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# *In vivo* assay for the identification of potential inhibitors of epithelial-mesenchymal transition

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We designed and developed a simple and reproducible screening assay to explore the ability of a number of small compounds, interfering with evolutionary conserved signalling pathways, to inhibit epithelial mesenchimal transition (EMT) *in vivo*. EMT is an important process during development by which fully polarized epithelial cells acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility. EMT mechanism is reactivated in tumor progression, tissue invasion, metastasis and the acquisition of resistance to therapy.[1,2] Targeting EMT, therefore, represents an important strategy for cancer treatment.

Sea urchin embryos provide a useful system to study EMT progression, just few hours after fertilization. The process is well characterized. Prior to ingression, the future primary mesenchyme cells (PMC) are indistinguishable from the neighbouring epithelial blastomeres, but their regulatory apparatus is actively preparing new molecular programs by which cells are going to turn on the expression of mesenchyme-specific molecular markers. Thus, at the right time PMCs, previously adherent to the adjacent epithelial cells via cadherin and adherens junctions, loose cell adhesion, enter the basal lamina and move through the blastocoele. Later, after migration to the proper *loci*, the specified PMCs will give rise to the embryonic skeleton.[3]

So far, potential EMT inhibitor compounds have been identified using a carcinoma cell line specifically induced to undergo EMT by the activation of growth factor signalling pathways.[4] Nevertheless, this approach restricts the research to bio-molecules only affecting the selected growth factor induced signals and in some cases the reporter cell lines are not responsive to all exogenous growth factors known to be EMT inducers.

Here, we propose an *in vivo* screening assay, using sea urchin embryos (Figure 1); we picked out a selection of pharmacologically active compounds from a commercial library (LOPAC[1280]TM, Sigma-Aldrich) and tested their ability to inhibit EMT in embryos. These molecules are known to interfere with some evolutionarily conserved signalling pathways: P38 mitogen-activated protein kinase (MAPK), platelet-derived growth factor receptors (PDGF-R) epidermal growth factor receptor (EGFR) tyrosine-protein kinase (Src), Glycogen synthase kinase 3 (GSK-3). We set up the experiments as follows: two different batches of *Paracentrotus lividus* embryos at zygote (just post-fertilization) or hatching blastula (12h post-fertilization and 4h prior PMC ingression) stages were incubated in multiwell plates in the presence of different concentrations of the selected drug. Treated or control embryos were then monitored under an inverted microscope and scored for timely precise PMC formation, number and migration capability. Embryos were then photographed, phenotipically classified and in some experiments assayed for the expression of specific antigens. We obtained evidence that some of these compounds inhibit EMT and prevent the expression of mesenchymal specific molecular markers. We propose this low-, medium-throughput Sea Urchin embryonic EMT Assay (SU-EMTA) as an affordable and useful method to screen a high number of compounds, with potential anti-metastatic activity.

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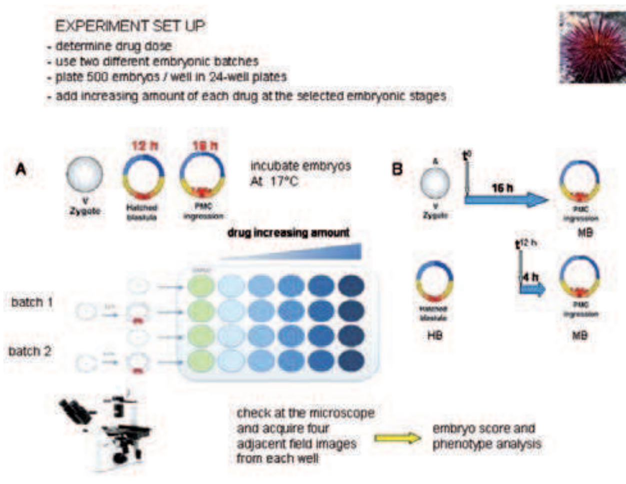


**Figure 1. A) Set up of a standard epithelial mesenchimal transition assay on sea urchin embryos; B) drug addition at fertilization (zygote) for 16 h or at hatching blastula stage (HB) for 4 h. Phenotype analysis at mesenchyme blastula stage (MB) and later (36 h after fertilization).**



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