Sprouting and lumen formation during angiogenesis
A cell-based computational model

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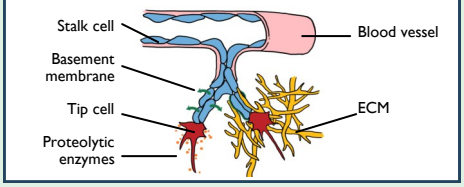
Introduction

Angiogenesis is the formation of new blood vessels from existing vessels. It is a complex process that involves many interconnected mechanisms that are poorly understood. Computational models enable us to comprehend the interplay, functioning and significance of these mechanisms. We developed a computational model, based on the *in vitro* model of Koolwijk et al. (1996), to unravel the mechanisms that drive sprouting and lumen formation. This systems biology approach links conceptual, experimental and computational models to gain insight in angiogenesis.

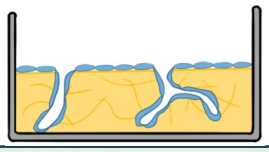
Conceptual model

During sprouting, the tip cell leads migration and stalk cells follow. The extracellular matrix (ECM) is remodeled and a lumen (hollow space) is formed inside the sprout.



Experimental model

A monolayer of endothelial cells is seeded on a 3D fibrin matrix. Upon stimulation with TNFα and VEGF and/or bFGF, sprouts grow into the fibrin matrix and form capillary-like tubular structures (Koolwijk et al. 1996).

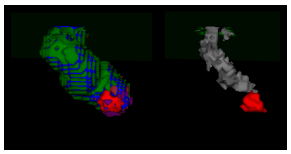


Validation

Predictions

Computational model

A cell-based model, based on the Cellular Potts Model, represents the experimental model of Koolwijk et al. (1996). It allows individual cells to move, depending on biological relevant constraints as cell size and adhesion.



watch movie

of sprouting and lumen formation by vacuolation

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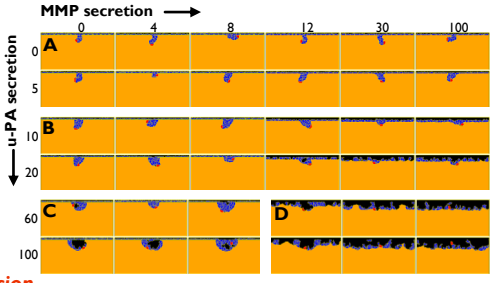
Sprouting

Proteolysis and sprout morphology

Capillary-like tubular structures (sprouts) are formed upon stimulation with TNFα and VEGF and/or bFGF, which induce proteolytic activity. Increased proteolytic activity results in the formation of round cyst-like structures and high proteolytic activity induces a uniform degradation of fibrin (monolayer lowering).

We hypothesize that the level of proteolytic enzyme secretion as well as the distribution of secretion over different cell types is responsible for these sprout morphologies.

We examined sprout morphology for different secretion levels of u-PA (degrades fibrin) and MMP (degrades BM) for stalk cells (blue), expressed in percentage of the maximal level as secreted by the tip cell (red). This resulted in different sprout morphologies (figure below): sprouts (A), solid cyst-like structures (B), hollow cyst-like structures (C) and monolayers (D). Both u-PA and MMP are (indirectly) induced upon stimulation, thus the sprout morphologies on the diagonal of the figure are biologically most probable and correspond to the experimental observations.



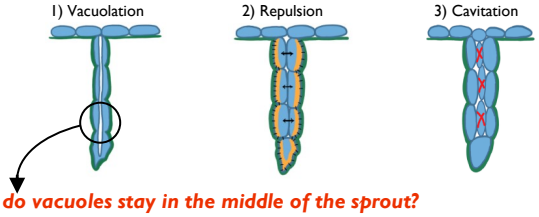
Discussion

Using this simplistic model, the experimental observations are well reproduced. The model will be extended to examine regulatory networks in proteolysis, such as inhibition and the interplay of local and diffuse proteolytic enzymes. Besides proteolytic degradation, other biomechanical and biochemical interactions with the matrix will be modeled in more detail.

Lumen formation

How is a lumen formed in a sprout?

Three alternative hypotheses explain lumen formation in sprouts (Lubarsky et al. 2003). 1) Vacuoles are formed intracellularly and these grow and fuse with vacuoles from neighboring cells to create a tube. 2) Cells in the sprout polarize and the apical membranes subsequently repulse each other to create a lumen. 3) A lumen is created by apoptosis of cells in the middle of the sprout.

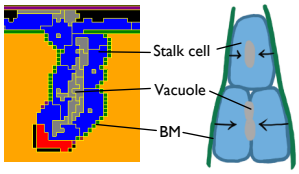


How do vacuoles stay in the middle of the sprout?

Integrins have been implicated in regulating formation and maintenance of vacuoles (Bayless et al. 2000).

We propose that the basement membrane (BM) regulates formation and localization of vacuoles, through integrin signaling.

By keeping the vacuoles from the BM, the vacuoles will stay in the middle of the sprout for different cell orientations within the sprout: overlapping cells and head-to-head orientation.



Discussion

The current computational model of cell hollowing shows that vacuoles can stay in the middle of a sprout for different cell orientations. To better understand this localization mechanism, modeling at a molecular level is necessary. Membrane polarization should be included to allow preferential vesicle fusion to the apical membrane and modeling the cytoskeleton allows expansion of lumens due to cell shape changes upon reorganization of the cytoskeleton.

Future work

Several hypotheses explain lumen formation: vacuolation, repulsion and cavitation. ***We propose that these three lumen formation hypotheses can be explained by a single mechanistic framework,*** involving membrane polarization, preferential fusion of vesicles to the apical membrane, apical membrane repulsion and cytoskeleton remodeling. We aim to develop a computational model that shows different predominant lumen formation mechanisms, while based on a single mechanistic network, as a result of different cell orientations in the sprout. Therefore, we will extend the current computational models to a ***multi-scale*** model, including molecular mechanisms (e.g. membrane polarization), cell behavior (e.g. shape changes) and matrix interactions (e.g. proteolytic degradation).

Acknowledgements

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