

Visiting Fellowship – Report on ‘Investigating the effects of distinct embryonic mammary progenitor/stem cell types on breast cancer cell behaviour.

By Dr. Efthymios Hadjimichael, Ptychio Iatrikes, MSc

Visiting supervisor: Dr Beatrice Howard, Breast Development Group, Breast Cancer Research Division Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, 1st June 2015 – 20th November 2015

Background:

Cancer has been suggested to result from development gone awry [1,2]. Many signalling pathways have been implicated in both embryonic mammary morphogenesis and carcinogenesis, providing support for this idea [3]. Embryonic stem cell signature genes are often activated in multiple types of aggressive tumours, suggesting that the activation of embryonic genes might contribute to the poorly differentiated characteristics of some tumours. Embryonic mammary cells are highly plastic and in an undifferentiated state [4], so one could envisage that their presence within differentiated cell populations could lead to tumour formation in a similar manner as observed upon transplantation of undifferentiated embryonic stem (ES) cells into mice, which leads to teratoma formation. Embryonic mammary epithelial cells (EMEC) signature activation in breast cancers is predictive of poor breast cancer patient outcome suggesting clinical relevance [5]. Conversely, the microenvironment of embryonic cells can suppress the tumorigenic phenotype of cancer cells [6,7]. Functional studies of embryonic mammary mesenchyme (MM), a key component of the EMEC microenvironment, demonstrated its profound role in directing and maintaining normal mammary epithelial tissue differentiation and architecture [8]. MM can restore some features of differentiated tissue to mouse mammary tumours *in vivo* [9-11]. Studies of the EMEC microenvironment could be useful for identifying stromal factors and cell surface receptors that promote mammary differentiation and mammary progenitor/stem cell survival or expansion and may lead to new possibilities for breast cancer therapies.

Aims:

The aim is to investigate the effects of embryonic mammary progenitor/stem cells on breast cancer cell behaviour and progression.

Results:

Embryonic mammary cells (from a pool of embryonic mammary cell types that show high clonogenic activity) and mammary tumour cells from the 4T1 mammary tumour series were grown together using three different techniques: 3D co-culture, confrontation assay, and *in vivo* by mammary fat pad injection.

In 3D co-cultures the embryonic cells showed distinct types of morphogenetic behaviour when grown with the three different cell lines from the 4T1 mammary tumour series. With the 67NR cell line (a non-invasive, non-metastatic cell line), the embryonic cells remained mixed with tumour cells and are rarely found at the periphery of the spheroid when grown in 3D co-culture (Fig. 1C). In contrast, in co-cultures with either the 4T07 cell line (invasive but not metastatic; Fig. 1A) or the 4T1 cell line (invasive and metastatic; Fig. 1B), the embryonic mammary cells were found located on the margins of the tumour spheroid at the basal position. In this assay, differential response in cell behaviour of the embryonic cells to the distinct tumour cell types is apparent. Results from the 3D co-culture studies suggest that cell-repulsive signalling may lead to differential localisation of cell populations

In confrontation assays, spheroids were formed separately from embryonic cells and cancer cells (4T1; Fig. 2). The embryonic and tumour spheroids were placed adjacent to each other, and allowed to grow and interact for several days. No invasion was observed from either cell population. Instead a distinct border was observed between the two types of spheroids where the spheroids had grown together indicative of some interaction between the two spheroids. In this assay, cell-cell interactions have already been established in the two cell populations leading to the formation of distinct spheroids or 3D “tissues”. Within the time-course of this assay, the cell-cell interactions do not lead to invasive growth that is measurable by other *in vitro* assays and *in vivo* studies. Embryonic cells may promote tumour growth through interactions with microenvironmental components that are not represented in this assay, including cells of the immune system, vasculature, lymphatics, fibroblasts, and pericytes.

Embryonic mammary cells accelerated mammary tumour growth when co-injected with the 4T1 cell line into the mammary fat pad when compared to injection of 4T1 cells alone. Resected tumours from these studies are being analysed to determine if the tumours that formed display any distinct features

Conclusions:

These functional studies allowed me to conclude that there is an interaction between the embryonic mammary progenitor cells and 4T1 mammary tumour cells. This interaction depends on the cell type, which correlates with the biological behaviour of the cancer cells with regards to their invasive and metastatic phenotypes (Fig. 1). Despite the fact that the cancer cells did not demonstrate invasion when confronted with embryonic cells *in vitro* (Fig. 2), the cancer cells displayed accelerated tumour growth *in vivo* when co-injected with the embryonic cells into the mammary fat pad (Fig. 3).

How Closely Have the Original Aims been Met:

Most of the original aims have been met. The findings generated with the support of the visiting fellowship award provide a solid base for future investigations with regards to the effect of the embryonic microenvironment in breast cancer progression.

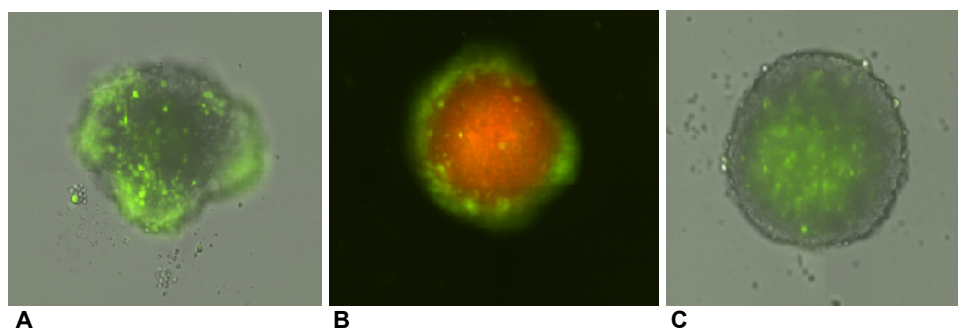


Figure 1: Co-culture of embryonic cells and cancer cells. **A:** Co-culture of embryonic cells (green) and 4T07 cells (grey). **B:** Co-culture of embryonic cells (green) and 4T1 cells (red). **C:** Co-culture of embryonic cells (green) and 67NR cells (grey).

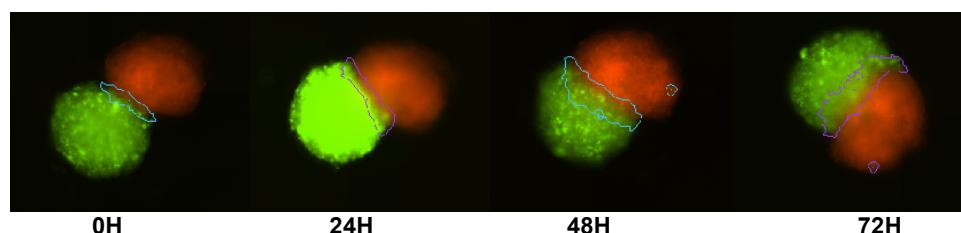


Figure 2: Confrontation assay of embryonic cells (Green) and cancer cells (4T1, red, H=Hours).

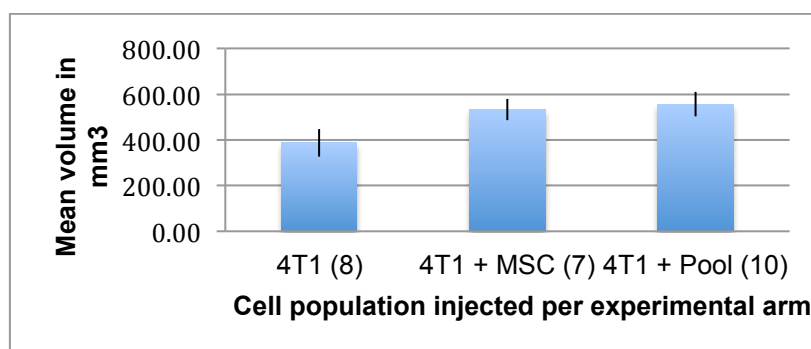


Figure 3: Results from *in vivo* mammary fat pad injection. Left and right mammary fat pads of 15 mice were injected with 4T1 cells only (5 mice), 4T1 and mesenchymal stromal cells (MSC) (5 mice) and 4T1 and embryonic cells (Pool) (5 mice). Each column represents the average tumour volume of both mammary fat pads. For the calculation of the average each tumour that was visible and measurable was included in the analysis. There was significant difference in the mean volume between 4T1+Pool and 4T1 alone ($P=0.019$, 95% CI: -403, -42.). The difference in the mean volume between 4T1+MSC and 4T1 was not significant (NS $P=0.052$, 95% CI: -341, 1).

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