33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Towards Multiscale Systems Modeling of Endocardial to Mesenchymal Transition

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***Abstract*— Cell behavior during endocardial to mesenchymal transition (EMT) was simulated using the cellular Potts formalism in Compucell3D. The processes of loss of endocardial cohesion and invasion into the extracellular matrix (ECM) were stimulated by changing surface energy parameters. The simulations match *in vitro* results which suggest that endocardial motility on the surface of collagen gel can be induced separately from 3D invasion of the gel, via Notch signaling in the absence of BMP2. A principle by which the rate of mitosis would regulate the monolayer was demonstrated; suggesting a route for Vascular Endothelial Growth Factor (VEGF) control of EMT.**

A conceptual model of the system of protein interactions during EMT was assembled from multiple studies. A route for subcellular models to be formalized as Systems Biology Markup Language (SBML) differential equations is indicated. Scale linking would be achieved through Compucell3D periodically integrating the SBML models for each cell during a simulation run, and updating parameters for protein concentrations assigned to individual cells. The surface energy parameters for the cells would be recalculated at each step from their simulated protein concentrations. Such scale linking opens up the potential for complexity to be gradually introduced, while maintaining experimental validation.

I. Introduction

The embryonic heart tube is composed of an inner layer of endocardium, an outer layer of myocardium and a middle layer of extra-cellular matrix termed cardiac jelly (Fig. 1). In two restricted areas of the heart tube - the outflow tract (OFT) and atrioventricular canal (AVC) - endocardial cells undergo EMT and invade the cardiac jelly. These restricted swellings are termed 'endocardial cushions' and are precursors for the heart valves and membranous septa.

The endocardial cushions begin to grow at embryonic day 26 (E26) in humans [1]. At the same time, the heart tube begins looping (see Fig. 2). Looping is completed by E28. The vessels come into alignment with their respective ventricles in a process termed 'wedging' [2].

The AVC endocardial cushions fuse around E32 which completes the atrioventricular septation process. Fusion of the cushions also gives rise to the leaflets of the mitral and tricuspid valves, and contributes tissue to both atrial and ventricular septation. The OFT endocardial cushions also fuse at E32, forming the OFT septum. This divides the aorta from the pulmonary artery, and is helical in shape, due to the rotation of the OFT (see Fig 2).

As the endocardial cushions play a role in forming much of the inner structure of the heart, it is apparent that abnormal EMT is a factor in many different types of congenital heart disease. These include valve, outflow tract and inter-ventricular septal defects. The importance of EMT during heart formation has led to a wide array of *in vivo* and *in vitro* research efforts to develop a caricature of the protein and genetic interaction involved (Fig. 1).

In the endocardial cushions of the embryonic heart, Notch and TGF-β act to synergysitcially downregulate VE-Cadherin, the main protein of endothelial cohesion. TGF-β and BMP2 act to upregulate the expression of integrins, which bind to fibronectin in the extra-cellular matrix (ECM) and are essential for mesenchymal invasiveness. Notch signaling is essential for delineating the cushion forming regions. In the endocardium, Notch is active in these regions, and inactive in the atrial and ventricular regions, while in the myocardium this expression pattern is reversed.

In the cushion forming regions, myocardium secretes BMP2 and TGFβ. Endocardium in these regions is Notch1 active, and the combination of these mechanisms stimulates EMT. Notch is downstream of BMP2 and TGF-β, and this provides redundant pathways. While Snail1 is a direct target of Notch and BMP2, Snail2/Slug can be targeted by TGF-β or synergistically by both TGF-β and Notch [3]. Both Snail1 and Snail2 bind to and repress the VE-Cadherin promoter.

High VEGF acts to inhibit EMT, and this could be due to increased endocardial proliferation maintaining the integrity of the endothelial layer. The interaction of VEGF, NFAT and Notch is another aspect of the multiscale complexity.

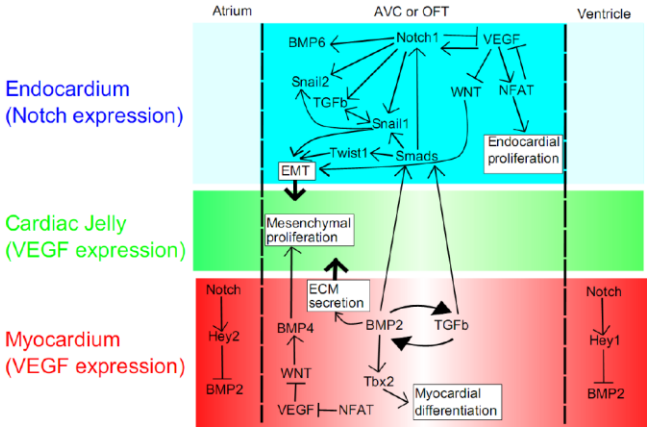

Fig. 1. Major protein interactions during EMT in endocardial cushion growth. After [1] and [4].

Manuscript received April 15, 2011. This work was supported in part by EPSRC Grant EP/E018521/1 "Bridging the Gap" award to R. Summers for an immersive secondment for J-M Schleich.

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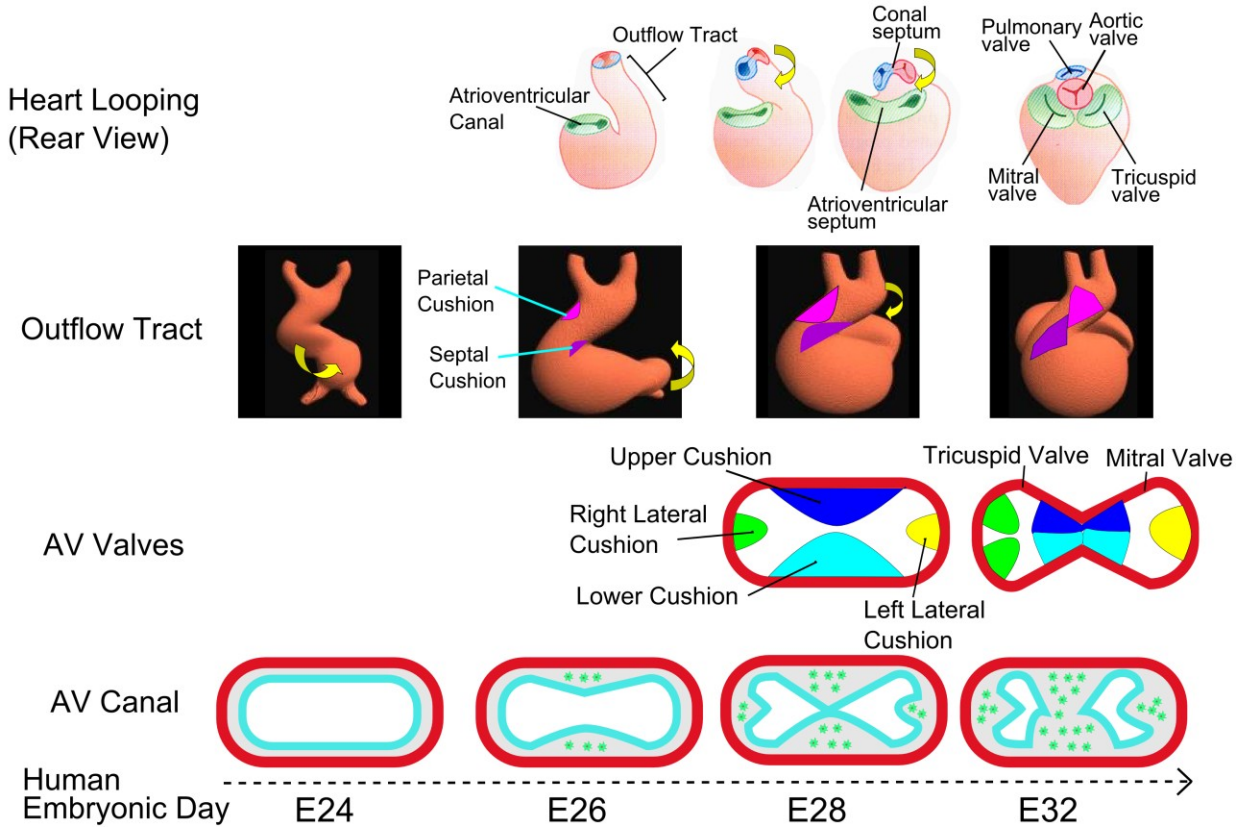

Fig. 2. Detail of endocardial cushion growth and fusion. EMT from E26 leads to cushion growth in the AVC and OFT. Fusion occurs at E32. In the OFT, parietal and septal cushions fuse forming the conal septum, which divides the aorta from the pulmonary artery. The conal septum is helical due to the rotation of the OFT. Upper and lower AVC cushions form the atrioventricular septum, the mitral valve and the tricuspid septal valve. The mitral valve lateral cusp forms from the AVC left lateral cushion, while the lateral cusps of the tricuspid valve forms from the AVC right lateral cushion. Heart looping and wedging brings the aortic valve into position between the mitral and tricuspid valves. After [2].

A recent *in vitro* study of endocardial cells cultured on a collagen gel demonstrated that 2D scattering of cells on the surface could be induced independently of 3D invasion into the gel [1]. This was achieved by constitutively activating Notch1 in the ventricular explants, without treatment with TGFβ2 or BMP2. Treatment with TGFβ led to similar 2D scattering and anti-TGFβ2 both counteracted this and maintained the monolayer in Notch1 activated cells. Treatment with BMP2 induced both 2D and 3D invasiveness of wildtype cells (Fig. 3). This suggests that the actions of both TGFβ and Notch1 in reducing endocardial adhesion are independent of factors that induce 3D invasion (including increased endocardial-matrix adhesion).

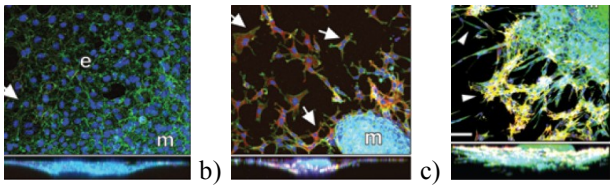

Fig. 3. *In vitro* mouse embryo ventricular endocardial explants. a) Wild type (untreated) tissue remains in a monolayer b) Notch activated cells scatter on the surface c) BMP2 treatment causes wild type cells to both scatter on the surface and invade the gel [1].
Key: e: endocardium m: myocardium

II. Methods

Cellular Potts models (CPM) simulate cell behaviours as terms within a generalised Hamiltonian energy, H [2]. Cells occupy multiple lattice sites, and thus have size, shape and surfaces that may be adjacent with other cells. During a simulation step, lattice copy attempts at cell surfaces will occur with a probability so as to reduce H. This includes interactions between cells; and between cells and the ECM. CPM can also include constraints on cell volumes, surface areas, chemotaxis and mitosis. In these simulations, H is given by the boundary energy between two neighbouring cells per unit area, and the volume constraints:

$$H = H_{Boundary} + H_{Volume}$$

$$H = \sum_{i,j} J\left[\tau\{\sigma(\bar{\imath})\}, \tau\{\sigma(\bar{\jmath})\}\right]\left[1 - \delta\{\sigma(\bar{\imath}), \sigma(\bar{\jmath})\}\right] + \sum_{\sigma} \lambda_{vol}(\sigma)[v(\sigma) - V_t(\sigma)]^2$$

Where for cell σ, λ_{vol} is the volume constraint, V_t is the target volume, and for neighbouring lattice sites $\bar{\imath}$ and $\bar{\jmath}$, J is the boundary coefficient between two cells (σ, σ') of given types $\tau(\sigma), \tau(\sigma')$, and the boundary energy coefficients are symmetric: $J[\tau(\sigma), \tau(\sigma')] = J[\tau(\sigma'), \tau(\sigma)]$, and the Kronecker delta is $\delta_{x,y} = \{1, x = y; 0, x \neq y\}$.

CPM provide a good representation for any mechanism where cell rearrangement is principally determined by differences in adhesion. This is because surface energy is a good (inverse) analogue of the overall adhesive force between cells. CPM have been widely used for modeling developmental mechanisms. It is thus an appropriate formalism for modeling EMT, and was used for modeling the *in vitro* EMT described in Section 1, as well as a more abstract scenario investigating mitosis. Compucell3D [3] is the most widely used modeling environment for developing CPM. It is open source and extensible, enabling the sharing of results. Compucell3D was used for all simulations described in this paper.

Compucell3D includes a type 'medium' by default, which is often treated as the ECM. In this case, it is treated as the space above the culture, with no intrinsic surface energy. The simulations also include endocardial cells (ECs) and ECM. An assumption is that EC-EC adhesion is stronger, in the wild type situation, than EC-ECM adhesion, which is stronger than ECM-ECM adhesion. The contact energy with the surrounding space is taken to be higher between EC-medium than ECM-medium, due to the lower deformability of cell membranes compared to ECM. Therefore, to simulate a wild type ventricular explant on collagen gel 'ECM' the following energy hierarchy is assumed:

$$J_{EC,medium} > J_{ECM,medium} > J_{ECM,ECM} > J_{EC,EC} > J_{medium,medium} = 0$$

An initial layout of 100 cells in a circular monolayer was generated and simulated for 1000 Monte Carlo Steps (MCS). By giving these initial volumes of 100 voxels, and target volumes of 400 voxels, a randomised arrangement of regular sized cells was generated. From parameter searching, it was found that the parameters in set 1 (Table 1) ensured this system remained in equilibrium, and cells did not scatter or invade the ECM. This suggests the energy hierarchy assumption above is reasonable. Set 2 corresponds to a loss of endocardial cohesion (increase in $J_{EC,EC}$). Set 3 corresponds to a gain in EC-ECM adhesion (reduction in $J_{EC,ECM}$). Set 4 corresponds to both effects simultaneously.

TABLE I
Surface energy parameters J, in $10^{-15}Kg^{1}s^{-2}$
KEY: EC-Endocardial Cell; ECM-Extra Cellular Matrix

Surface Energy *J*	EC, Medium	ECM, Medium	ECM, ECM	EC, ECM	EC, EC	Medium, Medium
Set 1	16	14	8	4	2	0
Set 2	16	14	8	4	10	0
Set 3	16	14	8	1	2	0
Set 4	16	14	8	1	10	0

A further abstract scenario was used for investigating mitosis. An endocardial monolayer was defined as occupying the entire midplane between two layers of medium. The mechanisms by which epithelial cells in a monolayer regulate mitosis are not precisely known. For the simulations, it was assumed that mitosis is regulated by some form of contact inhibition. The Compucell3D NeighbourTracker plugin and Mitosis stoppable were adapted such that a simulated cell will undergo mitosis if it meets the condition that the surface area it shares with the medium is greater than the surface area it shares with other endocardial cells. It must also have a volume greater than 200 voxels, in order to prevent excessive mitosis of small cell fragments. Surface energy parameters were adapted so that the medium would now represent ECM (Table 2).

TABLE II
SURFACE ENERGY PARAMETERS FOR MITOSIS SIMULATIONS

Surface Energy *J*	EC, Medium	EC, EC	Medium, Medium
Set A	16	2	0
Set B	4	10	0
Set C	2	2	0
Set D	2	10	0

III. RESULTS

The base case scenario (Fig 4a) using parameters from set 1 (Table 1) was perturbed by adopting the parameters in sets 2-4 and running the simulation for a further 1000 MCS in separate experiments.

With set 2, ECs scattered on the surface of the matrix without invading it (Fig. 4b). With set 3, the ECs invaded the ECM, but without delaminating from each other (results not shown). With set 4, all ECs delaminated from each other, and some invaded the matrix (Fig. 4c).

The mitosis scenarios were analogous to these results. Without mitosis, set A maintained the monolayer, while set B caused the cells to delaminate from one another in 2D, set C caused them to cluster together in the medium and set D caused 3D invasion after 1000 MCS. The inclusion of contact-inhibited mitosis caused the endothelial monolayer to prevail under set B and C, as each daughter cell would inherit a target volume of 400 voxels, causing the endothelial layer to rapidly replace gaps (Fig. 5). The monolayer failed under the conditions of set D, and endocardial cells quickly fill the entire lattice, due to the conditions for mitosis specified (not shown). In this model, mitosis prevents breakdown of the monolayer for reduced EC-EC adhesion or increased EC-ECM adhesion separately, but not in combination.

Although in this simulation mitosis is treated as a lumped variable that occurs instantaneously, the results demonstrate a plausible mechanism by which VEGF could control the level of EMT. EMT ceases in the endocardial cushions as VEGF expression increases, and this could be due to an increase in the level of endocardial contact-inhibited mitosis.

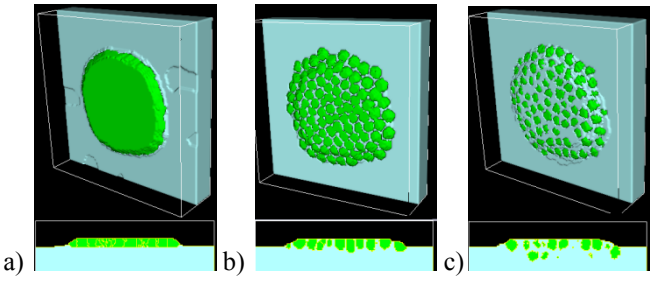

Fig. 4. CPM simulations of *in vitro* EMT. a) Endothelial monolayer on the surface of collagen gel. b) With reduced EC-EC adhesion, cells scatter on the surface, but do not invade the gel. c) With reduced EC-EC adhesion and increased EC-ECM adhesion, some cells invade the matrix (full EMT).

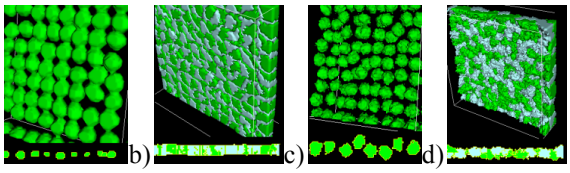

Fig.5. Mitosis simulations. a) Cells separate in 2D under set B. b) Monolayer prevails under set B if mitosis is included. c) Cells migrate in 2D and 3D under set C. d) Including mitosis rescues monolayer integrity for set C. Daughter cells are illustrated in a different shade to show how mitosis preserves the endocardial layer.

IV. Discussion

In the simulations presented, the surface energy parameters are set globally to illustrate the effects of relative changes in type dependent adhesion. This illustrates the principle by which changes in adhesion between endocardial cells, and endocardial cells and ECM orchestrate EMT.

Work currently in progress implements the EMT simulations as multiscale simulations by assigning SBML models to each cell in the Compucell3D model, and solving these periodically. This can be used, for example, to calculate the concentration of VE-Cadherin in a simulated cell, as a function of the initial concentrations of TGF-β and Notch. This concentration is then extracted using a Python steppable and the Compucell3D AdhesionFlex plugin to calculate cell-cell adhesion on an individual basis. Rather than setting surface energy as a global parameter, this allows the explicit inclusion of redundancy and crosstalk in the signaling pathways that determine the concentrations of adhesion proteins. Cellular feedback mechanisms emerge via self-organization, as cells move and create new signals.

Other types of juxtacrine signaling can also be included in the model. Notch signaling is crucial to EMT, and operates via lateral induction to create the fields of Notch activated endocardial cells in the cushion forming regions. Mutations in Notch signaling components such as JAG1 (which encodes the Jagged1 ligand for Notch) are implicated in endocardial cushion defects, and congenital heart defects generally. It would therefore be revealing to develop a multiscale model of endocardial Notch signaling, to formalize the mechanisms by which altered signaling leads to altered cushion phenotypes. This would investigate how different pathway manipulations lead to reduced or enlarged cushion areas, and hyperplastic or hypoplastic cushions.

Simulations with mitosis can similarly be extended to set the level of contact inhibited mitosis as a function of a VEGF concentration for each cell. This would be extracted by solving a subcellular model of VEGF regulation, with the possibility to include the complex feedback between VEGF, NFAT, Notch, VE-Cadherin and calcium.

Scale linking allows modularization of modeling tasks. Repositories of validated reaction kinetics models in SBML format, such as the Biomodels database [6], provide expanding resources for protein level modeling. These can be developed on and refined to create a model for the particular domain of interest.

Furthermore, with the gradual introduction of multiscale complexity, model validation can be achieved from experimental data at both levels. For example measurements of cell numbers, sizes and distances migrated provide validation for cell level simulations, while levels of protein expression over time provide validation for subcellular models of reaction kinetics.

V. Conclusion

The CPM simulations demonstrate some correspondence with the *in vitro* experiments on which they are based. In particular, in both cases it was possible to induce 2D scattering of endocardial cells independently of 3D invasion into the ECM. In the *in vitro* experiment this was accomplished through Notch activation of the endocardium. Alongside simulation results, this supports the hypothesis that Notch primarily acts to reduce endocardial cohesion.

In the simulation with set 3, it was possible for the endocardial cells to invade the matrix, but still remain attached together. This effect has not been observed in any *in vitro* experiments. This could be because it is not possible to isolate an increase in EC-ECM adhesion from a decrease in EC-EC adhesion, due to the nature of the signalling pathways. Notch is downstream of BMP signalling, and therefore inducing increased EC-ECM adhesion via introducing BMP would additionally reduce EC-EC adhesion (Fig. 1).

The simulations also indicated a possible role of contact-inhibited mitosis in controlling EMT, which would provide an explanation for EMT restriction at high levels of VEGF. This hypothesis could be tested *in vitro*, which would provide further refinement for the model.

Acknowledgment

We would like to thank Randy Heiland and Maciek Swat of the Biocomplexity Institute, Indiana University, for user support and guidance with the Compucell3D simulation environment.

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