-grade DCIS have been associated with the highest rate of local recurrence (25–30%), low-grade tumours have very low recurrence (0–5%), while intermediate-grade tumours have a recurrence rate somewhere between (10–15%) in a median of 12 years follow-up [211]. Moreover, high-grade tumours recur within a shorter time than the low-grade ones (for instance the median times are 88, 42, and 23 months in grades I, II and III, respectively, in [212]).

As grading is not directly based on molecular expression profiles, it may be asked whether grades are associated or not to the expression of specific sets of tumour markers, and more precisely if they are correlated to the distinct tumour cell phenotypes described above for BCC lines and largely retrieved in tumours. It has been repeatedly reported that most ER+ tumours are of low-grade. Inversely, high-grade tumours are mainly ER− (see for instance references [129–131]). Unsurprisingly, several markers whose expression is positively correlated to that of ER in BCC lines (and, frequently, in tumours) have also been associated to low-grade. They are mentioned in Table 2, panel A. This is the case for *CDH1*, *DSP*, *MDM2*, *NME1*, *PGR*, *TFF1*, and *TJP1*. Inversely, high-grade tumours are characterised by the expression of markers more related to the ER− profiles in BCC lines and/or in tumours. They are mentioned in Table 2, panel B, and include *CDKN2A*, *SERPINE1*, *PLAU*, *HXB*, *EGFR*, *CDH3*, *STMN1*, *RARB* (transcript 2), *ERBB2*. None of the genes positively correlated to ER in BCC lines and/or in tumours was found to be associated with high-grade/poorly differentiated carcinomas. None of the genes negatively correlated to ER in BCC lines and/or in tumours was found to be associated with low-grade/well-differentiated carcinomas.

High-grade DCIS cells also highly expressed several genes encoding extra-cellular matrix proteins and various growth factors, which likely contributes to the collagen- and fibroblast-rich stroma surrounding these lesions.

The case of *ERBB2* is of particular interest. *ERBB2*-over-expressing (ERBB2+) BCC lines (BT-474, MDA-MB-361, MDA-MB-453, SK-BR-3) express markers that make them closer to the

well-differentiated luminal epithelial-like phenotype than to the mesenchymal-like one (see above). On that basis, one would have expected to find most of the ERBB2+ tumours in the low- or at least the intermediate-grade categories. However, while a fraction of ERBB2+ are indeed of intermediate-grade, most of them are of high-grade. For instance, in three studies involving hundreds of cases, *ERBB2* amplification was found in 0%, 10%, and 33%; 3,9%, 20,4%, and 38,9%; 1%, 18%, and 28% of grades I, II and III, respectively [170, 171, 173]. Her-2/*neu* expression generally reflects *ERBB2* amplification. Discrepancies between *ERBB2* over-expressing tumours and cell lines are further discussed in the micro-array section.

Macroscopic homogeneity of breast tumours – Stable 'portrait' during progression

According to a common view, progression from primary to metastatic tumour is accompanied by the sequential acquisition of phenotype changes, thus allowing BCC to invade, disseminate, and colonise distant sites. Based on *in vitro* data, it has notably been proposed that BCC *in vivo* might undergo a transition from the luminal epithelial-like to the mesenchymal-like phenotype. Along the same lines, it has been repeatedly suggested that tumour progression is characterised by a shift from well differentiated/low-grade to poorly-differentiated/high-grade category. Nevertheless, most investigations have revealed that progression is not accompanied by major changes in marker expression or grade.

Progression to invasiveness and markers/grade. If changes in phenotype/grade were frequent during progression from *in situ* to invasive carcinoma, it should then be easy to find in a significant part of invasive tumours both luminal epithelial-like and mesenchymal-like markers, and both low- and high-grade compartments. Logically, mesenchymal-like markers and high-grade areas should be more often observed in the invasive than in the *in situ* tumour compartment. In fact, most studies examining this point have revealed a striking similarity between both parts of breast carcinomas [138, 213–218]. For instance, histopathological grading and tumour marker (p53, Her-2/*neu*, Ki-67, ER, PgR, bcl-2 and angiogenesis) expression were compared in 194 pure DCIS, 127 small invasive lesions, and 305 lesions with both an invasive and *in situ* component. Grade concordance was high between *in situ* and invasive components of the same tumour. All markers were found to correlate with grade rather than with invasiveness. No marker was clearly associated with the progression from *in situ* to invasiveness. The expression of tumour markers was almost identical in the two components of mixed lesions [213]. The DNA content and the expression of Her-2/*neu* were simultaneously examined in non-invasive and invasive phases of primary breast cancers, by image analysis. DNA content in the intra-ductal and invasive components was virtually identical. Expression of Her-2/*neu* was similar in both growth phases, implying identity of the Her-2/*neu* genotype [214]. In a study of 102 patients, a 67% concordance in grade was found between *in situ* and infiltrating components [215]. Another study of 64 cases indicated an 86% grade concordance between both components [216]. These studies, and others [217, 218], indicated a strong correlation between the grade of type of DCIS and the grade of infiltrating carcinoma in which both components were present.

It is thus striking that patterns of grade or the other markers did not seem to change during the transition from *in situ* to invasive carcinoma. Invasive cancer seems to occur independently of tumour grade. This is further supported by comparative genetic hybridisation data (see below).

Recurrence, metastasis. Metastatic and recurrent BCC appear late in tumour progression. They are believed to have accumulated alterations since their initial transformation event. On the other hand, metastatic cells may colonise various tissues often highly different from the breast after having completed all steps of a complex process including local invasion, intravasation, resistance to blood pressure, adhesion to blood vessels and extravasation. This suggests that they have sequentially acquired specific adaptive properties. All this supports the hypothesis that metastatic and recurrent cells could have a phenotype significantly different from that observed in the primary tumour. This supposition is of high importance, since many BCC lines originated from metastatic cells.

Attempts have been made to compare the expression of various markers and/or histological grade in primary tumours and their corresponding metastases and/or recurrences. It was shown that KRT8 and KRT19 expression was similar in both primary carcinomas and their lymph node (LN) metastases [219]. In an immuno-histochemical (IHC) study of 38 LN

metastases and their corresponding primaries, a very good concordance was found for Ki-67 (85%), ER (96%), PgR (82%), p53 (76%), Her-2/*neu* (84%) [220]. In another comparative IHC study involving 102 LN metastases, an 80% concordance was found for ER [221]. Investigations of a total of 31 LN, 35 lung, 25 skin, 1 liver, and 2 contralateral breast metastases revealed good concordance with primaries for ER, PgR, p53, and Her-2/*neu* [222–224]. This was also the case for ER, PgR, and EGFR evaluated in 26 LN and 2 distant metastases. In the latter study, expression of ER and EGFR was inverse, regarding the individual tumour cells in both primary tumours and metastases [225]. By ligand-binding assay, it has been estimated that no more than 20% of the ER+ primary tumours will produce ER− metastases. It has even been established that the expression of the frequent ER-alpha variant transcripts is conserved in primary tumours and their matched, concurrent LN metastases [118]. A good concordance was also found for grade. For instance, a study of 102 primaries and lymph node metastases revealed that both had the same grade (I, II, or III) in 79% of cases [215].

Along the same lines several studies have examined grade and marker (ER-alpha, ER-beta, PgR, p53, Her-2/*neu*, and TFF1/pS2) expression in recurrent breast cancers [110, 215, 223, 226, 227]. Concordance was found in most cases. For instance, in an analysis of 116 cases of recurrence, only four patients were found to have developed poorly differentiated DCIS or grade III invasive carcinoma after well-differentiated DCIS [226]. Regarding ER-alpha and ER-beta, their expression was even found higher in recurrence than in the corresponding primaries [110]. In a study of six cases of recurrence, histological type was the same as the initial one. There was concordance on ER, PgR, TFF1/pS2, Her-2/*neu*, and p53 between recurrence and primary [227]. In a study of 49 primaries and recurrences, a 78% grade (I, II, or III) concordance was found; in 36 patients who developed both metastasis and recurrence, grade concordance between them was also 78% [215]. In an analysis of 84 patients for which axillary metastases and/or local and/or regional recurrence(s) were found, 78% and 81% concordance were demonstrated between primaries and their metastases and first recurrences, respectively. In the cases where successive (up to six) recurrences were found, there was still a 74% concordance between the last recurrence and the initial tumour sample [212].

Concluding remarks on phenotype studies

A series of phenotypic traits distinguishing between BCC lines – and clearly associated to the ER status – also appear to discriminate between tumours, and their expression status largely overlaps histological grade classification. On the other hand, breast tumour phenotype/grade does not appear to significantly change during tumour progression from *in situ* carcinoma to secondary site colonisation.

As a consequence, BCC lines, even derived from metastases, are expected to have largely maintained the phenotype that they had in primaries. This was notably demonstrated in a study of 18 cell lines of the HCC series, all obtained from primary carcinomas [2]. These cell lines, which had been cultured for a median period of 25 months (range, 9–60 months), were compared to their corresponding archival tumour tissues. Immuno-histochemical analysis revealed a very good correlation on ER (87%), PgR (73%), Her-2/*neu* (93%), and p53 (100%) expression. An excellent correlation was also found between the morphological/differentiation features of the primary tumours and their corresponding cell lines. Most of the tumours (15 of 18, 83%) were poorly differentiated, and the corresponding cell lines grew as monolayers of cells devoid of obvious organisation or secretory activity. These cultures consisted of medium and large-sized cells with high-grade nuclear atypia and the presence of occasional multinucleated cells. Three (17%) of the tumours were moderately differentiated and demonstrated duct-like structures. The corresponding cell lines also were moderately differentiated and formed hollow or solid duct-like structures as well as hollow spherical fluid-filled morula-like structures lined by a single layer of epithelioid cells. The nuclei of the moderately differentiated cell lines demonstrated considerably less nuclear atypia than cell lines derived from poorly differentiated tumours [66].

For cell lines other than the HCC, there have been few data in the literature comparing their phenotype to that of the tumours from which they originated. The ER−/PgR− BRC-230 [3], GI-101 [22], HDQ-P1 [26], HH315 & HH375 [27], MDA-MB-231 [42], and MFM-223 [44] issued, sometimes after metastasis or recurrence, from tumours described as 'Grade III' or 'poorly-differentiated'. This is in agreement with the fact that fully negative steroid receptor status has been associated to the high-grade/poorly-differentiated tumour phenotype. In contrast, the ER+/PgR+ GCS [23], the ER−/PgR+ IBEP-1, and ER+/PgR− IBEP-2 [31] were derived from well-differentiated tumours.

There was no reported case of ER+ and/or PgR+ cell lines obtained from grade III/poorly-differentiated tumours, or of the ER−/PgR− line derived from a well-differentiated primary.

While most data reported here highly suggest that an irreversible EMT is unlikely to affect BCC during tumour progression, we can not exclude the occurrence of a transitory, reversible phenotype drift in these cells during the metastatic process. In an experimental murine model it was previously shown that the epithelial glycoprotein-2 (EGP-2, also known as Ep-CAM gene *TACSTD1*)-expressing HT29 colon cancer cells, produced lung metastases. While larger metastases positively stained for EGP-2, this was not found for smaller metastases. It was concluded that a transient loss of EGP-2 could occur during the migratory and early post-migratory period of HT29 cells [228]. Such transient modifications have not yet been clearly demonstrated in metastasising BCC. Whether these cells may express some degree of phenotypic plasticity remains, however, an open issue.

Genetic studies on BCC lines and tumours

Karyotype and cytogenetic studies on BCC lines and tumours

The apparition and progression of breast cancer is accompanied by multiple genetic changes. These include single nucleotide mutations, amplifications or deletions of single genes, insertions and translocations, gains and losses of entire, or parts of, chromosomes and chromosome arms, and eventually gross changes in chromosome number (aneuploidisation). Ploidy shift is believed to be a late genetic event, in such a way that even cancer cells appearing as diploid may be genetically altered to a significant extent [229].

The most easily observable genetic alteration, chromosome number change, has long been evaluated in tumours, revealing both inter- and intra-tumoural heterogeneity. In a study of 1 27 000 breast tumours, about half were found to be diploid or near diploid, the others exhibiting various types of aneuploidy [230]. In an analysis of 256 patients, 384 modal chromosome numbers were detected, ranging between 29 and 211. Seventy-four percent of these modal numbers were between 41 and 50, 19% between 51 and 80. Only 3% were lower than 41 and 4% higher than 80 [231]. In contrast, as shown in Table 1, a majority of BCC lines were found to have a modal chromosome number between 51 and 80, and only a few between 41 and 50. In several cases, a different modal number was found in the same cell line by different groups, reflecting instability. The high number of near-triploid cell lines might be due to their mostly metastatic origin, as a significant proportion of tumours – and more often when they have a high-grade – are believed to progress to near-triploidy [232]. Alternatively, cells with higher chromosome numbers could have a selective advantage *in vitro*. In their comparison of 18 BCC lines and their corresponding archival tumour tissues, Wistuba et al. [66] found that despite a poor correlation in the ploidy indexes (PIs) of the cell lines and tissues, a high correlation existed for the two ploidy categories defined (diploid: PI = 0.9–1.1, aneuploid: PI > 1.1). This may appear surprising, since the cell lines had been cultured for a relatively long period (9–60 months, median 25). However, they had been directly established from primaries, thus at a relatively early step in the progression.

Although the modal chromosome number is generally higher in BCC lines than in tumours, the patterns of whole chromosome gains and losses are very similar in both groups. Our cumulative study of breast tumours series [53, 233–238] emphasised that trisomy most frequently concerned chromosomes 4, 18, 19, and X, while monosomy was observed mainly for chromosomes 7, 19, 20, and X. By analysing a set of relatively small and partly overlapping BCC lines series [52, 53, 58, 239, 240], the same variations were observed, except that loss of chromosome X was found less frequently in cell lines (data not shown).

Besides whole chromosome number changes, DNA gains or losses larger than 10 megabases have been detected in tumours and cell lines by comparative genomic hybridisation (CGH). In general, CGH analyses supported the view that with regard to major DNA alterations, BCC lines are representative of tumours [52, 58, 231, 234, 236, 239–241]. For instance, Larramendy et al. [240] compared the HCC series of cell lines [2] and the tumours from which they originated. Although the cell lines showed more DNA copy number changes than the primary tumours, all aberrations, except one found in a tumour, were always present in the corresponding cell line. That the HCC cell lines retained the properties of their parental tumours for lengthy culture periods was confirmed by another study comparing allelic losses at 18 chromosomal regions frequently deleted in breast tumours, using 51 polymorphic micro-satellite markers [66].

In Table 3, we have compared the frequency of DNA gains (panel A) or losses (panel B) affecting the 10 most involved chromosome arms in a cumulative

Table 3. Chromosome arms most frequently altered in breast tumours (CGH analysis), as compared to five series of BCC lines. A: DNA gains; B: DNA losses. Italics: rank order of frequency for the mentioned alterations in the cumulative set of tumours and in each BCC lines series

(A)

Gains	1q	8q	17q	20q	16p	11q	12q	7q	6q	3q
Tumours	50%	44%	24%	23%	22%	21%	16%	15%	14%	14%
($n = 493$)	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
[52]	87%	87%	73%	80%	47%	73%	53%	60%	13%	20%
($n = 15$)	*1*	*1*	*4*	*3*	*8*	*4*	*7*	*6*	*10*	*9*
[53]	40%	70%	65%	55%	15%	45%	35%	65%	20%	30%
($n = 20$)	*6*	*1*	*2*	*4*	*10*	*5*	*7*	*2*	*9*	*8*
[58]	64%	73%	27%	27%	18%	27%	27%	64%	18%	45%
($n = 11$)	*2*	*1*	*5*	*5*	*9*	*5*	*5*	*2*	*9*	*4*
[239]	72%	72%	34%	55%	13%	32%	24%	47%	24%	39%
($n = 38$)	*1*	*1*	*6*	*3*	*10*	*7*	*8*	*4*	*8*	*5*
[240]	89%	100%	67%	83%	33%	44%	28%	50%	22%	67%
($n = 18$)	*2*	*1*	*4*	*3*	*8*	*7*	*9*	*6*	*10*	*4*

(B)

Losses	16q	8p	13q	1p	11q	17p	22q	6q	Xp	Xq
Tumours	24%	20%	20%	20%	18%	16%	16%	14%	11%	11%
($n = 493$)	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
[52]	13%	53%	40%	40%	60%	13%	0%	47%	40%	47%
($n = 15$)	*8*	*2*	*5*	*5*	*1*	*8*	*10*	*3*	*5*	*3*
[53]	35%	40%	40%	55%	25%	35%	30%	35%	20%	20%
($n = 20$)	*4*	*2*	*2*	*1*	*8*	*4*	*7*	*4*	*9*	*9*
[58]	18%	45%	45%	18%	36%	27%	9%	27%	55%	45%
($n = 11$)	*8*	*2*	*2*	*8*	*5*	*6*	*10*	*6*	*1*	*2*
[239]	29%	55%	29%	39%	39%	24%	26%	26%	39%	42%
($n = 38$)	*6*	*1*	*6*	*3*	*3*	*10*	*8*	*8*	*3*	*2*
[240]	6%	44%	50%	11%	11%	6%	6%	39%	50%	56%
($n = 18$)	*8*	*4*	*2*	*6*	*6*	*8*	*8*	*5*	*2*	*1*

set of breast tumours [53, 234–238, 242] and in five partly overlapping BCC lines groups [52, 53, 58, 239, 240]. The most spectacular difference was the higher frequency of losses at Xp and Xq in cell lines, as compared to tumours. This is rather paradoxical, in view of the fact that loss of the whole X chromosome was found to be higher in tumours (see above). We did not find any data in the literature revealing such divergence between cell lines and tumours. We suggest that, contrasting with other chromosomes, loss of, or at, X might not have been systemically examined in tumours and cell lines. Another possibility is that extended losses at both Xp and Xq could have been counted as X losses. With the exception of Xp and Xq, the most evident discrepancy between tumours and cell lines was with loss at chromosome 16q (panel B). While this arm is the most frequently affected by DNA loss in tumours, this is clearly not the case in BCC lines. It has been shown that DNA loss at 16q is less frequent in high-grade ductal carcinoma [236, 243] and in ER− tumours [234]. The data on BCC lines are thus in agreement with the fact that ER− negativity is more prevalent in cell lines than in tumours, and that the ER− lines, which are generally less epithelioid than the ER+ ones, are believed to originate essentially from high-grade, poorly-differentiated tumours [2]. Together, these observations on 16q constitute a strong argument against the theory supporting a frequent tumour progression from low- to high-grade and from ER+ to ER− status. Indeed, it appears unlikely that grade III tumours could arise from grade I tumours through a process involving regain of 16q.

Besides similar observations on 16q, Richard et al. [244] also noted a higher frequency of 7q gains in ER− carcinomas and of 3q gains in ER− and high-grade carcinomas, as well as a lower frequency of 16p gains in ER− tumours and of 22q losses in ER− and high-grade carcinomas. As shown in Table 3, similar trends were observed in BCC lines, as compared to tumours. This is an additional argument supporting the idea that the panel of available BCC lines is enriched in cases originating from ER− and high-grade carcinomas.

The occurrence of DNA alterations was higher in BCC lines than in tumours. Alterations reported in Table 3 were on average about two-fold more frequent, for gains as well as for losses, in cell lines than in tumours. In a study examining a higher number of chromosome arms, cell lines exhibited on average 2.5 more changes, as compared to uncultured tumours [239]. This could be partly explained by the fact that many available BCC lines are ER− and are believed to have originated from high-grade tumours. Indeed, DNA changes were found to be 1.7-fold more frequent in ER− than in ER+ tumours [244], while between 1.5- and 2.6-fold more genetic alterations were observed in grade III/high-grade than in grade I/low-grade samples [233, 235, 236, 244]. For instance, amplification of *ERBB2*, *TOP2A*, *MYC*, and *CCND1*-containing regions was more frequently found in high- than in low-grade tumours. However, major amplifications in pure *in situ* carcinoma and in intra-ductal carcinoma with an invasive component did not differ [245]. That no specific gross DNA alteration was associated with invasion was confirmed by analysis of a series of lobular and ductal tumours submitted to CGH following micro-dissection [246].

In some tumours, defects in mismatch repair enzymes lead to errors in the replication of simple nucleotide repeat (SNR) segments. This condition is commonly known as micro-satellite instability (MSI) because of the frequent mutations of micro-satellite sequences. Although the MSI phenotype is well recognised in some colon, gastric, pancreatic, and endometrial cancers, reports of MSI in breast cancers or BCC lines have been rare. For instance, in an evaluation of SNR in the *TGFBR2*, *IGFIIR*, *BAX*, and *E2F-4* genes, which are frequently mutated in tumours with MSI, no mutation was found in any of the 30 BCC and 61 primary tumours examined [247].

DNA alterations affecting specific genes have also been compared in tumours and cell lines. For instance, p53 gene (*TP53*) gene mutation status in exons 5–10 was determined in the HCC series of cell lines and their corresponding archival tumour tissues. A 75% concordance was seen [66]. More generally, point mutations identified in tumours were also often found in at least one or some cell lines, and inversely, as reported by several studies. Thus, such mutations do not appear to prevent the obtention, or to result from the *in vitro* culture, of BCC lines.

For some genes, including *FHIT* and *PTEN* [89, 248], intragenic homozygous deletions observed in BCC lines were not found in tumours. The investigators suggested that their screening methods were unable to detect this kind of lesion in primary tumours, which are invariably heterogeneous and contaminated with normal cells.

Besides a series of gross or localised DNA changes that are frequent and thus easily observed by common analysis techniques in both tumours and cell lines, BCC lines may also exhibit DNA alterations not encountered in tumours. Whether this results from *in vitro* culture of BCC, or rather reflects the selection, during the cell line isolation process, of alterations occurring in tumours but to a low frequency and only in small sub-populations, is not always easy to establish. For instance, it has been shown that despite the presence in both BCC lines and tumours of several recurrent, high level amplifications, such as 1q32, 8p11 (containing *FGFR1*), 8q23 (*MYC*), 11q13 (*CCND1*, *EMS1*), 17q12 (*ERBB2*), 17q23, 17q24 (*TOP2A*), and 20q13 (*ZNF217*), other amplifications sites have been identified in BCC lines, involving, for instance, 1p13, 7q21, 7q31, 9p23, and 11p13 [239]. These latter changes could arise preferentially during the *in vitro* cell culture. Indeed, that cultured BCC may undergo DNA changes is attested by CGH analyses of different stocks of MCF-7, showing variable sensitivity to estrogens, or of cells resistant to tamoxifen or to thymidylate synthase inhibitors. In most cases, the observed multiple genetic variations were not associated to common regions of gain or loss. However, the link between gene expression changes due to specific DNA alterations and phenotypic changes (for instance the acquisition of a resistance) was not always evident, suggesting that most of these alterations could be non-specific changes providing only occasionally a decisive advantage to the cells [67, 68, 249].

Obviously, genetic variability is not restricted to cultured BCC and also characterises tumour cells *in vivo*. From analyses of tumours it appears clearly that a number of gains or losses of chromosomal material occurs at a low frequency at many different sites. This is

in accordance with the concept of micro-heterogeneity in breast cancer. Multiple karyotypically related as well as unrelated clones (i.e., no single chromosomal abnormality is shared by them) have been found in a high proportion of breast carcinomas, suggesting that genetic mechanisms are crucially involved in the generation of small cell-to-cell and clone-to-clone variations in tumours [231, 250]. Thus, invasive breast cancer is a disease with multiple cytogenetic sub-clones and since no specific DNA alterations has been associated to invasion (see for instance [246]), it is concluded that complex patterns of non-specific changes are acquired during tumour progression. Accumulation of these minor (low frequency) alterations could ultimately overcome the mechanisms preventing cell aggressiveness. It has been found that the number of genomic aberrations is higher in tumours that give rise to recurrences [233]. Moreover, analysis of distant metastases (brain) showed that they were characterised by an accumulation of various genetic alterations and increased LOH frequency at all loci examined [251].

Extension of genetic analyses: micro-array studies

During the last few years, gene expression studies have gained considerably from the introduction of massive and simultaneous analysis tools. For instance, DNA micro-arrays have allowed us to measure the level of up to thousands of mRNAs in BCC and tumours [150, 252–261]. Data currently available confirm the existence of a limited number of distinct classes for BCC lines and breast cancers, based on their pattern of gene expression.

BCC lines. Cluster analysis of micro-array data (reviewed in [257]) showed that MDA-MB-231, BT-549, and Hs578T BCC lines could be grouped together, along with a primary breast stromal/fibroblast cell strain (HMS32) and an immortalised stromal cell line (UTSW), according to their similarity in patterns of gene expression. To this group was associated a so-called 'stromal/mesenchymal' gene expression signature, characterised by the high expression of genes encoding smooth muscle actin (gene *ACTA2*), vimentin (*VIM*), fibrillin (*FBN1*), biglycan (*BGN*), chains of collagen types I, III, V, and VI, lysyl oxidase (*LOX*) and LOX-like2 (*LOXL2*), the angiogenesis-associated interleukin-8 (*IL8*) and thrombospondin 1 (*THBS1*), urokinase receptor (*PLAUR*), connective tissue growth factor (*CTGF*), combined with the low expression of genes typical of epithelial cells. A different group contained the ER+/ERBB2− MCF-7 and T-47D, and the ER+ or −/ERBB2+ BT-474 and SK-BR-3 BCC lines, as well as an SV40 immortalised breast epithelial line (HB2). This group was characterised by the high expression of genes associated to luminal breast epithelial cells, including those encoding ER, keratins 8 (*KRT*8), 18 (*KRT18*), and 19 (*KRT19*), cell junction proteins cadherin-1 (*CDH1*), claudin-4 (*CLDN4*), claudin-7 (*CLDN7*), desmoplakin (*DSP*), and plakoglobin (*JUP*), selenium-binding protein 1 (*SBP1*), tumour-associated calcium signal transducer protein 1 (*TACSTD1*) and by the absence or low-level expression of the mesenchymal/stromal signature. This group was thus qualified as 'luminal epithelial-like'.

By cDNA micro-array studies, gene expression profiles (GEP) discriminating between weakly and highly invasive BCC lines were identified [259]. A common GEP was found for the ER− MDA-MB-231, BT-549, Hs578T, and MDA-MB-435s BCC, which were demonstrated to be highly invasive *in vitro*, confirming previous data [113]. This GEP was characterised by the high expression of genes encoding vimentin (*VIM*), chains of collagen I and VI, c-jun (*JUN*), glutathione S-transferase pi (*GSTP1)* and various proteolysis-associated genes (*TIMP*, *MMP14*, *SERPINE1*). Another GEP was common to the weakly invasive ER+ MCF-7, T-47D, ZR-75-1, BT-20 (the latter does not express the ER, but contain its mRNA; in contrast, it over-expresses EGFR (see above) and to the *ERBB2*-over-expressing BT-474, SK-BR-3, MDA-MB-361, and MDA-MB-453 BCC lines. This GEP was characterised by the high expression of *KRT18* and *KRT19*, *GATA3*, *ARHB*, *IGFBP5*, *JUP*, *RARA* (encoding RARalpha1), and *PIG7* (LPS-induced TNF-alpha factor).

A search for these GEPs in previously non-investigated BCC lines concluded the high invasiveness of SUM1315 and SUM159PT lines, which are both ER−/PgR−, and the low invasiveness of SUM44PE and SUM52PE lines. These are respectively ER+/PgR+ and ER−/PgR−, but *ERBB2*+, which relates them to luminal epithelial-like cells (see above). This GEP-based inference was validated by migratory and invasion studies of these cell lines on Matrigel, in Boyden chambers.

Thus, micro-array studies on widely-used BCC lines confirmed not only the previous investigations of Sommers et al., but also the subsequent marker analyses which correlated the expression of a series of genes to either the luminal epithelial-like or the

mesenchymal-like phenotype (see Table 2). This confirmation was notably found for *ARHB*, *GATA3*, *IGFBP5*, *JUP*, and *KRT18*, as well as for *GSTP1*, *MMP14*, *SERPINE1*, and *VIM*. Micro-array studies also underlined the link between invasiveness and phenotype, suggesting that the ability to invade reconstituted membranes *in vitro* could require complex interactions between several molecules rather than the expression or the extinction of one or a very few genes. While the discussion of this point is beyond the scope of the present paper, it is for instance well-known that the efficient activity of the matrix metalloproteinases and the plasminogen activator systems needs the ordered co-operation of numerous factors.

Tumours. Cluster analysis of micro-array data from series of breast tumours led to the identification of a major 'luminal epithelial-like/ER+' subtype, comprising 60–65% of tumours. It was distinguished by high expression of a set of genes including the ER-alpha gene (*ESR1*) and genes either regulated by estrogens (*LIV-1*, *TFF1*, *TFF3*), or previously identified as co-expressed with ER (*GATA3*, for instance). Other interesting genes were *IGFBP5* and those encoding the promoter-binding proteins hepatocyte nuclear factor 3 (*HNF3*) and X-box protein 1 (*XBP1*) [253, 257, 258]. Three additional subtypes, all characterised by low or no ESR1 expression, were found: a 'normal breast-like', grouping some tumours with samples of normal breast tissue; a 'basal/myoepithelial-like', comprising about 15–20% of tumours, and notably expressing high levels of keratins 5 (*KRT5*) and 17 (*KRT17*); an 'ERBB2+' group, characterised by the high level of expression of several genes in the *ERBB2* amplicon at 17q22.24 including *ERBB2*, *GRB7*, *MLN64*. Most tumours expressing a strong luminal epithelial signature were of low grade, while the majority of tumours expressing mainly the other signatures were of high grade.

Tumours expressing a basal/myoepithelial gene signature, based on micro-array studies, are expected to include the fraction of ductal carcinomas that are not pure myoepithelial cell carcinomas but are of high-grade and for which a basaloid/myoepithelial cell differentiation and steroid receptor-negativity has been demonstrated by immuno-histochemistry (see for instance [262]). Myoepithelial differentiation, high-grade and ER-negativity are also characteristic of certain metaplastic carcinomas (spindle-cell carcinomas and matrix-producing carcinomas), but also of invasive ductal carcinomas with large central acellular zone [263].

That ER status reflects major differences in tumour gene expression patterns and phenotypes was further illustrated by Gruvberger et al. [256]. They analysed a series of breast carcinomas by cDNA micro-array. Using artificial neural networks as well as standard hierarchical clustering techniques, the number of genes which discriminated tumours (or BCC) according to their ER status was high. Moreover, only a small proportion of these discriminator genes were known to be regulated by estrogens (in MCF-7 cells), suggesting that mechanisms underlying *ESR1* gene expression are indeed common to many genes. Among the genes positively correlated to ER were *GATA3*, *IGFBP2*, *TFF1* and *TFF3*, among those negatively correlated to ER were *CDH3*, *HMGIY*, *LCN2*, *EGFR*. The fact that ER+ and ER− tumours have highly different gene expression patterns suggests that EMT is infrequent in tumour cells, as it is expected to require a considerable number of genetic events.

In a micro-array analysis of 115 breast tumours, including 18 samples from carriers of BRCA1 mutations [260], the latter were strongly associated with a basal-like signature, in agreement with the fact that BRCA1 tumours generally lack expression of *ESR1* and *ERBB2* [264, 265]. Of interest, HCC1937, the only BCC line known to have been established from a germ-line BRCA1 mutation carrier (IDC, primary tumour, grade III), is ER− and PgR−, with only very low levels of Her-2/*neu* [76].

Comparison of genetic profiles of BCC lines and tumours. The luminal epithelial/ER+ signature was most strongly expressed by about 60–65% tumours and by the luminal epithelial-like cell lines (MCF-7, T-47D, BT-474, SK-BR-3). The ER+/ERBB2− luminal epithelial-like MCF-7 and T-47D BCC lines appear thus as acceptable models for ER+/ERBB2− luminal epithelial-derived tumours.

In addition to the luminal epithelial/ER+ signature, the ER+/ERBB2+ BT-474 and ER−/ERBB2+ SK-BR-3 cell lines also expressed the ERBB2+ signature found in ERBB2+ tumours. They seem thus to be pertinent models for ER+/ERBB2+ and ER−/ERBB2+ tumours, respectively. There are, however, some striking differences between ERBB2+ cell lines and tumours. ERBB2+ cell lines (also including MDA-MB-361 and MDA-MB-453), no matter what their ER status, were closer to the luminal epithelial-like/ER+/ERBB2− MCF-7 and T-47D than to the mesenchymal-like/ER−/ERBB2− MDA-MB-231, Hs578T, and BT-549 cell lines, based on their gene expression pattern, phenotype and weak

in vitro invasiveness. In contrast, most ERBB2+ tumours, although expressing an attenuated luminal epithelial signature, are highly aggressive and associated with a poorer prognosis, as compared to most luminal epithelial-like/ER+/ERBB2− tumours. Moreover, most ERBB2+ tumours are of high grade (II and III), thus exhibiting a poorly differentiated aspect, while a majority of luminal epithelial-like/ER+ tumours are highly differentiated and of low grade. By applying a statistical treatment of micro-array data excluding the genes composing the ERBB2+ signature, Sørlie et al. [258] found that the ERBB2+ tumours merged with the tumours, mainly ER−, expressing strong basal/myoepithelial-like and normal-like signatures, not with the luminal epithelial-like/ER+ ones. Differences observed between cell lines and tumours could reflect specific interactions between tumour cells and the normal cells present in their surrounding stroma. We believe, however, that these observations deserve the study of additional ERBB2+ cell lines (for instance the UACC812 and UACC893).

BCC lines exhibiting a stromal/mesenchymal signature are the most intriguing of all. They do not appear to clearly express any of the four gene signatures found in tumours. The absence of the luminal epithelial-like and ERBB2+ signatures in these cell lines is in agreement with data reported in several parts of the present paper. On the other hand, these mesenchymal-like cell lines are not clearly related to either 'basal/myoepithelial-like' or 'normal-like' tumours, the specific signatures of which seem to be rather expressed by the normal, or artificially transformed, mammary epithelial cells cultured *in vitro*. It is a fact that these cell lines, for which a luminal epithelial origin is unlikely (they have a low level or no keratins 8/18/19), do not express a series of markers specific to basal and/or myoepithelial cells, such as keratins 5, 14 and 17, maspin/proteinase inhibitor 5 (*SERPINB5*) and myoepithelium-expressed proteinase inhibitor (*SERPINI2*); however, a series of genes composing the basal signature, or having been described in myoepithelial cells, are expressed at relatively high amounts in these Hs578T, BT-549, and MDA-MB-231. They include *FGF2*, *TIMP1*, *ITGA6* (encoding alpha 6 integrin) and *THBS1*.

Among the mesenchymal-like cell lines, Hs578T have been described as originating from a carcinosarcoma – a stromal-like tumour. Despite this, an epithelial nature has been suggested for these cells, as they express casein and indicators of secretory activity [29]. The origin of BT-549, as mentioned by the ATCC on the basis of a communication by Coutinho and Lasfargues, is 'papillary, invasive ductal carcinoma', a non-frequent type. As suggested by Ross and Perou [257], MDA-MB-231 might represent a de-differentiated cell type that has lost expression of the signature of its tissue of origin. These cells were obtained from a pleural effusion from a patient who had developed a 'poorly-differentiated tumour tending toward papillary configuration and tubule formation', while also having an intra-ductal carcinoma [42].

Several markers identified in MDA-MB-231 and/or Hs578T cell lines and negatively correlated to the luminal epithelial-like/ER+ phenotype have also been found in a majority of ER− tumours (Table 2). In most cases, the expression was observed in tumour cells themselves, but frequently also, in certain normal cells contained in the tumour stroma. This suggests both that the Hs578T and MDA-MB-231 are representative of tumour cells, composing at least a fraction of ER− tumours, and that they are related to stromal cells. In culture, these tumour cells could have lost part of their normal or basal/myoepithelial gene signature.

To further complicate the discussion on representativeness, it must be mentioned that the ER−/ERBB2− MDA-MB-435S BCC line, isolated as breast cancer cell line, might be of non-breast origin. In a clustered analysis of cDNA micro-array data obtained in 60 tumour cell lines, MDA-MB-435S cells were found to cluster with melanoma cells, rather than with other breast cancer lines [254]. Melanoma cells are frequently highly aggressive/metastatic, a feature that they share with MDA-MB-435S cells [266]. As shown by a more recent study using PCR and immunohistochemistry, MDA-MB-435S cells do not express pS2 (*TFF1*), mammaglobin (*MGB1*), and prolactin-inducible protein (*PIP*), which are typical of breast cancer cells. In contrast, they express several genes commonly transcribed in melanocytes: *RXRG* (retinoid X receptor gamma), *TYR* (tyrosinase), *ACP5* (acid phosphatase, type 5), and *DCP* (dopachrome tautomerase/tyrosine related protein 2), which are not found in Hs578T, MCF-7, T-47D, BT-474, and MDA-MB-453 BCC [267].

Since the accuracy of the widely-used ER−/ERBB2− BCC lines (Hs578T, BT-549, MDA-MB-231, MDA-MB-435S) as models for ER−/ERBB2− tumours remains a matter of debate, it is suggested that future studies should examine additional cell lines.

Epigenetic alterations – hypermethylation

Besides genetic changes – point mutation, deletion, translocation, or amplification – epigenetic alterations

Table 4. A list of genes for which promoter hypermethylation has been demonstrated in BCC and in tumours

Gene name	Gene product name(s)	Tumours	BCC lines
APC	Adenomatosis polyposis coli	H	H
ARHI	Ras homolog gene family, member I	H	H
ASC (TMS1)	Apoptosis-associated speck-like protein containing a CARD	H	H
BRCA1	Breast cancer 1, early onset	H	H
CCND2	Cyclin D2	H	H
CDH1	Cadherin 1, epithelial cadherin (E-cadherin)	H	H
CDH13	Cadherin 13, H-cadherin (heart)	H	H
CDKN2A	Cyclin-dependent kinase inhibitor 2A (p16)	H	H
ESR1	Estrogen receptor 1	H	H
FABP3	Fatty acid binding protein 3 (MDGI)	H	H
FHIT	Fragile histidine triad gene	H	H
GJB2	Gap junction protein, beta 2, 26kD (connexin 26)	H	H
GPC3	Glypican 3	H	H
GSN	Gelsolin (amyloidosis, Finnish type)	H	U
GSTP1	Glutathione S-transferase pi	H	H
HIC1	Hypermethylated in cancer 1	H	H
HOXA5	Homeo box A5	H	H
HSHIN1	High in normal-1	H	H
IL6	Interleukin-6	H	U
KLK10	Kallikrein 10	H	H
NME1	Protein expressed in non-metastatic cells 1 (NM23A)	H	H
PGR	Progesterone receptor	H	H
PLAGL1	Pleiomorphic adenoma gene-like 1	U	H
PLAU	Plasminogen activator, urokinase	H	U
PRDM2(1)	PR domain-containing protein 2 (RIZ1), transcript 1	H	H
PRKCDBP	Protein kinase C, delta binding protein (SRBC)	H	H
PRSS8	Protease, serine, 8 (prostasin)	H	U
RARB(2)	Retinoic acid receptor, beta (transcript 2)	H	H
RASSF1(A)	Ras association (RalGDS/AF-6) domain family 1 (transcript A)	H	H
SERPINB5	Maspin/Proteinase Inhibitor 5	H	U
SFN	Stratifin	H	H
SLC19A1	Solute carrier family 19 (folate transporter), member 1	H	U
SNCG (BSCG1)	Synuclein, gamma (breast cancer-specific protein 1)	H	U
SYK	Spleen tyrosine kinase	H	H
TFF1	Trefoil factor 1 (pS2, BCEI)	H	H
TGFB3	Transforming growth factor, beta 3	U	H
TIMP3	Tissue inhibitor of metalloproteinase 3	H	H
WT1	Wilms tumor 1	H	H

H: Hypermethylated.
U: Status unknown.
For references, see [270].

have also been suggested to play a role in the initiation and progression of breast carcinomas. These are heritable modifications of gene expression that do not involve mutation. The concerned genes are structurally intact, but their expression is reduced or absent, which is related to methylation in their promoter region or deacetylation of histones that interact with their promoter region, or both mechanisms. For instance, hypermethylation of CpG islands in and around promoter regions may contribute to the transcriptional inactivation of at least 100 tumour-related genes in many types of cancer [268] [+ website www.missouri.edu/ ∼hypernet]. The heritability of methylation states and the secondary nature of the decision to attract or exclude methylation support the idea that DNA methylation is adapted for the cellular

memory [269]. Hypermethylation could participate to the development and the preservation of a specific cell phenotype, by definitely silencing ('bolting') specific sets of genes.

Table 4 lists the genes for which CpG island methylation has been found in breast tumours, BCC lines, or both. More detailed information on these genes is provided in the review of Widschwendter and Jones [270]. As shown in the Table 4, there was no gene for which CpG island methylation was found in BCC lines (at least part of them) but not in tumours, and inversely. Regarding their pattern of gene CpG island methylation, cell lines and tumours appear thus very close.

A number of hypermethylated genes are associated with the distinctive phenotypes observed in BCC lines. For instance, *ESR1*, *PGR*, *CDH1*, *SYK*, *GPC3*, *TFF1*, are expressed in many luminal epithelial-like cell lines, but not in mesenchymal-like lines. In contrast, *CDKN2A*, *GSTPI*, *PLAU*, are preferentially found in mesenchymal-like rather than in luminal epithelial-like cell lines. In most cases, these genes, when silent, can be re-expressed by treatment of BCC with the demethylating agent 5-aza-cytidine. That many genes for which promoter methylation has been shown are directly or inversely correlated to the ER was demonstrated by Yan et al. [271]. These authors performed a methylation profile analysis of 7776 CpG islands, which led to the identification of CpG island clusters that can significantly distinguish ER−/PgR− from ER+/PgR+ breast tumours. Thus, epigenetic events might significantly contribute to stabilise the phenotype – luminal epithelial-like or mesenchymal-like – of BCC. Since it appears that the tumour cell phenotype is unlikely to change often during the process of tumour progression, hypermethylation is not expected to play a key role in this progression. From this point of view, it is significant that spontaneous change in CpG methylation pattern of genes has, to our knowledge, never been observed in BCC lines *in vitro*. This does not mean, however, that methylation/demethylation is not involved in tumourigenesis. Indeed, some genes expressed in tumours and BCC lines, including maspin, are silent in normal epithelial cells and/or normal breast tissue.

Dialogue between tumour cells and their cellular environment

A full-blown cancer phenotype is characterised by features such as sustained cell proliferation, disregard of growth and differentiation controlling signals, evasion of apoptosis, immortalisation, and ability to invade surrounding tissue and induction of angiogenesis. Pre-invasive cells have acquired almost all of these features, but are not yet invasive. Since no major (high-frequency) and gross phenotypic/genotypic differences are seen between *in situ* invasive, and metastatic compartments of tumours, progression to invasiveness and metastasis could rather result from the accumulation by *in situ* carcinoma of various minor and localised genetic or epigenetic events. This would eventually dysregulate the molecular balances governing cell adhesion, migratory ability, proteolytic and/or angiogenic activity. Such evolution is suggested by the known micro-heterogeneity of tumour tissues, the higher amount of DNA alterations in BCC lines (most of which originated from metastases) and the genetic instability of these lines, as illustrated by BCC lines variants.

Acquisition of invasiveness could proceed from additional events. Tumour size might play a role. A growing *in situ* tumour is believed to exert a mechanical stress on its neighbouring basement membrane. Moreover, the accumulation of BCC in a confined space might lead to local concentrations of various secreted molecules (for instance metalloproteinases) able to overcome the resistance developed by the surrounding normal cells (for instance through secretion of metalloproteinases inhibitors). That tumour evolution is highly dependent on interactions (by direct contact or through paracrine signalling) between BCC and other cell types present in their vicinity, is now widely accepted. BCC modulate stromal cells activities. In turn, the stromal micro-environment in which BCC develop profoundly influences many steps of tumour progression. In various experimental tumour models, the micro-environment affects the efficiency of tumour formation, the rate of tumour growth, the extent of invasiveness, and the ability of tumour cells to metastasise [272]. Among the cell types with which BCC may interact are normal breast epithelial cells, blood cells, notably lymphocytes and macrophages, vascular endothelial cells, and, at metastatic sites, specialised cells from brain (i.e., astrocytes), lung, liver, bone (osteoblasts and osteoclasts) and bone marrow [273–278]. Detailed studies on the implication of normal cells in invasion have notably examined myoepithelial cells and stromal fibroblasts.

It has been suggested that myoepithelial cells constitute 'natural tumour suppressors' [279]. These cells surround the mammary ducts, deposit

extra-cellular matrix material and express high amounts of several proteinase inhibitors (TIMP-1, maspin, myoepithelium-expressed proteinase inhibitor, protease nexin-II/amyloid beta precursor protein/BACE2) and angiogenic inhibitors. In contrast, their expression of proteinases and angiogenic factors is low [280]. Myoepithelial cells appear thus at least partly responsive for limiting invasive behaviour. The loss of this cell type, seen only in invasive tumours, should permit subsequent invasion and tumour progression [281, 282]. The mechanisms by which BCC may reduce the amount of myoepithelial cells in their neighbourhood remains largely unknown to date.

BCC also have paracrine interactions with the surrounding stromal fibroblasts. In tumours, the latter are often phenotypically different from normal fibroblasts. For example, they may develop smooth muscle differentiation (myofibroblasts) with increased motility into collagen gel [283]. Myofibroblasts, which comprise a predominant stromal cell type in breast tumours, are often seen in close association with the myoepithelium surrounding carcinoma *in situ*, and such associated cells express several extra-cellular matrix-degrading proteases, including MMP-2, MMP-11, MMP-13, and MT-MMPs. Under the influence of BCC, stromal (myo)fibroblasts can increase their production of various components of the urokinase (uPA) system, such as uPA itself, uPA receptor (uPAR), uPARAP [284], and of MMPs [285]. Since BCC themselves are able to produce proteolysis-related molecules (uPA, uPAR, and various MMPs in mesenchymal-like cells, matriptase/ST14 in luminal epithelial-like cells) [152], this may lead to a considerable local degradation of matrix and cancer progression [286].

The complexity of the dialogue between BCC and other cells indicates that BCC lines, even considered as representative models of transformed cells *in vivo*, are clearly insufficient to understand the various aspects of tumour biology. However, comparison of appropriate panels of BCC lines to panels of tumours, notably by micro-array analysis, could allow us to distinguish gene expressions due to transformed cells from those associated to their normal surrounding cells. Such data should further be confirmed by histological techniques, which remain more than ever necessary.

Tumourigenicity of BCC lines in animal models

While various animal recipients (including rats, cats and dogs) have been used to study the tumourigenicity and metastatic ability of BCC lines [292], nude mice are the most popular model.

Data on the tumourigenicity of most BCC lines reviewed in this paper are presented in Table 5. In general, the cited references are the initial articles describing the cell lines. When reported, the histology of the tumours produced in animals was often consistent with that of the original tumour (not detailed here).

The three ER+ BCC lines most widely used in mice are MCF-7, T-47D, and ZR-75-1. It has been shown that they require some degree of estrogenic supplementation for tumourigenesis in nude mice, even with inocula containing as much as 20×10^6 cells. Tumours did not appear in the absence of oestrogen. In general, treatment of animals bearing established tumours with oestrogen withdrawal resulted in cessation of tumour growth, but not in tumour regression. Growth resumed upon oestrogen replenishment, even after prolonged (weeks) deprivation, indicating that the cells remained viable. The oestrogen-induced growth of MCF-7, T-47D, and ZR-75-1 tumours was generally inhibited by an appropriate dose of anti-oestrogen (tamoxifen, LY156758, ICI 182,780...) [293–296]. Tumour take was shown to be variable (amount of oestrogen requested, growth speed) when using different MCF-7 sub-lines [297].

While ER+ tumours frequently invade locally and metastasise in patients, tumours obtained from ER+ BCC in nude mice are poorly invasive and rarely, if ever, are metastatic. More aggressiveness has been observed with the two ER− BCC lines most used in mice, MDA-MB-231 and MDA-MB-435. The latter is the most metastatic, producing lung and lymph metastases in a high proportion of nude and SCID mice, with a low incidence of metastases in muscle (chest wall and thigh), heart and brain.

Tumourigenicity of other BCC lines has also been tested in nude mice. CAMA-1, BT-20, BT-474, BT-483, HMT-3909, MDA-MB-468, MDA-MB-134 and MDA-MB-361 were all tumourigenic. In contrast, Hs578T, SK-BR-3 and MDA-MB-453 appeared essentially unable to easily grow in mice [see notably references 298–300]. HBL-100 and HMT-3522, of non-malignant origin, were reported as non-tumourigenic [298]. In fact, it has been shown that these cell lines could progressively become tumourigenic [25, 28].

It is of interest that the non- or very poorly tumourigenic MDA-MB-453 and SK-BR-3 cell lines both over-express *ERBB2*. In a study of 433 human breast carcinomas hetero-transplanted in nude mice,

Table 5. Tumourigenicity of BCC lines

Cell line	Tumourigenicity	References
21MT	90% tumour take in nude mice (10^7 cells injected s.c.)	[6]
21NT	Non-tumourigenic in nude mice (10^7 cells injected s.c.)	[6]
21PT	30% tumour take in nude mice (10^7 cells injected s.c.)	[6]
BOT-2	In nude mice (5×10^6 cells injected s.c.)	[8]
BRC-230	100% tumour take in nude mice (10^6–3×10^7 cells)	[3]
BrCa-MZ-01	In nude mice (10^7 cells injected s.c.)	[9]
BrCa-MZ-02	In nude mice (10^7 cells injected s.c.)	[9]
BSMZ	67–100% tumour take in nude mice (10^7 cells injected s.c.). Latency: 4 weeks.	[10]
BT-20	See text	
BT-474	See text	
BT-483	See text	
BT-549	100% tumour take in nude mice (2×10^6 cells injected s.c.).	[288]
CAL-18A	17 tumours/20 sites in nude mice (2×10^6 cells injected s.c.). Latency: 3 weeks	[13]
CAL-18B	Non-tumourigenic (0 tumours/10 sites) in nude mice (2×10^6 cells injected s.c.)	[13]
CAL-51	4 tumours/6 sites in nude mice (2×10^6 cells injected s.c.). Latency: 10 days	[14]
CAMA-1	See text	
DU4475	80–100% tumour take in nude mice (1–10×10^6 cells injected s.c.). Latency: 10 days	[16]
EP	In mice immuno-suppressed with anti-thymocyte serum [ATS, reference 287] (2.5×10^6 cells injected s.c.).	[19]
HBL-100	Progressively tumourigenic	[25]
HDQ-P1	Non-tumourigenic after 8 weeks in nude mice (up to 10^7 cells injected s.c.)	[26]
HMT-3522	Progressively tumourigenic	[28]
Hs578T	Non-tumourigenic (0/20) in mice immuno-suppressed with ATS [reference 287] (5×10^6 cells injected intra-dermically).	[29]
Ia-270	Tumourigenic in nude mice (3×10^6 cells injected s.c.)	[30]
IBEP-1	Tumourigenic in 5/5 nude mice (5×10^6 cells injected s.c.). Latency: 11 weeks	[31]
IBEP-2	Tumourigenic in 5/5 nude mice (5×10^6 cells injected s.c.). Latency: 14 weeks	[31]
IBEP-3	Tumourigenic in 4/5 nude mice (5×10^6 cells injected s.c.). Latency: 16 weeks	[31]
IIB-BR-G	100% tumour take in nude mice (10^7 cells injected in the m.f.p.)	[32]
KPL-1	100% tumour take in nude mice (1–10×10^6 cells injected in the m.f.p.). Latency: 1–2 weeks.	[33]
KPL-3C	100% tumour take at late passages in nude mice (5×10^6 cells injected in the m.f.p.).	[34]
KPL-4	Tumourigenic in 10/10 nude and SCID mice (10^7 cells injected in the m.f.p.)	[35]
LCC15-MB	100% tumour take in nude mice (5×10^6 cells injected in the m.f.p.)	[36]
MA11	See text	
MCF-7	See text	
MDA-MB-134 VI	Tumourigenic in nude mice (10^8 cells), see also text	[4]

Table 5. (*continued*)

Cell line	Tumourigenicity	References
MDA-MB-157	Tumourigenic in nude mice (10^8 cells)	[4]
MDA-MB-175 VII	Tumourigenic in nude mice (10^8 cells)	[4]
MDA-MB-231	See text	[4]
MDA-MB-330	Non-tumourigenic in nude mice (10^8 cells)	[4]
MDA-MB-361	Tumourigenic in nude mice (10^8 cells), see also text	[4]
MDA-MB-435S	See text	[4]
MDA-MB-436	Non-tumourigenic in nude mice (10^8 cells)	[4]
MDA-MB-453	Non-tumourigenic in nude mice (10^8 cells), see also text	[4]
MDA-MB-468	Tumourigenic in nude mice (10^8 cells), see also text	[4]
MT-1	See text	
MW	Tumourigenic at low passages in mice immuno-suppressed with ATS [reference 287] (2.5×10^6 cells injected s.c.).	[19]
PMC42	Only the original cells yielded a tumour in nude mice. None of the subsequent inocula (at least 10^6 cells injected s.c.) yielded any tumour growth.	[289]
SK-BR-3	See text	
SUM-149	Tumourigenic in nude mice (1.5×10^6 cells injected in the m.f.p.)	[97]
SUM-159	About 90% tumour take in nude mice (4×10^6 cells injected s.c. or 10^6 cells injected in the m.f.p.)	[290]
T-47D	See text	
UISO-BCA-1	100% tumour take in nude mice ($1–5 \times 10^6$ cells injected s.c.)	[49]
UISO-BCA-2	100% tumour take in nude mice ($1–5 \times 10^6$ cells injected s.c.)	[49]
VHB-1	Tumourigenic (4/6 sites) in nude mice stimulated by oestradiol (3×10^6 cells injected s.c.)	[50]
ZR-75-1	See text	
ZR-75-30	Tumourigenic in nude mice ($5–8 \times 10^6$ cells)	[291]

The nude phenotype is associated to the *nu* (Unigene *Foxn1* = 'Forkheadbox N1') mutation. *nu/nu* mice are essentially athymic. T-cells are lacking and B-cell maturation is also defective. However, nude mice are not totally immuno-deficient, as they possess an elevated natural killer (NK) cell activity, while the level of lymphocyte-activated killer (LAK) cells appears normal.

SCID = severe combined immuno-deficiency. The *scid* (Unigene IL2RG = 'interleukin-2 receptor, gamma chain') mutation produces mice with significantly smaller lymphoid organs. The differentiation/maturation of lymphocytes is defective, and both pre-B and b-cells are undetectable. The few remaining T-cells appear non-functional. In contrast, macrophages, NK and LAK precursor cells are essentially normal.

s.c.: subcutaneously; m.f.p.: mammary fat pad (see text for further explanations).

it was shown that 64% of the growing carcinomas exhibited *ERBB2* amplification, a percentage approximately twice that found in unselected populations of tumours [301]. This suggests that the establishment of *ERBB2*-over-expressing BCC in mice could specifically benefit from the presence of native extra-cellular matrix, in agreement with other data of the present paper indicating that the behaviour of these cells might be different *in vitro* and in tumours *in vivo*.

It has been repeatedly shown that tumour take is higher when BCC are inoculated with Matrigel or fibroblasts. Matrigel is a solution of tissue basement membrane matrix extracted from the Engelbreth–Holm–Swarm (EHS) mouse tumour. It is rich in extracellular matrix proteins, with laminin, collagen IV, heparan sulphate proteoglycans, entactin and nidogen as major components. Various growth factors are also present, including TGF-beta, FGF and plasminogen activators. Matrigel polymerises under normal physiological conditions to produce a reconstituted, biologically active matrix that is effective for the attachment and differentiation of cellular material.

Co-injection with Matrigel or fibroblasts appeared to be of benefit for various cell lines, including BT-474, MCF-7, T-47D, MDA-MB-231 . . . [299, 300, 302]. This underlines the importance of interactions between BCC and their environment (cells and various basement membrane molecules) in tumour establishment.

There is now considerable evidence supporting the importance of the implantation site for BCC. While many BCC lines will grow subcutaneously (s.c.), and frequently at other sites, it appears that the most appropriate site for their implantation is the mammary fat pad (m.f.p.) [296, 300, 303]. Orthotopic transplantation can significantly increase the take rate of tumours from various cancers, and occasionally facilitate metastatic spread that will not occur from a s.c. site. Thus, the specificity of tumour–host and tumour–stromal interactions appears determinant in the progression of breast cancers.

While tumours may arise from BCC injected s.c. or at m.f.p., they are rarely metastatic. When cells disseminate from these sites, they are mainly found in lungs. To study the ability of BCC to colonise various organs, additional injection routes have been used. They include intravenous injection, for instance in the lateral tail vein (to obtain metastasis to the lung), injection into the spleen (metastasis to the liver), direct or intracarotid artery injection (metastasis to the brain) and injection into the left-ventricle of the heart (metastasis to bone marrow and bone) [300].

Examples of tumourigenic and metastatic studies in mice

Animal models have extensively allowed examination of the contribution of genes to the tumourigenic and/or metastatic ability of BCC. Only a few examples will be cited here. For instance, the influence of chromosomes 6, 11 and 17 on the cell phenotype of MDA-MB-231 and MCF-7 has been studied by transfer of intact chromosomes to these cell lines. Chromosome 6 induced alterations of *in vitro* growth properties and suppressed the tumourigenicity of MDA-MB-231 cells. Spontaneous reduction of the transferred chromosome allowed mapping of the tumour suppressor gene(s) to region 6q21–q23 and/or 6q26–q27. Clones MCF-7/H6 underwent a senescence process. Chromosome 11 had no effect on MDA-MB-231 cells, although it suppressed tumourigenicity of MCF-7 cells. A MCF-7/H11 clone lacking the short arm of the transferred chromosome retained tumourigenicity; however, tumour cell growth was significantly reduced. These results suggest that each chromosomal arm may contain genes important for the suppression of MCF-7 tumourigenic properties. No viable clone maintaining an intact chromosome 17 was obtained in either MDA-MB-231 or MCF-7. Only one MDA-231/H17 clone contained the long arm of the transferred chromosome 17. Interestingly, this clone lost the ability to induce tumours in nude mice, indicating that at least one gene mapping to the long arm of chromosome 17 could suppress the tumourigenic phenotype [304, 305].

Other studies in mice have used BCC transfected with various genes. For instance, *CDH1* and *IGFBP5*, two genes associated to the luminal epithelial-like phenotype, were shown to reduce tumourigenic and/or metastatic abilities of MDA-MB-231 BCC when transfected into these cells [306, 307]. Inversely, *CYR61, LOR-1*, *VEGFC*, and *VIM*, which are associated mainly to the basal-like phenotype of breast tumours, were shown to increase the tumourigenic and/or metastatic potential of transfected MCF-7 [117, 308–310].

Animal models have also been employed to evaluate anti-tumour strategies. For instance, it has been shown that a combination of murine anti Her-2/*neu* monoclonal antibodies (Mabs) was more effective than the individual Mabs in treating s.c. tumour nodules of BT-474 in SCID mice, confirming *in vitro* studies [311]. The protein kinase Akt-3 is highly expressed in MDA-MB-231 but not in MCF-7 and T-47D BCC [158, 312]. To test the potential role of this molecule in hormone-independence, Akt-3-expressing MCF-7 were obtained. These cells were found to produce tumours in mice in the absence of E_2 that were approximately equivalent in size to control cells in mice given E_2. Moreover, the formation of tumours by the Akt-3 cells was greatly suppressed by E_2, but stimulated by the partial anti-oestrogen tamoxifen, while unaffected by the pure anti-oestrogen ICI 182,780 [313]. Previous studies using MDA-MB-231 cells stably transfected with ER had shown that the lung metastases developed by these cells in nude mice were inhibited three-fold by estradiol [314]. Akt-3 could play a role in these growth-inhibiting effects.

Metastasis studies have revealed the preference of some cell lines or sub-lines for specific colonisation sites. For instance, in an analysis of two cell lines injected to various sites in nude rats, it has been shown that MA-11 BCC had a propensity to produce brain metastases, while MT-1 appeared particularly able to develop in bone marrow [37, 65].

The metastatic progression of most breast cancers involve bone marrow invasion and bone alteration, which results in a considerable morbidity. As a consequence, numerous studies have aimed to understand the mechanisms leading to bone colonisation. Such *in vivo* investigations in mice generally used BCC injections into the left-ventricle of the heart. Initial studies had revealed that the MDA-MB-231 BCC were able to cause dramatic osteolysis in a few weeks in mice. The molecular bases of this effect have been extensively explored. For instance bone-seeking and brain-seeking clones of MDA-MB-231 BCC have been isolated, by repeated sequential passages in nude mice and *in vitro* of metastatic cells obtained from bone and brain metastases, respectively. In culture, bone-seeking clones produced greater amounts of the parathyroid hormone-related peptide (PTHrP, gene *PTHLH*), well-known for its osteolytic properties [75]. In another study using mice, however, interleukin-8 (*IL8*), but not PTHrP was correlated to bone metastasis and osteolysis by MDA-MB-231 [315]. More recently, MDA-MB-231 sub-populations with elevated metastatic activity were selected, and the genes over-expressed in these sub-populations were determined by micro-array analysis. It was shown that most of these genes encode secreted and cell surface proteins. Two of them, interleukin-11 (*IL11*) and connective tissue growth factor (*CTGF*), encode osteolytic [180] and angiogenic [316] factors whose expression was further increased by the pro-metastatic cytokine TGF beta. Data on IL11 are in agreement with a previous article reporting that bone metastases were more frequently observed in patients with IL11-positive than in those with IL11-negative primary tumours [317]. Other genes over-expressed in MDA-MB-231 sub-populations with elevated metastatic activity notably included those coding for the bone homing 'chemokine (C-X-C motif) receptor 4' (*CXCR4*), the 'matrix metalloproteinase-1' (*MMP1*), and the 'disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 1' (*ADAMTS1*). The potential co-operative importance of osteopontin (*SPP1*) was also underlined. Over-expression of the bone metastasis gene set was superimposed on a gene expression signature already present in the parental breast cancer population, suggesting that metastasis requires a set of functions beyond those underlying the emergence of the primary tumour [318].

Typically, ER+ patients tend to have bone metastases more frequently [319, 320]. Since MDA-MB-231 BCC are ER−, their accuracy as the best model for investigating bone metastasis may be discussed, more especially as ER+ tumours represent more than two-thirds of all breast carcinomas. ER+ breast tumours have been known to develop osteoblastic or mixed (osteolytic/osteoblastic) bone metastases [321]. In agreement with this, and contrasting with the purely osteolytic MDA-MB-231 cells, the ER+ MCF-7, T-47D, and ZR-75 BCC tend to induce osteoblastic lesions, while ER+ BT483 cells cause rare mixed osteoblastic and osteolytic metastases after heart inoculation into female nude mice [322, 323]. While factors responsible for the formation of osteoblastic metastases are under investigation, recent data suggest an important role of the BCC-secreted endothelin-1 (ET-1, gene *EDN1*) [322].

Both the osteolytic (MDA-MB-231) and the osteoblastic (MCF-7) models have been used to demonstrate the protective effect of bisphosphonates on bone [323]. The osteolytic model has also allowed us to demonstrate the ability of osteoprotegerin (OPG, gene *TNFRSF11B*) to inhibit tumour-induced osteoclastogenesis [324].

To close this section on tumourigenicity, it must be mentioned that green fluorescent protein-expressing BCC, notably including MDA-MB-435 cells, have been recently produced. They may allow whole-body optical imaging of metastasis in mice [325–327].

Conclusion

BCC lines have allowed considerable advances in the knowledge of most aspects of cancer biology. In the course of years, tumours themselves have been increasingly studied. From comparison of data obtained in both cell lines and tumours, the pertinence of the first as models for the second has been evaluated. Three questions needed to be answered:

To what extent are individual tumours genetically and phenotypically heterogeneous?

In other words: is it justified to believe that a single BCC line might accurately represent any cancer cell present in the tumour from which it originated, at any step of its progression?

There is a common belief that breast tumours are heterogeneous. However, most data of genetic and phenotypic (grade, markers expression) nature indicate that the 'portrait' of a tumour does not undergo striking alterations during progression. Rather

unexpectedly, *in situ* and invasive components of carcinomas appear to be very similar, and this similarity has also been repeatedly retrieved in metastases, regardless of their localisation, and in recurrences. In fact, at any step in their progression, breast tumours may be viewed as collections of cell sub-populations exhibiting the same general pattern of gross recurrent genetic alterations and sharing the same major phenotypic features. Micro-heterogeneity exists, due to minor (low frequency) changes, generally restricted to the small sub-populations. While 'dramatic' phenotype alterations accompanying progression have been suggested, as illustrated by the EMT hypothesis, we do not believe them to occur frequently *in vivo*. Tumour progression, including metastasis, does not appear to result mainly from clonal expansion of only one cell sub-population, as has been previously proposed. It is rather believed to proceed from the complex co-operation among several sub-populations generated by a series of partly divergent DNA alterations. Dialogue between tumour cells and their surrounding normal cells also appear to be important. It results from what precedes the BCC being extracted from tumour tissue, even from metastasis and may be considered, at the time of their isolation, as having kept the main features of any tumour cells *in vivo*.

Do BCC cultured in vitro *maintain the characteristics they had in tumours?*

As freshly isolated BCC may be considered to be representative of BCC *in vivo*, their prolonged culture in artificial conditions *in vitro* has been thought to modify their properties. Clearly, variants of BCC lines may develop either spontaneously or by selection against various compounds. This variation is facilitated by genetic instability. However, although most of the variants described are genetically different from the initial population, it is often difficult to associate their specific properties to specific and recurrent DNA alterations. Different mechanisms seem to underlie the acquisition of a given property (for instance, resistance to anti-estrogens) and whether they are the same in BCC lines and tumours remains a matter for investigation and discussion. On the other hand, the *in vitro* evolution of BCC lines is infrequently accompanied by deep genetic/phenotypic changes. For instance, examples of EMT *in vitro* are rare, and attempts to obtain ER− cells from ER+ BCC have been largely unsuccessful. *In vitro* as *in vivo*, the main features of BCC appear to be widely intangible.

Do the panel of widely used BCC lines accurately reflect the variety of tumour phenotypes?

Attempts to classify BCC lines and tumours have identified a few distinct phenotypes and underlined the power of ER(-alpha)/ESR1 and Her-2/neu/ERBB2 as discriminators. From these studies, the ER+/ERBB2− MCF-7 and T-47D cell lines may be considered as reasonably acceptable models for tumour cells in luminal epithelial-like/ER+/ERBB2− tumours. ERBB2+ BCC lines (BT-474 and SK-BR-3, but also MDA-MB-361 and MDA-MB-453) appear to be more closely related to the luminal epithelial-like/ER+/ERBB2− lines than ERBB2+ tumours are to luminal epithelial-like/ER+/ERBB2− tumours. This suggests that either the available cell lines are not the best representatives of tumour cells *in vivo*, or that the phenotype of these ERBB2+ cells is significantly altered when they develop interactions with their surrounding tumour stroma. To resolve this question, it would be of interest to analyse the 'portrait' of additional ERBB2+ cell lines. While most widely used ER−/ERBB2− BCC lines (BT-549, Hs578T, MDA-MB-231 and MDA-MB-435S) have a stromal/mesenchymal-like genotype/phenotype, they do not clearly express the normal-like and basal/myoepithelial-like gene signatures found in most ER−/ERBB2− tumours. There are pro and con arguments regarding the representativeness of these cell lines. Here again, recourse to additional ER−/ERBB2− lines could allow the discussion to progress.

Despite the existence of a few major tumour classes, based on phenotype/genotype, each carcinoma is unique. The same is true for BCC lines. Besides the widely used ones, the recourse to less investigated cell lines is expected to improve our knowledge of breast cancer biology. For instance, PMC42 cells are of high interest, as they express both secretory and myoepithelial markers, suggesting that they are derived from basal cells able to give rise to these two distinct lineages. Cell lines originating from specific metastatic sites (i.e., MDA-MB-361 issued from the brain), or from rare types of breast cancers (lobular, medullary, inflammatory – such as the SUM-149 BCC–,. . .) also deserve further investigation. Besides the BRCA1-mutated HCC1937 BCC, there is a need for additional cell lines originating from patients with germ-line mutations. Studies relative to ER activity and induction of target genes (including

PGR) should examine ER+/PgR− and ER−/PgR+ cell lines. Among the cell lines that have been little studied, BT-20 and MDA-MB-468 are peculiar in that they have an amplified *EGFR* locus, an alteration rarely observed in tumours. *FGFR1* amplification, as seen in MDA-MB-134, is more frequent in carcinomas. Whether the over-expression of Her-2/*neu*, EGFR, and FGFR1 may alter the BCC phenotype in a similar way remains largely unknown and merits additional investigation.

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