# Artificial Life in the Fight against Cancer

## Extended Abstract of Invited Keynote Lecture

Katie Bentley [1,2]

1. Computational Biology Laboratory, Centre for Vascular Biology Research,
Beth Israel Deaconess Medical Center, Harvard Medical School
99 Brookline Avenue, Boston, MA, 02215, USA.

2. Vascular Biology Laboratory, London Research Institute,
Cancer Research UK, 44 Lincolns Inn Fields,
London, WC2A 3LY, UK

*Abstract—*

Blood vessel growth is a fascinating and important example of an adaptive, morphologically plastic network formation process driven by complex interactions between individual cells in the vessel and between the cells and their dynamic extracellular environment. Under normal conditions this can generate a well-adapted hierarchical branching structure. However, in tumors, blood vessels become maladapted, leaky and bulbous, resulting in increased hypoxia and tumour cell metastasis. A method to switch tumor blood vessels back to a normal network could reduce metastasis and thus represents a significant goal in cancer therapy. However, studying human disease and the abnormalities that lead to pathological phenotypes is a monumentally difficult task. Probing the inner workings of *in vivo* systems present numerous technical challenges, though boundaries continue to be broken. Further, the *normal* fundamental mechanisms controlling development are of course not fully understood, let alone their perturbation by environmental changes in disease.

Artificial Life (ALife) aims to instantiate and study biological principles of organisation in new media in order to exploit different methods to test the system uniquely available in that medium. Thus ALife can perfectly complement cutting edge *in vivo* research giving vital temporal, spatial and organisational understanding of the process, if we work together with biologists, to build data driven models, and test emergent properties.

Using this integrated ALife/*in vivo* experimental approach, we have made advances in the understanding of blood vessel growth. The emergent properties of the embodied, agent-based model we developed, when put into a disease environment, have led to the discovery of a novel switch in cell communication which is changing the way we think about tumour malformations.

***Keywords—artificial life; cancer; simulation; angiogenesis, morphogenesis, agent-based modelling***

## I. INTRODUCTION

Angiogenesis, the growth of new blood vessels from pre-existing vasculature in response to oxygen deprived tissue signals such as the release of vascular endothelial growth factor (VEGF), is a complex spatial/temporal multiscale process. Individual, migratory endothelial cells are selected within the pre-existing vasculature to lead new vessels by a process of "lateral inhibition" [1]. During lateral inhibition cells signal to their directly connected neighbor cells and can suppress certain cellular states leading over time to patterns of alternating "on-off" state-patterns and dynamics along strings and surfaces of connected cells.

The principal lateral inhibition receptor-ligand signaling pathway is Notch (receptor)-Delta (ligand), which has been well studied mathematically in other systems, e.g. [2]. This Notch signaling pathway was found to determine the selection of alternating tip vs inhibited, or "stalk cell" fates during angiogenesis, which then leads to the regularly spaced branches required in the newly growing vascular network [1].

In [3] we characterized, and subsequently modeled, this process into the following simplified pathway. The process depends on negative feedback; each cell increases its Dll4 production (a Delta ligand) in response to VEGF. Cells then use their Dll4 ligands to activate Notch reecptors on a neighbor cell. Active Notch receptors cause the down regulation of the VEGFR-2 (and other receptors for the VEGF growth factor), which then in turn lowers the cells overall Dll4 production, making it less able to inhibit a neighbor, and therefore on its way to being totally inhibited itself.

The process is known to have the property that small differences in the cells levels of receptor activation will be

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amplified through the negative feedback such that though they initially experience similar levels of the growth factor, amplification of noise in the system drives some cells over time to more greatly inhibit their neighbors than the level of inhibition they feel. Eventually these become the tip cells.

The production of stable alternating patterns as opposed to oscillations or noisy behavior is highly dependent on initial conditions and indeed time delays in the signaling pathway such as the rate of production of proteins. See [1,2,3,4] for further discussions of the curious dynamics during lateral inhibition.

Unpicking the temporal dynamics of this lateral inhibition signaling in real blood vessels, and the effect on tip cell selection and subsequent vessel branching is of course not straight forward using traditional experimental techniques, let alone investigating how different tissue environments such as tumours would feedback and affect these dynamics. Thus, we developed a 3D agent-based model "the memAgent-Spring Model" to simulate and explore the environmental feedback on tip cell dynamics and branch determination [3, 4].

Fig. 1. The memAgent-Spring Model. Tip cells migrate away from the pre-existing vessel by growing long thin filopodia protrusions in response to growth factor gradients in the environment. Lateral inhibition generates an alternating selection of these tip cells (pink) from an initially homogeneous cell population, under normal conditions. Neighboring cells become inhibited (blue). Each cell is comprised of thousands of memAgents connected by springs into a surface mesh allowing for complex 3D shape changes and local interactions.

## II. Methods

In the "memAgent-Spring" model agents represent sections of the cell membrane and through local rules they are able to interact with their environment and signal with other agents to control localized shape changes in the cell, driving migratory filopodia protrusions to aid movement and to trigger reception activation in neighboring cells (such as lateral inhibition). Springs following Hooke's law connect the agents and maintain a cohesive tensile cortex to the cell, representing the actin cytoskeleton cortex beneath the membrane. See Fig.1.

In parallel, and with constant feedback to the model development researchers in the Vascular Biology Laboratory, Cancer Research UK also performed *in vitro/ex vivo* embyoid body sprouting assays and monolayer cultures using mouse Embryonic stems cells and bEND5 endothelial cells. We also investigated *in vivo* with time-lapse studies in the zebrafish and in the mouse retina.

## III. Results

The model has made numerous predictions, principally we observed in the model a catastrophic switch in Notch signaling dynamics upon elevated VEGF growth factor levels in the environment surrounding the vessel. Instead of creating a stable alternating selection of tip then stalk cells, which would generate regular branching of the network. The cells instead synchronously oscillate – where all cells are tip cells then they all fully inhibit each other and act as a string of inhibited stalk cells, and so on, preliminary *in vitro/in vivo* evidence indeed shows this could underly the abnormalities in real vessels under high growth factor conditions such as found in tumours [3].

Recently we observed an unexpectedly high level of dynamic rearrangement in growing vessels, cells constantly shuffle and rearranging their positions [5]. The memAgent-Spring model was then overhauled to include and investigate this new added dimension to the complexity, as during cell rearrangement, neighbor relations and connectivity will of course change, altering the Notch signalling dynamics between cells as the move in the vessel to find new neighbors, with implications for functional, stable branch determination. These model refinements, in light of new data, then created a surge of new insights and predictions. Principally, that Notch signalling may be involved in regulating cell rearrangements and maintaining regular vessel diameter, giving new explanations for the morphogenesis of tumour vessel malformations [5].

## IV. Discussion

The Artificial Life simulation paradigm provides a powerful platform with which to investigate complex spatial/temporal systems and can perfectly complement the current *in vitro/in vivo* methods available in order to uncover unforeseen mechanisms and insights. Close collaboration in the wetlab and open communication to an almost "symbiotic" degree can

further enhance this investigatory power, as recently discussed in this review [6].

Further, building on the bottom up nature of agent-based modeling, we can show the memAgent-Spring Model and this integrated approach is highly extendable, producing a plethora of insights in diverse applications such as bone fracture healing, intricate temporal effects of endothelial Notch gene expression mutations and entirely new vascular pathways such as VEGFR- 3/VEGF-C signalling [7-9].

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