Computational model and microfluidic platform for the investigation of paracrine and autocrine signaling in mouse embryonic stem cells

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***Short Abstract* — Autocrine and paracrine signaling mechanisms are traditionally difficult to study due to the recursive nature of the process and the sub-micromolar concentrations involved. This has proven to be especially limiting in the study of embryonic stem cells that might rely on such signaling for viability, self-renewal, and proliferation. To better characterize possible effects of autocrine and paracrine signaling in the setting of expanding stem cells, we developed a computational model assuming a critical need for cell-secreted survival factors. This model suggested that the precise way in which the removal of putative survival factors could affect stem cell survival in culture. We experimentally tested the predictions in mouse embryonic stem cells by taking advantage of a novel microfluidic device allowing removal of the cell-conditioned medium at defined time intervals. Experimental results in both serum-containing media and N2B27 media confirmed computational model predictions, suggested existence of unknown survival factors with distinct rates of diffusion, and revealed an adaptive/selective phase in mouse embryonic stem cell response to a lack of paracrine signaling. We suggest that the described computational/experimental platform can be used to identify and study specific factors and pathways involved in a wide variety of paracrine signaling systems.**

***Keywords* — Autocrine, paracrine, mouse embryonic stem cells, viability, computer model, microfluidics, survival factors**

I. Purpose

AUTOCRINE and paracrine signaling is an important aspect of normal and abnormal cell biology and has been shown to be essential in the development of mouse embryonic stem cells (mESCs).[1] Unfortunately, understanding autocrine signaling is severely limited due to the recursive nature of the process, and its action at sub-micron dimensions.[2] Current mathematical and computational models of autocrine and paracrine signaling generally focus on the spatial signaling range. Such simulations are based on either single-cell approximations[2] or compartmental models.[3-5] This type of simulation is ideally suited to address questions of the spatial range of autocrine signaling, but is not useful for determining the overall effect that autocrine and paracrine signaling might have on the viability of mESCs.

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We introduce both a computational and experimental model of autocrine and paracrine signaling in cell cultures. Our particular emphasis in this study is on the ability to experimentally control the removal of molecular factors secreted by cells into the surrounding media, thus affecting the degree and efficiency of autocrine and paracrine regulation of cell functions. The model is explored to predict the effect of partial removal of the secreted signaling factors on cell survival, so that a close match between the modeling predictions and experimental validation can be achieved.

II. Conclusion

We demonstrated that mESCs viability is dependent on soluble factors secreted by mESCs and not present in the supplied cell media, and that the viability can therefore be varied as a function of the medium replacement (wash) rate. Furthermore, we showed that mESCs undergo an adaptive and/or selective process in response to artificially decreased paracrine signaling. The described experimental platform is designed to allow further study of soluble factors and pathways in varied cell types, beyond the more specific application presented here. With the use of media supplementation, pharmacological inhibitors, and various live cell markers, a large array of additional cellular responses can be examined in a high throughput fashion.

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