Breast Epithelial Cell Lines

Coming soon to the ECACC General Collection are a range of breast cell lines, derived from the same originating patients. These cell lines are offered as an immortalised 'normal' line (BPE1, BPE2, BPE3, BPE4 and HME2, HME3 and HME4), a transformed line (BPLE1, BPLE2, BPLE3, BPLE4 and HMLE2, HMLE3 and HMLE4), and a tumourigenic line (BPLER1, BPLER2, BPLER3, BPLER4 and HMLER2, HMLER3 and HMLER4).

Tumour phenotype is influenced by many factors including, but not limited to genetic and epigenetic alterations and the systemic environment which in turn has an influence on the tumour stroma. The analysis by RT-PCR and Western blot of normal cell populations reveals various tumour cells are essential for the complete understanding of tumour phenotypes including differences in histopathology, tumourigenicity and metastatic behaviour.

The heterogeneity amongst human breast cancer is thought to be largely due to the variety of cell types within the mammary cell population. However, it is less clear whether transformation of neighbouring epithelial cells residing within a single organ can lead to different tumour types. Work carried out by Dr Ince Tan and colleagues indicated that the propagation of normal human breast epithelial cells *in vitro* (breast primary epithelial progenitor cells (BPEC's) and human mammary epithelial cells (HPEC's) remained partially differentiated along luminal and myoepithelial pathways (Figure 1).

To determine the influence of the 'normal' cell phenotype on derived 'engineered' cell types, normal breast cells were transformed in three consecutive steps using retroviral vectors expressing hTERT, SV40 LT/st and H-ras. The gene expression vectors were sequentially introduced to recipient cells in individual steps in the following order: pmig-GFP-hTERT, pBABE-zeo-SV40-ER and pBABE-puro-H-ras V12. The addition of hTERT immortalised the cells in cell culture (creating the BPE and HME family of cells). The addition of SV40 ER LT/st transformed the cells (creating the BPLE and HMLE family of cells), which formed anchorage independent colonies but not tumours in immunocompromised mice. The H-ras renders the cells tumorigenic in an immunocompromised mouse model (the BPLER and HMLER family of cells).[1]

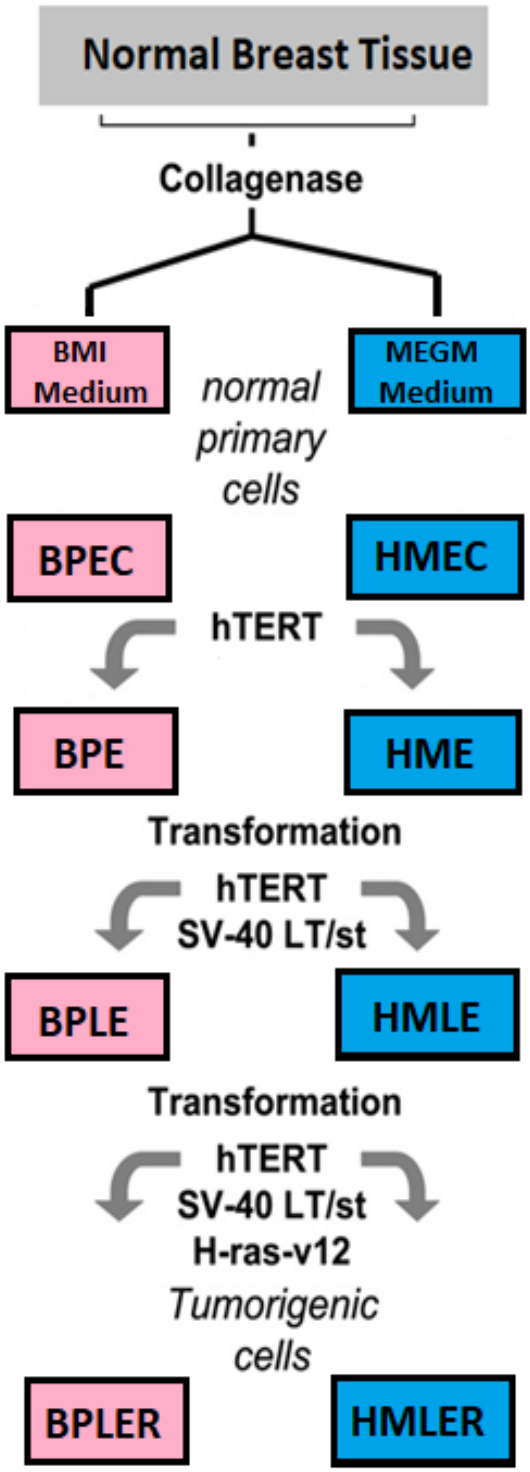

Figure 1. Schematic steps for the creation of the two tumourgenic breast cell types.

Immortalised breast epithelial/myoepithelial cell lines (containing only the pmig-GFP-hTERT vector) are referred to BPE and HME and fully transformed derivatives as BPLER and HMLER following the introduction of vectors encoding hTERT (E), SV40 early region LT/st (L) and H-ras (R).*

mRNA gene expression was compared between the normal and hTERT expressing cells against a set of previously reported luminal- and myoepithelial-specific human breast genes, with results showing ≥2-fold higher level in the hTERT expressing cells relative to their corresponding normal cell type. The same was determined for the tumorigenic cells, known as BPLER (Breast Cancer Stem Cell lines, but sometimes also referred to as Tumour-Initiating Cells (T-ICs) and HMLER (non-stem tumour cell line (nsTC)); following the introduction of the SV40 LT/st and H-ras vectors. The T-ICs are thought to be responsible for tumour initiation, metastasis and resistance to chemotherapy.[2]

Similar responses as above were also identified as determined by RT-PCR, immunofluorescence and immunoblots. Both cell types (BPLER and HMLER) lack ESR1 (Oestrogen Receptor alpha 1), PGR (Progesterone receptor) and ERBB2 expression (an oncogene which is overexpressed in breast cancer. They also expressed the following basal markers KRT5 (Keratin 5), KRT14 (Keratin 14), KRT17 (Epithelial Keratin 17) and EGFR (Epidermal Growth Factor Receptor).[2] However, BPLER expressed intermediate levels of both E-cadherin (a known tumour suppressor which is expressed in 'normal' tissue) and vimentin mRNA (a myoepithelial marker which is highly expressed in high-grade ductal breast cancers and tumours with low oestrogen receptor levels). They contain a mix of luminal-myoepithelial phenotype and maintain telomerase activity *in vitro*.

HMLER cells expressed ~20-fold more vimentin and negligible amounts of E-cadherin. These are both consistent with their retrospective phenotypes. The HMLER cells consist of a more differentiated myoepithelial phenotype and do not have telomerase activity.[3] Triple negative tumours (tumours which are negative for oestrogen receptors, progesterone receptors and HER2) show increased EGFR expression and ultimately poor prognosis and survival.

Epigenetic changes are observed in specific histone deacetylases (HDACs); which are chromatin-modifying enzymes that are involved in a variety of cell biology processes, including apoptosis, mitosis, differentiation, autophagy, migration and angiogenesis. There is growing evidence that HDAC1 and HDAC7 are over expressed in the BPLER cells at significantly higher levels at the protein level compared to the HMLER family of cells. Interestingly, this was not consistent at the mRNA level suggesting that these differences are only found at the protein level. [3]

The BPLER family of cells are grown in a chemically defined media (known as BMI media) which was developed by the lab of Dr. Ince Tan at the University of Miami, Fl, USA. The HMLER family of cells are grown in commercially available MEGM media and as such encourages 'normal' human breast epithelial cells to grow as epithelial progenitor cells and myoepithelial cells respectively.[2] As part of the transformation process with the SV40 LT/st, the p53 tumour suppressor is inactivated. CD44 is a key tumour-promoting factor in those cells who lack p53 function.[4] When injected into immune-compromised mouse hosts, the BPLER cells yield tumour xenografts resembling invasive ductal adenocarcinomas (with as few as 10-100 of the transformed cells injected). These possess the ability to metastasise and were identified in the lungs 10 weeks' post-injection. The HMLER cells in comparison showed much reduced metastasis after the same length of time.[1][4] Histomorphology of these xenografts from the injection site indicated squamous cell carcinoma, a rare form of breast cancer.[2] The more malignant of the two cell line families (BPLER) also showed elevated levels of Heat-Shock Factor 1 (HSF1) as shown by HSF1 staining compared to the HMLER tumours.[5]

* The Plasmid Maps for the vectors referenced in this report can be found here:

pmig-GFP-hTERT: GSE6885
pBABE-zeo-SV40-ER: GSE6885
pBABE-puro-H-ras V12: GSE6885, GSE48444

Related Cell lines

ECACC Catalogue Number	Cell Name	ECACC Catalogue Number	Cell Name
20012027	BPE1	20012038	BPLER4
20012028	BPLE1	20012039	HME2
20012029	BPLER1	20012040	HMLE2
20012030	BPE2	20012041	HMLER2
20012031	BPLE2	20012042	HME3
20012032	BPLER2	20012043	HMLE3
20012033	BPE3	20012044	HMLER3
20012034	BPLE3	20012045	HME4
20012035	BPLER3	20012046	HMLE4
20012036	BPE4	20012047	HMLER4
20012037	BPLE4		

References

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