Asymmetric Cell Fate Decisions in Colon Cancer Stem Cells

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***Short Abstract* — Asymmetric division is a mechanism for stem cells to self-renew and to generate different cell types. Using colon cancer stem cells (CCSCs) generated from patient specimens, we identified a novel microRNA-dependent, coherent feed-forward loop (FFL) that is capable of generating asymmetric cell fate. Further computational analysis and quantitative experimental characterizations revealed that the FFL enhances the robustness of asymmetric cell fate decisions. Disruption of the FFL leads to more symmetric cell fate and less asymmetric cell fate. Our study explains previous reports that p53 mutations led to symmetric self-renewal and proliferation of cancer stem cells.**

***Keywords* — Asymmetric division, feed-forward loop, cell fate decision, robustness, colon cancer stem cell.**

I. Purpose

Stem cells constantly make critical cell fate decisions in order to establish and control cell population diversity. To maintain tissue homeostasis, stem cells must be capable of self-replicating indefinitely (self-renewal) as well as differentiating into other cell types (pluripotency). A variety of stem cells can asymmetrically divide to produce one daughter cell like itself, able to self-renew, and another daughter cell unlike itself, able to go down a path of differentiation. So how do these stem cells manage to robustly make cell fate decisions during development and tissue homeostasis? Will the subversion of these mechanisms lead to uncontrolled proliferation and cancer?

II. Materials and Methods

Human colon cancer stem cell (CCSCs) lines were generated using specimens from early stage colorectal patients [1]. Cells were cultured as spheres in ultralow-attachment flasks in stem cell media. To perform the 2-cell immuno-fluorescence (IF) assay, CCSCs were synchronized with Nocodazole, plated as single-cells on fibronectin and assayed after one cell division (~3 days).

III. Results

Using IF, we discovered that CCSCs asymmetrically segregate the cell fate determinant NUMB and the stem cell marker ALDH1 during mitosis [2]. In contrast, common colorectal cancer cell lines (e.g. HCT116) only partition NUMB symmetrically. Asymmetric segregation of NUMB and ALDH1 has never been previously reported in any solid tumor cells.

Further studies revealed a novel coherent feed-forward loop (FFL) mechanism that regulates CCSC asymmetric division. NUMB has been known to promote Notch receptor endocytosis, but we discovered that the tumor repressor protein p53 and the microRNA miR-34a form an alternative pathway with NUMB to downregulate Notch expression. Therefore, the daughter cell that inherits the asymmetrically segregated NUMB will downregulate Notch and starts to differentiate, while the other daughter cell will remain as a stem cell. RNA fluorescent in-situ hybridization (FISH) on miR-34a demonstrated the first reported case that a microRNA is asymmetrically segregated and acts as a cell fate determinant during mitosis.

Steady state analysis illustrated that, by removing the need for precise NUMB levels, the FFL increases noise margins to tolerate cell-cell variations. Dynamic analyses including ODEs and frequency domain analysis demonstrated that the sign-sensitive delay of the FFL filters out noise and perturbations to enhance the robustness of asymmetric cell fate decisions, especially in the presence of intercellular Notch interactions [3]. Quantitative characterizations of cell fate after miR-34a constitutive expression and knockdown validated our computational predictions that disruption of FFL in either way statistically leads to more symmetric divisions. Our findings helped explain reports that p53 mutations, which are common in late stage tumors, gives rise to symmetric self-renewal and stem cell proliferation [4].

IV. Conclusion

Stem cells possess microRNA-dependent mechanisms to enhance the robustness of asymmetric cell fate decisions. Disruption to these mechanisms in late stage tumors can lead to the proliferation of cancer stem cells.

References

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