Investigation of Epithelial to Mesenchymal Transition in breast cancer stem cells

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Introduction

What is Epithelial to Mesenchymal Transition (EMT)?

When epithelial cells transform into mesenchymal cells, they detach from the basement membrane, become elongated, more motile and invasive.[1] This plays an important role during development, but also initiates metastasis for cancer progression.[1] EMT is regulated by several signalling pathways, which fundamentally result in down-regulation of E-cadherin, a calcium-dependent cell adhesion molecule.[2] (Figure 1)

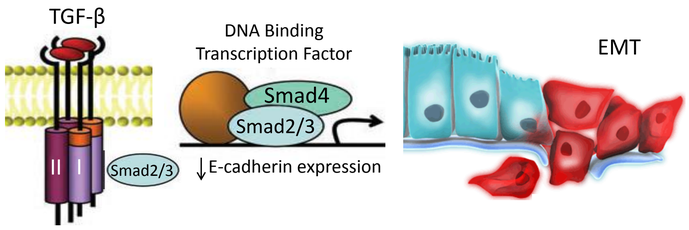


Figure 1: The TGF-β signalling pathway results in down-regulation of E-cadherin and initiation of EMT.[3]

What are cancer stem cells (CSCs)?

CSCs possess unlimited regeneration and differentiation capabilities, which enable them to drive tumor proliferation and metastasis.[1] Side population (SP) cells are a putative CSC population that express high levels of ABC transporters, which confers the ability to efflux chemotherapeutic reagents.[4]

Current cancer treatments may reduce tumor bulk, whilst leaving some CSCs behind that promote tumor recurrence.[4] Therefore targeting of cancer SP cells may be crucial for effective tumor treatment.[4]

Why is EMT important in CSCs?

EMT has been hypothesised to allow CSCs to become more migratory and invasive.[1] Recent evidence from breast cancer cell lines suggests that induction of EMT generates cells with stem cell-like properties but also in complete depletion of the SP population.[5] Furthermore, a high prevalence of SP cells is associated with the triple negative breast cancer subtype.[6]

Understanding the mechanisms involved in the generation of SP cells through EMT could prove useful for identifying therapeutic targets for the treatment of metastasis and drug resistance in particular breast cancer patient subgroups.

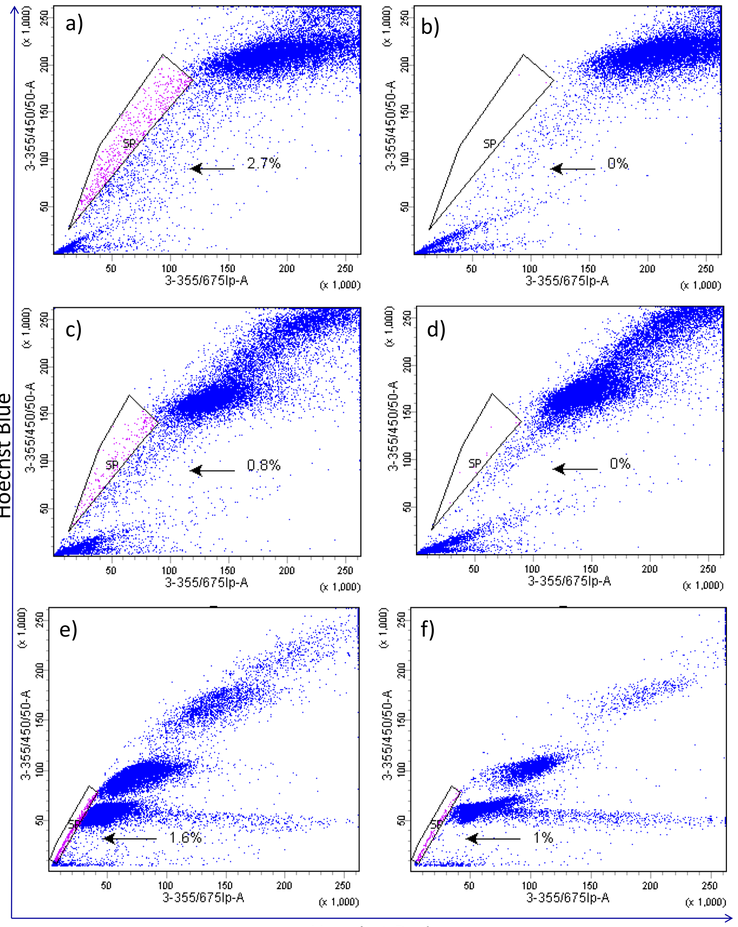
References

[1] Weinberg R, 2013. *The Biology of Cancer* 2nd ed. Garland Science. [2] Saxena M et al, 2011. Cell Death and Disease; 2: e179. [3] Figure adapted from: *Cellular stress, TGF-6, and EMT*. SABiosciences, 2012. http://www.sabiosciences.com/pathwaymagazine/minireview/fibrosis.php [4] Britton KM et al, 2011. Cancers; 3: 2106-2130. [5] Yin L et al, 2008. Cancer Res; 68: 800-807. [6] Britton KM et al, 2012. Cancer Letters; 323: 97–105

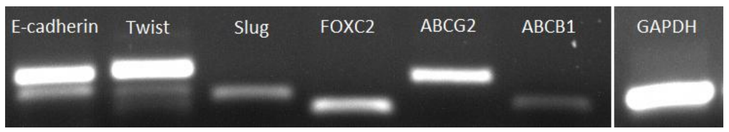
Methods

MCF-7 and MDAMB-231 cell lines: Tissue culture of MCF-7 and MDAMB-231 cell lines. Use of flow cytometry combined with Hoechst 33342 dye efflux to identify the SP and non-SP populations.

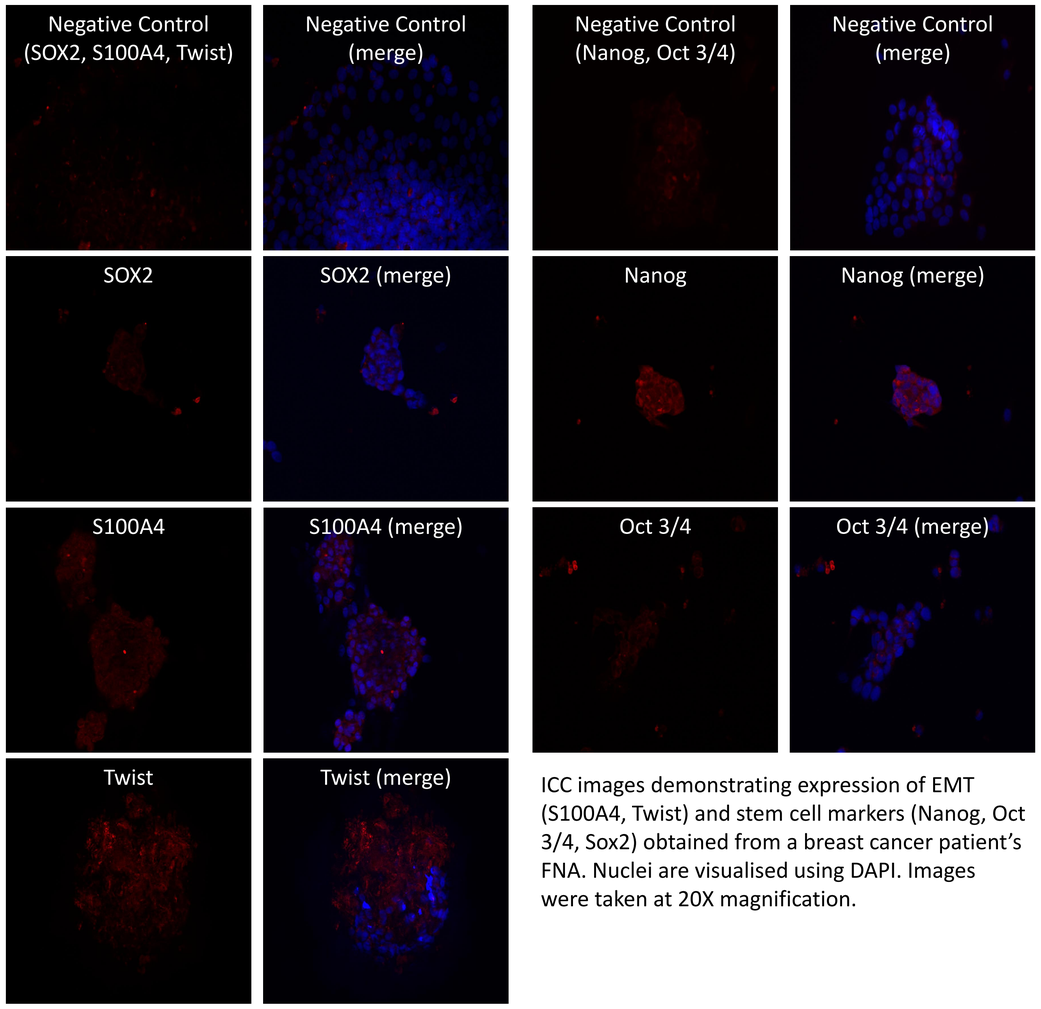
FNA sample: Processing of a fine needle aspirate (FNA) sample from a palpable breast tumor. Use of flow cytometry with Hoechst 33342 dye to identify the presence of a possible SP phenotype. PCR to examine expression of EMT markers and ABC transporters. Immunocytochemistry (ICC) to examine expression of EMT and stem cell markers.



Identification of SP cells from a) MCF-7 c) MDA-MB-231 e) breast cancer patient's FNA. The SP phenotype was confirmed by addition of FTC in b) and d) and verapamil in f). Dead cells were excluded by the addition of propidium iodide prior to analysis.



PCR investigation of a breast cancer patient's FNA, showing expression of EMT markers (E-cadherin, Twist, Slug, FOXC2) and ABC transporters. GAPDH used as a control.



ICC images demonstrating expression of EMT (S100A4, Twist) and stem cell markers (Nanog, Oct 3/4, Sox2) obtained from a breast cancer patient's FNA. Nuclei are visualised using DAPI. Images were taken at 20X magnification.

Results & Conclusions

Flow Cytometry We successfully identified a distinct SP population in both cell lines and the FNA sample. This was confirmed using FTC in the cell lines, but verapamil only partially blocked ABC transporter function in the FNA sample.

PCR analysis shows gene expression of several EMT markers in this breast cancer patient, with higher levels of ABCG2 than ABCB1, which could explain the incomplete inhibition with verapamil.

ICC The cancer cells obtained from the FNA sample exhibited sphere formation in culture. ICC revealed that there is expression of several EMT and stem cell markers in this breast cancer patient.

Investigation of a breast cancer patient's FNA has revealed expression of EMT and stem cell markers, suggesting that this patient may have a metastatic, multi-drug resistant form of cancer. It would be interesting to explore whether SP cells have undergone EMT and whether EMT promotes generation of CSCs. Association of these data with the patient's clinical condition could provide an insight into the contribution of EMT to SP function in metastatic and aggressive subtypes of breast cancer.