MOLECULAR & CELLULAR ONCOLOGY
2018, VOL. 5, NO. 1, e1403531 (3 pages)
https://doi.org/10.1080/23723556.2017.1403531

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AUTHOR'S VIEW

Crack the state of silence: Tune the depth of cellular quiescence for cancer therapy

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ABSTRACT
The regulation of cellular quiescence underlies numerous physiopathological phenomena. We recently found that quiescence depth can be tuned as to adjust a dimmer switch, by altering the expression of genes in the Retinoblastoma (Rb)-E2f pathway. Reducing quiescence depth may wake dormant cancer cells and make them susceptible to treatment.

ARTICLE HISTORY
Received 23 October 2017
Revised 25 October 2017
Accepted 26 October 2017

KEYWORDS
cellular quiescence; quiescence depth; cell cycle entry; Rb-E2F pathway

Quiescence is a sleep-like cellular state in which cells are dormant but retain their ability to re-enter the cell cycle and divide upon growth signals. This "reversibility" is a salient feature that distinguishes quiescence from other coma-like irreversibly arrested cellular states such as terminal differentiation and senescence. The reactivation of quiescent cells, including stem and progenitor cells in the body, is fundamental to tissue repair and homeostasis. Quiescence also protects cells against cellular stress and toxicity.[1] In this regard, dormant cancer cells present a therapeutic challenge by often evading drug treatments, which may later lead to cancer relapse.

Quiescence is not a homogeneous but rather a heterogeneous cellular state.[2] There are remarkable variations among quiescent cells in their likelihood to re-enter the cell cycle upon growth stimulation, which we term quiescence depth. *In vitro*, when cultured longer under quiescence-inducing signals (e.g., serum starvation or contact inhibition), cells progress into "deeper" quiescence, from which they require longer time and stronger serum stimulation to resume proliferation.[3] Meanwhile, considerable heterogeneity in quiescence depth exists among cells in the same population under the same culture conditions.[4] The heterogeneity of quiescence depth is also seen *in vivo*. Muscle stem cells and neural stem cells upon collateral or neural injury respectively become "alerted" and move to shallow quiescence, exhibiting an increased likelihood to proliferate upon the next stimulation.[5,6]

Our recent work demonstrates that quiescence depth can be tuned continuously in the cell. Previously we have found that the Rb-E2f pathway (here Rb stands for retinoblastoma protein family, including Rb1, Rbl1/p107, and Rbl2/p130, and E2f stands for activator E2f transcription factors, including E2f1, E2f2, and E2f3a) functions as a bistable switch, converting graded and transient growth signals into an all-or-none E2f activation, which underlies the all-or-none transition from quiescence to proliferation.[7] By experimentally testing the predictions generated from a mathematical model, we now show that the E2f-OFF state of the Rb-E2f bistable switch can be adjusted to different levels, as to adjust a dimmer switch, by altering the expression of genes in the Rb-E2f pathway (Fig. 1A). For example, increasing Cdkn1a (cyclin dependent kinase inhibitor 1A, best known as p21) and Rb1 levels can serve as coarse- and fine-tuners, respectively, to drive cells into deeper quiescence. Likewise, increasing Ccnd1 (cyclin D1) and Myc (proto-oncogene c-Myc) levels can coarse- and fine-tune cells to shallower quiescence, respectively. Our analysis showed that cellular activities that directly affect the phosphorylation status of Rb have the strongest effect on the minimum serum strength required to activate the Rb-E2f bistable switch (i.e., the switch activation threshold), which in turn determines quiescence depth. Consistently, it has been recently shown that cyclin-dependent kinases (Cdks such as Cdk2) and Cdk inhibitors (e.g., p21) play critical roles in the cell fate choice between proliferation and quiescence after mitosis.[8] Together, these recent studies suggest that 1) the Rb-E2f bistable switch is pivotal to quiescence control; 2) the activation threshold of the Rb-E2f bistable switch underlies quiescence depth; and 3) quiescence depth can be adjusted to different degrees by various proteins in the Rb-E2f network, in a computer-model predicted manner.[3,9]

Many cellular factors and mechanisms may affect quiescence depth by impinging on the Rb-E2f bistable switch and modulating its activation threshold (Fig. 1A). One such example is cell growth. We recently found that prior cell growth affects the depth of quiescent cells, likely by affecting the sensitivity of the Rb-E2f bistable switch to serum growth signals.[4] Specifically, given the same immediate division history, quiescent cells that had more growth at the time of quiescence induction (i.e., those that had progressed further along the preceding cell cycle) exhibit a shallower quiescence depth. Further, we showed that the deterministic cell-growth memory, coupled with the stochasticity of the Rb-E2f bistable switch, quantitatively explains the quiescence heterogeneity in a population of isogenic cells under the same culture condition.[4] Similar to cell

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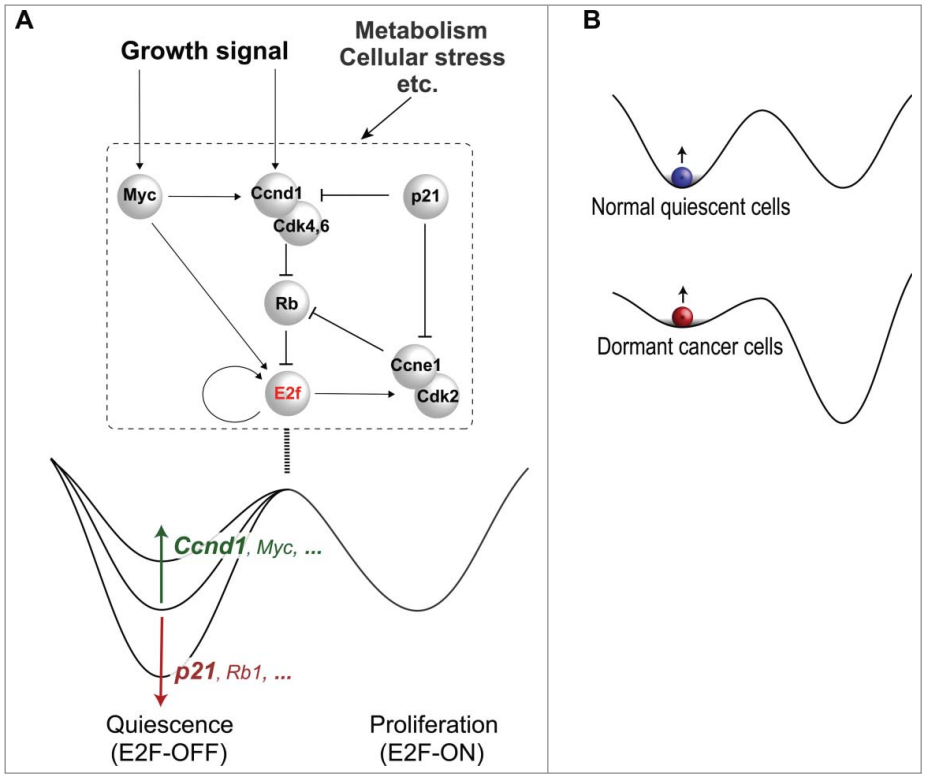

Figure 1. Tuning the depth of quiescence for cancer therapy. (A) Quiescence depth is controlled by the activation threshold of Rb-E2f bistable switch, which can be modulated by other cellular activities such as metabolic and stress responses. Examples of coarse and fine tuners of quiescence depth identified in the Rb-E2f pathway are shown in bold and regular fonts, respectively, to the right of the green and red arrows in the bottom graph. Myc, proto-oncogene c-Myc; Ccnd1, cyclin D1; Ccne1, cyclin E1; Cdk4,6, cyclin-dependent kinases 4 and 6; Cdk2, cyclin-dependent kinase 2; p21, cyclin dependent kinase inhibitor 1A; Rb, retinoblastoma protein family, including Rb1, Rbl1/p107, and Rbl2/p130; E2f, activator E2f transcription factors, including E2f1, E2f2, and E2f3a. E2f-OFF and E2f-ON, the OFF and ON states of the Rb-E2f bistable switch, respectively. (B) Dormant cancer cells, due to pro-proliferative mutations, presumably feature a shallower and less stable quiescent state than healthy cells, and thus will be awoken first and exposed to chemotherapy when quiescence depth is reduced.

growth, we anticipate that other quiescence regulatory activities, such as autophagy, DNA damage response, and stress response, to name a few, crosstalk with the Rb-E2f bistable switch and form an interconnected quiescence regulatory network (QRN). In the QRN, the Rb-E2f bistable module performs the necessary integration and conversion of various positive and negative growth signals into a binary E2f-OFF/ON output, while the other quiescence regulatory activities modulate the activation threshold of this Rb-E2f bistable module, which sets the ultimate metric of quiescence depth.

Elucidating the molecular mechanisms controlling quiescence depth may provide novel strategies to combat cancer, aging, and other hyper- or hypo-proliferation related diseases. As one example, dormant cancer cells, including many metastatic cells, evade chemotherapies that typically kill off actively dividing cells. These dormant cancer cells present a pressing therapeutic challenge as their revival often lead to cancer relapse and drug resistance. Cancer cells are, by nature, pro-proliferative with frequent mutations in the Rb-E2f pathway (e.g., the loss of Rb or Cdk inhibitors, or the amplification/overexpression of cyclins or Cdks). Thus, dormant cancer cells are likely poised at shallower quiescence than healthy cells due to altered Rb-E2f network dynamics (Fig. 1B).[3] Treatments that reduce the activation threshold of the Rb-E2f switch are then likely to first push dormant cancer cells but not healthy cells out of quiescence and expose them to chemotherapy. In this regard, we have recently identified two natural compounds purified from a medicinal mushroom *Ganoderma lucidum*. These two compounds, ergosterol peroxide and ganodermanondiol, were found to reduce quiescence depth by increasing basal E2f level, and appeared to preferentially target quiescent cancer cells (MCF7) instead of their normal counterpart (MCF10A) for apoptosis.[10] Further investigating the nature of quiescence heterogeneity, especially how the Rb-E2f switch interacts with other regulatory activities to control quiescence depth in different cell types under different conditions, will help the development of novel strategies to better target dormant cancer cells, and correspondingly, cancer metastasis and recurrence.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We apologize to colleagues whose work could not be cited due to space limitations. This work was supported by grants from the NSF (DMS-1463137 and DMS-1418172) and DARPA (WF911NF-14-1-0395).

Funding

NSF (DMS-1463137); DARPA (WF911NF-14-1-0395); NSF (DMS-1418172)

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