Interlocked control motifs regulate the adhesive pathway activity of beta-catenin

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***Short Abstract* — While beta-catenin is a key signal transducer in both canonical Wnt signaling and cell-cell adhesive junctions, the control motifs regulating its' activity remain unclear. Here an integrated *in vitro-in silico* approach was developed to quantify the dynamics and to identify control motifs associated with beta-catenin-induced gene expression following disruption of adhesive junctions. Collectively, the data suggest that (1) a positive feedback loop is used to restore the integrity of adhesive junctions and (2) a negative feedback loop is used to limit beta-catenin-induced gene expression.**

***Keywords* — Model-based inference, high content assays, Wnt signaling.**

I. Summary

A competitive fitness landscape within multicellular tissues limits emergence of malignant cells. Conceptually, this fitness landscape is comprised of intracellular and extracellular control mechanisms that aim to maintain tissue homeostasis in the presence of perturbations [1]. Critical extracellular control mechanisms are innate and adaptive immunity. Innate immunity initiates an anti-tumor response while adaptive immunity restores homeostasis. Interleukin-12 is an important cytokine that links innate to adaptive immunity. We have recently shown that malignant melanocytes secrete Wnt-inducible signaling protein 1 (WISP1) and that WISP1 exerts paracrine action on immune cells to inhibit their response to IL-12 [2]. While WISP1 was produced transiently in vitro, WISP1 is upregulated in most invasive breast carcinomas. WISP1 is induced following nuclear localization of beta-catenin. Beta-catenin is a pleotropic protein involved in the canonical Wnt signaling pathway and adhesive signaling pathways that involve homotypic cell-cell adhesion via cadherins. In contrast to the canonical Wnt signaling pathway, the role that beta-catenin plays as a signal transducer in cell-cell adherens junctions remains less clear. Here, our aim was to clarify the dynamics associated with beta-catenin induced gene expression in response to disruption of cell-cell adherens junctions. We used flow cytometry to quantify total copy numbers and confocal microscopy to quantify cellular localization of beta-catenin and E-cadherin following proteolytic cleavage of the extracellular portion of E-cadherin. ELISA quantified the functional response, in terms of cellular production of WISP1. We used model-based inference to quantify the strength of regulatory motifs, given the available data. The results highlight the different control structures that regulate the activity of beta-catenin by these two pathways. Prior work has shown that induction of a canonical Wnt signal enables beta-catenin to accumulate in the cytosol by disrupting a constitutively active APC/Axin destruction complex. In the adhesive pathway, beta-catenin can be stored as part of a multi-protein adhesive junction complex. Disruption of these adhesive junctions liberates this pool of beta-catenin to induce gene expression. Here, the dynamics of the adhesive pathway suggested two interlinked control motifs: a positive feedback loop to restore the integrity of adhesive junctions and a negative feedback loop to limit beta-catenin-induced gene expression.

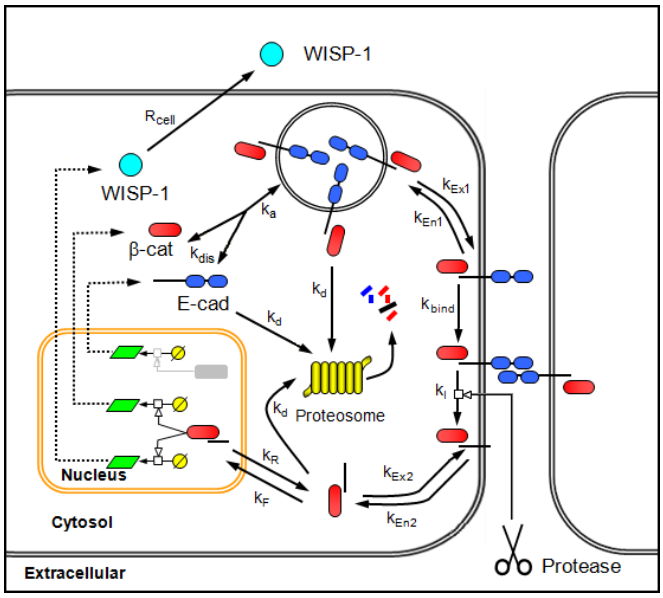

Figure 1: A schematic of the proposed control motifs associated with the cell-cell adhesive signaling pathway.

References

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[2] Kulkarni YM et al. (2012) *Integr Biol (Camb)* **4**, 925-36.

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