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A microRNA-operated switch of asymmetric-to-symmetric cancer stem cell divisions

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Abstract

Defective asymmetric cell divisions of stem and progenitor cells are associated with tumorigenesis by a largely unknown mechanism. A signalling axis involving Snail, microRNA-146a and Numb is now shown to regulate the switch between symmetric and asymmetric cell division in colorectal cancer stem cells.

Somatic stem and progenitor cells use distinct cell division strategies to generate differentiated cell types, and to renew the stem and progenitor pool. They can divide symmetrically to generate two equal cells that self-renew (symmetric self-renewing division) or differentiate (symmetric differentiating division). Alternatively, they can undergo asymmetric cell divisions (ACDs) to generate both self-renewing and differentiating progeny each time they divide (asymmetric self-sustaining divisions) (Fig. 1a). Symmetric self-renewing divisions amplify the stem cell compartment, whereas ACDs balance proliferation with differentiation and are used during functional development, such as neurogenesis, and in homeostatic tissue¹. Through their ability to undergo both cell division modes, stem and progenitor cells are able to respond to micro-environment changes such as those induced by injury². However, aberrant control of cell division mode carries a risk of enhanced tumorigenesis¹.

Our knowledge of ACD regulatory mechanisms largely originates from *Drosophila melanogaster* neuroblast studies¹, where a stepwise process during mitosis leads to the establishment of an apicobasal polarity axis along which the mitotic spindle orients. This polarization allows cell fate determinants with opposing functions to localize to opposite poles and to segregate unequally during cytokinesis, which promotes self-renewal in one daughter cell and differentiation in the other. Although evidence exists for ACDs in mammalian stem and progenitor cells, and cancer stem cells¹, how these integrate with regulatory mechanisms of adult mammalian tissue homeostasis and cancer is unclear. In this issue, Hwang *et al.*³ demonstrate that signals promoting epithelial-to-mesenchymal transition (EMT) in colorectal cancer cells also determine the cell division mode of colorectal cancer stem cells (CRCSCs).

COMPETING FINANCIAL INTERESTS

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In the normal intestine, intestinal stem cells (ISCs) expressing Lgr5 (leucine-rich-repeat containing G-protein-coupled receptor 5), and located at the bottom of intestinal crypts, self-renew and generate transit-amplifying cells that expand, migrate upwards and differentiate at the crypt–villus junction⁴. The cell division mode of ISCs has been intensely investigated, with one study reporting that about 40% of them divide in a perpendicular plane and generate progeny with distinct proliferation capacities, indicative of ACD (ref. 5). In contrast, clonal fate mapping experiments indicated that Lgr5⁺ cells divide symmetrically, and their progeny eventually acquire stem or transit-amplifying cell fate in a stochastic manner instructed by niche signals⁶. More recently, ISCs were found to partially switch from symmetric, self-renewing division in infancy to asymmetric divisions in larger crypts⁷. These conflicting results could potentially be attributed to different experimental settings. Taken together, the current research suggests that a sub-population of ISCs divides asymmetrically in Lgr5⁺ and Lgr5[−] progeny during a defined phase of crypt formation.

Disruption of ACD during the neoplastic transformation of mammalian stem and progenitor cells has been reported in premalignant oligodendrocyte progenitor cells, leukaemic progenitors and mammary stem cells, suggesting that ACDs act as a tumour suppressive mechanism by restricting proliferation and balancing it with differentiation¹. However, whether ACD disruption is involved in colorectal cancer initiation is unclear. Colorectal-cancer-initiating mutations target and activate the Wnt pathway⁸, with Wnt-transformed ISCs identified as the adenoma cell of origin⁹. As asymmetrically dividing ISCs seem to be temporally restricted to early postnatal stages⁷, and tumours develop in adolescent and adult genetically engineered mice⁹, whether ISC transformation is accompanied by loss of ACD remains unknown.

Nevertheless, insights into the control of cell division mode have been provided by studies on CRCSCs, which are thought to be crucial for tumour growth and progression based on their increased tumour-initiating capacity in immunocompromised mice¹⁰. Notch signalling promotes growth of ISCs and colorectal cancer cells, and self-renewal of CRCSCs¹⁰. These stem cells can be identified by their sphere-forming capacity and slow proliferation rates *in vitro*, and were shown to distribute Numb asymmetrically during division¹¹. Numb is a conserved regulator of cell fate that inhibits Notch signalling, thereby promoting differentiation at the expense of self-renewal¹. In CRCSCs, switching from asymmetric to symmetric Numb distribution following depletion of ID1 and ID3 (inhibitor of DNA binding 1 and 3) was shown to correlate with increased differentiation and decreased self-renewal¹¹. Moreover, xenograft experiments with ID1/3-deficient CRCSCs associated symmetric differentiating divisions with elevated chemosensitivity¹¹. Further work demonstrated that CRCSCs isolated from late cancers predominantly undergo symmetric self-renewing divisions, and linked the control of division mode to microRNA (miR)-34a (ref. 12). This miRNA, which has tumour-suppressive activity in solid tumours¹³, was distributed asymmetrically to the differentiating daughter cell in 10–20% of CRCSC divisions. miR-34a^{high} cells downregulated Notch signalling and differentiated into a non-cancer stem cell, whereas the miR-34a^{low} cells proliferated and self-renewed. These findings suggest that in early colorectal cancer, a cellular hierarchy headed by CRCSCs is established by consecutive asymmetric and symmetric distribution of miR-34a, and provide evidence that

miRNA thresholds may exert tumour-suppressive functions by affecting binary cell fate decisions.

The work of Hwang *et al.* now reveals another layer of miR- and Numb-dependent regulation of ACD in CRCSCs³. At late stages, tumour cells undergo EMT, a key developmental program that, when activated in cancer, promotes invasion and metastasis¹⁴. EMT-inducing transcription factors, such as Snail, have been linked to increased stem-like phenotypes of mammary carcinoma cells¹⁴. Hwang *et al.* now provide mechanistic insights into how Snail increases stem cell properties. They observed that CRCSCs derived from established colorectal cancer cell lines predominantly underwent symmetric cell divisions, distributing Snail and the cancer stem cell marker CD44 equally between daughter cells. Following Snail depletion, CRCSCs switched to ACD, distributing CD44 asymmetrically and opposite to differentiation markers such as BMP4 (bone morphogenetic protein 4) and cytokeratin 20. Increased ACD rates correlated with restricted self-renewal capacity, indicating that Snail is critical for symmetric self-renewing divisions. miRNA expression analysis of CRCSCs revealed that miR-146a was upregulated by Snail, and further experiments demonstrated that the Snail-dependent induction of miR-146a was indirect, functioning through the activity of β -catenin and TCF4 (transcription factor 4), two key mediators of canonical Wnt signalling. Functional analyses showed that miR-146a acts as a major effector for the Snail-induced CRCSC phenotype. miR-146a depletion reduced self-renewal, clonogenicity and xenograft tumour growth, whereas ectopic expression of miR-146a enhanced these abilities without affecting cell proliferation or viability. Furthermore, although ectopic expression of miR-146a failed to affect migration and to induce complete EMT, its downregulation promoted asymmetric self-sustaining divisions and attenuated metastasis. By identifying that this Snail- and miR-146a-dependent signalling network influences cell division mode, Hwang *et al.*³ show a divergence from the classical Snail-induced effects on EMT.

To address how miR-146a affects cell division mode, the authors combined computational target predictions with gene expression data from miR-146a-overexpressing cells, and identified Numb as a major miR-146a target. They further delineated a positive feedback loop, whereby miR-146a maintains Wnt signalling by blocking Numb-dependent proteasomal degradation of β -catenin. Accordingly, enforced Numb downregulation enhanced Wnt signalling and increased symmetric self-renewing divisions, whereas restoring Numb levels in miR-146a-expressing cells had the opposite effect and reduced stem-cell-related properties *in vitro* and tumour growth *in vivo*. In line with the previously identified role of Notch in promoting symmetric self-renewing divisions¹², the authors showed that pharmacological inhibition of Notch signalling in CRCSCs increased ACD rates. Unexpectedly, neither ectopic expression of Numb nor depletion of miR-146a repressed Notch signalling, indicating that Numb operates in this CRCSC context predominantly by targeting β -catenin (Fig. 1b). This result might explain the puzzling observation that ectopic Numb expression, which presumably occurred in both daughter cells, led to unequal segregation of Numb and restored ACD rather than induced symmetric differentiating divisions, as would be expected based on the function of Numb as a Notch inhibitor. The extent to which Numb promotes acquisition of differentiated fate in CRCSCs might be confounded by several factors, including isoform-specific effects, regulated

phosphorylation status of Numb, or Numb- and Notch-independent regulation of ACD and cell fate — and will be important to address in future studies. Using an inhibitor that blocks Wnt activity downstream of EGF (epithelial growth factor), the authors further showed that pharmacological disruption of the Snail–miR-146a axis increased asymmetric self-sustaining divisions and attenuated tumour progression. They also observed that symmetrically expanding CRCSCs were more resistant to Cetuximab, a common inhibitor of the EGF receptor. Finally, a Snail^{High}Numb^{Low} expression profile was found to correlate with Cetuximab resistance and poor prognosis in colorectal cancer patients.

The present study presents important mechanistic insights into the regulation of symmetric and asymmetric cell divisions and the role of Snail in increasing CRCSC numbers to promote tumour growth, and provides evidence that a cell division mode switch might contribute to metastasis. However, several open questions remain. For example, it is unclear how the Snail–miR-146a signalling axis and the associated increased symmetric cell divisions might promote metastasis. Moreover, it would be important to dissect the precise role of Numb and the pathways it regulates, including Notch and Wnt, in the control of CRCSC fate. Based on the present³ and previous studies^{11,12}, miR-NAs and ID1/3 emerge as important determinants of cell division mode, and it would be interesting to investigate whether these factors and the signalling networks they modulate also influence ACD in ISCs. In addition, as cancer stem cells are thought to mediate resistance to traditional therapies¹⁵, the present study raises the exciting possibility that pharmacological modulation of ACD rates could provide therapeutic opportunities in colorectal cancer.

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Figure 1.

Cell division modes used by cancer stem cells and their regulation in colorectal cancer. **(a)** Colorectal cancer stem cells (CRCSCs) undergo symmetric self-renewing divisions giving rise to two self-renewing daughter cells (red). The red curved arrows indicate self-renewal. **(b)** CRCSCs also undergo asymmetric self-sustaining divisions by localizing cell fate determinants with opposite functions (red and blue crescents) to opposite poles, thereby generating one self-renewing (red) and one differentiating (blue) daughter cell. **(c)** Symmetric differentiating divisions can also be induced in non-CSC colorectal cancer cells (blue); for example, by the presence of miR-34a (ref. 12). **(d)** Model of integrated regulation of cell division mode, tumour progression and metastasis. Left: miRNA-regulated Wnt and Notch signalling promotes symmetric self-renewing cell divisions of intestinal stem cells at the bottom of intestinal crypts. In non-neoplastic stem cells and CRCSCs from early tumour stages, Snail expression is low or absent, whereas Numb expression is high, leading to proteasomal degradation of β -catenin (β -cat) and attenuation of Wnt signalling. As a result, intestinal stem cells and early-stage CRCSCs divide asymmetrically. Right: Hwang *et al.*³ propose a positive feedback mechanism in late tumorigenesis, whereby the Snail-dependent action of nuclear β -catenin (β -catⁿ) and TCF4 induces miR-146a expression and the subsequent downregulation of Numb. This relieves the Numb-mediated degradation of β -catenin and enhances Wnt signalling, thereby increasing symmetric divisions and maintaining the self-renewing CRCSC phenotype. The Snail- β -cat-miR-146a axis promotes

tumour growth and metastasis, in part independently of the epithelial-to-mesenchymal transition (EMT).

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