Ovarian and Fallopian Tube Cell Line Collection

Coming soon to the ECACC General Collection are a range of cell lines of ovarian (OCE) and fallopian (FNE) cells, derived from the same originating patient. These cell lines are offered as an immortalised 'normal' line (OCE1, OCE2, FNE1, FNE2), a transformed line (OCLE1, OCLE2, FNLE1, FNLE2), and a tumourigenic line (OCLER1, FNLER1, FNLER2).

Traditionally, it is believed that ovarian cancers arise from the surface of the ovary, however recent evidence has shown the possibility of precursor lesions in the fallopian tube fimbria epithelia, by evidence of p53 mutations at fallopian sites. This suggests that some 'ovarian' cancers may in fact originate in the fallopian tube [1]. ECACC's panel of paired ovarian/fallopian carcinoma cell lines will allow the study of human ovarian and fallopian tumour pathology, while reducing the possibility of patient-to-patient variability, changes in drug response, and alterations in gene expression.

Cells from the ovarian and fallopian cell lines were harvested from two postmenopausal female donors aged 56 and 65, respectively, during surgical treatment for benign gynaecological conditions. These cells were cultured using FOMI media (formally known as WIT-fo), developed specifically for these ovarian and fallopian tube cell lines by the lab of Dr. Ince Tan at the University of Miami, Fl, USA. Immortalisation was carried out by transfection of the pmig-GFP-hTERT vector to produce a normal immortalised line (OCE1/2 and FNE1/2) [2].

Additionally, some cells were cultured in the FOMI media, immortalised by transfection of the pmig-GFP-hTERT vector, and transformed by the introduction of a vector encoding the SV40 ER LT/st region to produce transformed cell lines. Lastly, a proportion of cells were also immortalised as previously described and transformed by the introduction of vectors encoding the SV40 ER LT/st region and H-ras to confer tumorigenicity* [2]. Further analysis was conducted on the gene expression of both cell lines, where the gene expression profiles were compared to two previously established datasets, the Wu dataset and the Tothill dataset (2), to determine the

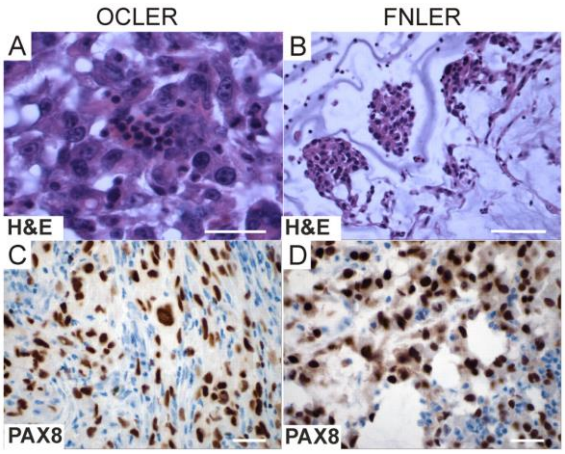

Figure 1: Tumour histopathology of OCLER and FNLER xenografts. Pax8 immunoperoxidase stain confirms that PAX8 expression is retained in xenograft mouse models.

different cancer histotypes (serous, endometrioid, clear cell, mucinous) present in both the ovarian and fallopian lines.

These cultures were confirmed by immunoblotting and immunofluorescence staining for various protein, such as PAX8, a novel differentiation marker, FOXJ1 a protein responsible for cilia formation, and CK7, an epithelial membrane marker, all of which are expressed in a cell type-specific manner in human ovary and fallopian tube epithelia (Figure 1). This confirmed that the OCE cell line is consistent with ovarian surface/inclusion cyst epithelium while the FNE cells are similar to those of non-ciliated epithelium found in the fallopian tube in mouse xenograft models when compared to normal human ovaries and fallopian tubes.

In most studies, the 'normal', pathology-free ovarian and fallopian tube epithelial cells and tumourigenic cells are derived from separate patients, however the OCE an FNE cell lines and their transformed counterparts are all derived from the same patients. Evidence from RNA microarray models suggest that tumour models may in fact behave differently from patient to patient, and that the intrinsic network of genes expressed by the specific 'normal' cells-of-origin may play an important part in determining malignant tumour phenotype.

Some studies show that 80% of serous carcinomas in two independent ovarian cancer gene expression datasets were classified as fallopian tube (FT)-like using the FNE versus OCE cell-of-origin signature, supporting the growing hypothesis that a larger proportion of high grade serous carcinomas may arise from the fallopian tube epithelium, rather than the ovarian epithelium. Furthermore, it was established that when comparing the FNE versus OCE cell of origin signature with previously published gene expression datasets for patient clinical outcomes; it was observed that patients with these fallopian tube derived tumours had significantly worse disease-free survival and poorer overall survival. The results from these studies using these two cell line groups strongly suggest that cell of origin may play a role in determining the associated tumour phenotype [2]. Using paired cell lines from the same originating source for *in vivo* and *in vitro* studies reduces the variability of cell-of-origin response and outcomes.

When comparing OCE and FNE cell lines to the tissue of origin, it was observed that similarity was 90-95% when comparing the samples genomic loss-of-heterozygosity, using reverse-phase protein array (RPPA). These breast and fallopian tumour cell lines retain the genomic landscape, histopathology and molecular features of the original source tumours, and therefore represent a significantly improved platform of human tumour pathophysiology [2]. Furthermore, these lines maintain their phenotype during long term culture, as confirmed by RPAA studies [1].

Additionally, these ovarian and fallopian cancer cell lines produce tumour xenografts with histopathology strongly resembling the original human tumours, when compared to other xenograft models, which tend to lack distinctive histopathologic features. Additionally, these developed cell lines display evidence of increased resistance to cisplatin and taxol when compared to standard ovarian cancer lines, which suggests that *in vitro* drug responses of these lines correlate closely with *in vivo* patient responses [1].

***** 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

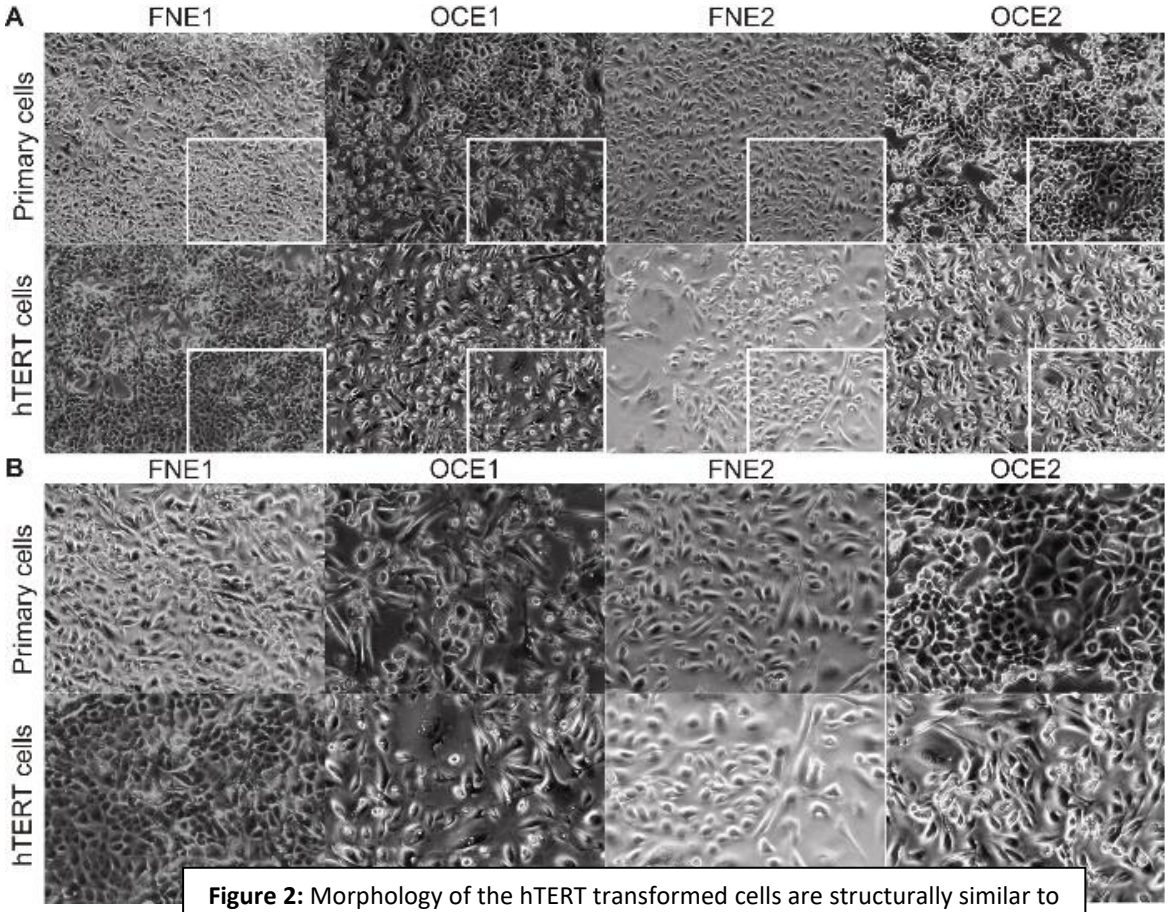

Figure 2: Morphology of the hTERT transformed cells are structurally similar to that of primary cells for both the ovarian and fallopian primary tissue.

Related Cell Lines

ECACC Catalogue Number	Cell Line Name	ECACC Catalogue Number	Cell Line Name
20012016	OCE1	20012021	FNE1
20012017	OCLE1	20012022	FNLE1
20012018	OCLER1	20012023	FNLER1
20012019	OCE2	20012024	FNE2
20012020	OCLE2	20012025	FNLE2
		20012026	FNLER2

References

1. Ince, T.A., Sousa, A.D., Jones, M.A., Harrell, J.C., Agoston, E.S., Krohn, M., Selfors, L.M., Liu, W., Chen, K., Yong, M. and Buchwald, P. (2015) Characterization of twenty-five ovarian tumour cell lines that phenocopy primary tumours. *Nature communications*, 6 **(1)**, pp.1-14. PMID: 26080861

2. Merritt, M.A., Bentink, S., Schwede, M., Iwanicki, M.P., Quackenbush, J., Woo, T., Agoston, E.S., Reinhardt, F., Crum, C.P., Berkowitz, R.S. and Mok, S.C. (2013) Gene expression signature of normal cell-of-origin predicts ovarian tumor outcomes. *PloS One*, 8 **(11)** PMID: 24303006