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Cancer Embryonic Stem Cell-like Attractors alongside Deficiency of Regulatory Restraints of Cell-Division and Cell-Cycle

Zhang Yue[1,2]*

[1]Research center, CHUM, Notre Dame Hospital, 1560 Rue Sherbrooke Est, Montreal, H2L 4M1, Canada
[2]Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard medical School, Boston, 02215, USA

Abstract

The attractive conception of cancer attractors has recently been proposed, but it remains hypothetic and awaiting for experimental confirmation. Further, it is essential to elucidate how cell cycle and cell division may smoothly integrate with it. It is well-known that many cancers show with embryonic stem cell-like signatures. Based on publicly available gene expression profiling data, one unique pattern for cancer embryonic stem cell-like attractors was identified alongside deficiency of Mi-2/NuRD and its partner's chromatin remodel systems (CRS) critical for germline-reactivated cancers, and particularly extendable to deficiency of regulatory components of cell-cycle and cell-division. Moreover, our results of Gene Expression Dynamics Inspector (GEDI) assays exemplified with the state of cell attractors of fruitfly *l(3)mbt* brain tumor reveals that cancer embryonic stem cell-like attractors might fall in between those of two-cell embryos and onset of differentiation states. Such stem–like attractors were further explored for its dynamics feature. The high-transcript abundance (HTA) within Self Organization Maps (SOMs) decrease during the progression of differentiation in wild-type animals and this trend can be significantly antagonized with a mutation in Polycomb homolog *mes-2/E(Z)* (enhancer of zeste homolog 2 (EZH2)) in *Caenorhabditis elegans*. Finally such features may contribute to a better understanding of carcinogenesis under cancer embryonic stem cell-like attractors theory and provide novel angles for cancer therapeutic interventions.

Keywords: Dynamics; Cell division and cell cycle; Cancer embryonic stem cell-like attractors; Germline gene; Chromatin remodeling system

Introduction

Many cancers have embryonic stem cell-like signatures [1]. Cell fates could follow different trajectories but reach same endpoints [2]. The theory of cell attractors initially incorporated the original concept of epigenetics from Dr. Waddington [3] and integrated systems biology. Huang et al. further elaborated the cell attractor theory of genetic regulatory network (GRN) with experimental example and then coined the hypothetic "cancer attractors" [2,4] and thus it caught good attentions [5]. Our CRS-linked cancer attractors may unify some controversies of different hypotheses of carcinogenesis [6,7]. The SNF2-like ATPase Mi-2β of the Nucleosome Remodeling Deacetylase (NuRD) complex inherently integrate the deterministic and stochastic biological characteristics [8]. This complex together with functionally related CRS may be the master of switch on/off the carcinogenesis [6] that can put the brake for cellular reprogramming [9]. Besides, unlike the failure of expected show-ups of many tumor suppressors, somatic mutations of chromatin remodeling components includes Mi-2/CHD4 have been reported in an increasing sequenced cancer types [10,11].

Recently, the dynamics of gene expression have gained more and more attention [12,13]. It was tempting to study the role of gene expression dynamics in carcinogenesis not only in cultured cells but also in multi-cellular organisms such as animals based on availability of published gene expression profiling data. Such dynamics could originate from the origin of life and the first "archaic" cell divided [14]. Kauffman et al. proposed the "metabolism-first" and gene expression dynamics based on "autocatalytic nets" which can be also viewed as precursors to attractors and then termed the aforementioned "cancer attractors" [4], from which one hypothesis raised is that the antecedent (or its pattern during the evolution) for cell division in modern multi-cellular cells may be among the keys to understand the carcinogenesis. Cell division may be critical for the quality of germline cell and other cell health via a wide range of cellular damage dilution during the embryogenesis, development and tissue regeneration [15].

However, Mi-2/NuRD and its partners CRS such as the Rb-MuvB-dREAM complex are evolutionally conservative and essential for the restraint of cell cycle gene expression and regulation [16]. Firstly, during embryogenesis after the two-cell stage, the Mi-2/ NuRD complex is supposed to remodel the chromatin and erase germline pluripotency [17] (Figure S1). Earlier studies have documented that mutations within functionally-related multiple components in the Mi-2/NuRD complex, particularly MEP-1/KLF-4 like and LET-418/Mi2β [18], dREAM/MyB-MuvB (MMB) components *l(3)mbt* [19] and the PcG-like MES-2/E(Z), cause ectopic expression of the germline genes. In *C. elegans*, the Mi-2/NuRD complex is required for maintenance of somatic differentiation (Figure S1). This complex is also required for maintenance and multilineage differentiation in the early mouse hematopoietic hierarchy [20]. On the other hand, cancer cells have some immature, germline or embryonic traits alongside deficiency of the Mi-2/NuRD complex and its related CRS. The ectopic expression of some cancer germline genes results in somatic tumors such as melanoma and brain tumors. Secondly, L3MBTL1, the human homolog of *Drosophila* L(3)MBT, is a transcriptional repressor that is found in a complex with Rb, a component of the dREAM-MMB complex that represses the transcription of particular suites of genes, including germline-specific genes. Thirdly, the component of p107 and its related family members Rb and p130 are critical regulators of the cell cycle and tumorigenesis with unique and redundant functions, and activation

***Corresponding author:** Yue Zhang, PhD. 1560 Sherbrooke Street East, Pavillon DeSève, Room Y2625, Montreal, Quebec, Canada, H2L 4M1, Tel: 1-514-890-8000; E-mail: zhanglee2006@gmail.com

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of the RB1 pathway triggers cell cycle arrest [21,22]. Lysine-specific demethylase 1 (LSD1) -deleted embryonic stem cells can proliferate normally and keep stem cell characteristics [23]. LSD1 associated interchromatin granules are hubs for interchromosomal interactions [24]. Furthermore, BMI1, a PcG repressor, is necessary for efficient self-renewing cell divisions in adult hematopoietic stem cells as well as adult neural stem cells [25,26]. Lastly, deacetylation of p53 modulates its effect on cell growth and apoptosis through the Mi-2/NuRD complex, and p53 can play a critical role in cellular reprogramming [27]. Mutation of all three RB1 family members leads to loss of contact inhibition and outgrowth of fibroblasts into spheres where cell–cell contacts predominate and such outgrowth triggers reprogramming to generate cells with the properties of cancer stem cells arrest [21,22]. The overall relationship among components associated with cell division and cell cycle are simplified and summarized in discussion. Besides, in cell attractors theory, cells eventually reach the attractors along with cell division and cell differentiation as well as the trajectories along its "quasi-potential energy" [28]. It remains unknown how cell cycle and cell division-related proliferation potential correlate with such trajectories. The cancer attractors hypothesis would be rejected or need modification if they could behave inconsistently with activity of Mi-2/NuRD and its related CRS whose deficiency has cancer embryonic stem cell-like attractors. However, our analysis was able to prove that they are roughly concord. Besides, the dynamic and chaotic gene expression noises in the progenitors cell may drive the differentiation [12] and may possibly lead to carcinogenesis.

Here we were first able to identify the pattern of cancer "embryonic stem cell-like" attractors in the cross-species model organisms, then particularly able to extend to deficiency of regulatory components of cell -cycle and cell -division. Further, we observed that the mosaic mini-clusters of genome-wide high transcript abundance of Self Organization Maps (SOMs) decrease during the process of differentiation and this can be significantly reversed with a mutation in *mes-2/E(Z)* in *C. elegans*. At the same time, an inverse relationship in the tendency of variation and dynamics of genome-wide GEP and plasticity of development was uncovered between *mes-2/E(Z)* mutants and wild-type animal controls concurrent with differentiation. Further challenge is to have more data to prove the hypothesis thoroughly; our analysis reveals that it is promising for cancer embryonic stem cell-like attractors to smoothly integrate with cell cycle and cell division. This work may first bring down the abstractness of current cancer attractors hypothesis to real assays and eventually facilitate a better understanding of epigenetic cellular reprogramming in carcinogenesis.

Methods

Gene expression dynamics inspector assays

Gene expression dynamics inspector (GEDI) assays were performed as previously described [29]. The dataset was run on GEDI, which is a Matlab R13 freeweb program using self-organizing maps (SOMs) to translate high dimensional Gene expression profiling data of time courses or sample classes into animated, coherent and robust 2D mosaic images. It facilitates the identification of interesting patterns of molecular activity simultaneously across the gene, time and sample space without prior assumption of any structure in the data, and then permits the user to retrieve genes of interest with their quantities [29]. Each tile of the mosaic (i.e. SOMs, gene mini-clusters) represents an individual SOM cluster and is color-coded to represent over-expression or under-expression of the cluster's genes, thus identifying the underlying gene pattern. Multiple samples can be evaluated together, thus linking their overall SOM pattern [29]. Datasets were downloaded from the GEO database (For mouse, including BMI1: GSE21912; Lysine specific demethylase 1 (LSD1): GSE21131; for *C. elegans*, including LET-418/Mi-2β, MEP-1/KLF-4 like; GFP: GSE216; *MES-2/E(Z)*: GSE14913); for the *Drosophila melanogaster,* such as lethal(3)malignant brain tumor *[L(3)MBT]*: GSM612783); except for the mouse GEPs of *MTA1* and p53 [30] and Mi-2β [24] , which were extracted from tables in their appropriate publications. In general, the representatives for each protein are taken from SOMs with at least three replicates of GEPs, showing the transcriptome patterns. Each map represents a transcriptome, with pixels representing the same genes in each pair of experimental and control maps. The GEDI assays were run together for comparison or independently for unique patterns for different sample classes. These images include a representation of either autonomous static GEDI SOMs for the same class (i.e. a single genotype and a single developmental stage), dynamic GEDI SOMs for developmental process (i.e. the same genotype but different stages), genotype-comparing GEDI SOMs for different genotypes at the same stage, or pseudo-dynamic GEDI SOMs for different genotypes with different stages generated from the corresponding raw data. The color represents the expression level: Red denotes high expression levels; blue denotes low expression levels.

Result

Embryonic stem cell-like cell attractors and dynamics of the global gene expression of the stem cells and progenitor cells as driver of cell differentiation

We first propose that two-cell embryo SOMs may serve as archaic "embryonic stem cell-like" cell attractors (Figures 1a, 1b

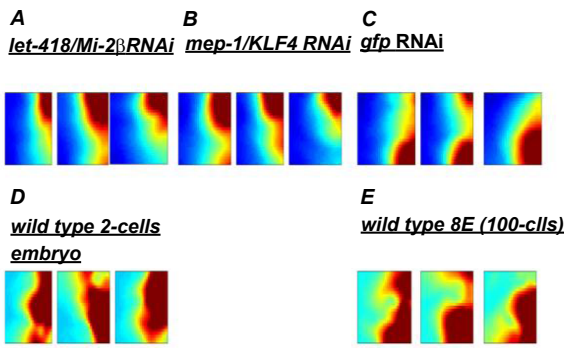

Figure 1: The SOMs of GEDI assays of the "embryonic stem cell-like" state and "differentiated" state in *C. elegans*.
The SOMs of global gene expressions for > 20,000 genes visualized by autonomous GEDI assay for (a,b) the "embryonic stem cell-like" state with an RNAi-caused defective Mi-2/ NuRD complex and (c) the "differentiated" state within the untreated control in animals; (d) two-cell embryos: ("embryonic stem cell-like") and (e) the 8E state ("differentiated") in wild-type animals. Pixels in the same location within each GEDI map contain the same mini-cluster of genes. The colour of pixels indicates the centroid value of the gene expression level for each mini-cluster in units of signal. The mosaic mini-cluster of the self-organization of stemness/early genes with high expression levels (i.e. "clouds" in red) becomes evident as they converge at the top right corner in an autonomous GEDI assay. Those of differentiation genes with high expression levels (i.e. "clouds" in red) become evident as they converge at the bottom right corner in an autonomous static GEDI assay.

a-c: The SOMs of the "embryonic stem cell-like" state produced by a defective Mi-2/ NuRD complex and the "differentiated" state in the control in *C. elegans*
d-e: The SOMs of the two-cell embryos ("embryonic stem cell-like") and 8E state 100 cells; ("Differentiated") in wild-type *C. elegans*

and 1d), the details discussed in our previously perspectives [6]. The "differentiation" state was initially defined on the process of GFP RNAi controls and 8E (100 cells, the onset of differentiation) in the SOMs of wild-type animals (Figures 1c and 1e) in that the distinct RNAi (*let-418* and *mep-1*) explored distinct trajectories, similar to the GRN cell attractors defined by a time-course during a drug treatment [2], but they finally converged sufficiently similar endpoints. To understand how dynamics of global gene expression of stem cell and progenitor cell drive the cell differentiation in multi-cellular *C. elegans* animals, we performed further systematic GEDI assays.

As previously, we had a focus on wild-type *C. elegans* whole animals at the two-cell, 2E (24 cells), 4E (50 cells) and 8E (100 cells) stages, the timeline for *C. elegans* embryogenesis. Again, all embryos within the same class (i.e. stage) had converging high-dimensional trajectories (across >20,000 genes) from the two-cells stage to the onset of differentiation. Some slight variation in the GEP within the same class was indeed observed, but their mini-clusters' distribution of over-expression genes (i.e. high transcript abundance, shown as "clouds" in red) remained rather similar. It is known that the embryonic blastomeres have developmental plasticity until the 2E stage and this characteristic is lost during gastrulation. At the ~8E stage, cells become committed to a cell fate. However, SOMs for "embryonic stem cell-like" states are clearly distinct from those for the "differentiated" state by means of autonomous static GEDI assay (Figures 2a-2d). In order to visualize the comparative distributions over-expression and under-expression during development and differentiation (i.e. the proxy of dynamics and noise in GEP) we made the dynamic GEDI assay by inputting all datasets for all stages of wild-type *C. elegans* for a systematic comparison. Strikingly, the tendency is that the mini-cluster mosaics of over-expression (i.e. high dynamics) gradually descended from an "embryonic stem cell-like" state to a "differentiation" state. In the latter states, differential gene expression remained at relatively low levels (shown as "clouds" in blue) (Figures 2e–2h). Next, we asked if the deficiency of any component of the Mi-2/NuRD complex and the related CRS could be responsible for maintenance of such relatively low expression levels in the differentiation state. In *C. elegans,* the PcG-like MES-2/MES-3/MES-6 complex has been proposed as promoting the ectopic expression of germline genes caused by a deficiency of LET-418/Mi-2 and MEP-1 through antagonizing the activities of the Mi-2/NuRD complex, so the characteristics of *mes-2/E(Z)* were of interest to investigate (Figures 2i-2n). The Polycomb Repressive Complex 2 (PRC2)/PcG in *C. elegans* , i.e. *MES-2/E(Z)*, is a homolog responsible for the repressive mark histone 3 lysine 27 methylation (H3K27me3), which is critical in cell cycle and carcinogenesis [31]. The GEDI SOMs are similar to either the *mes-2* mutant (8E) or the corresponding 8E wild-type (N2). Alongside the dynamic GEDI assay, the "embryonic stem cell-like" high dynamics GEDI patterns "recover" (Figures 2i-2k) in comparison with those of the wild–type (Figures 2f-2h), although they did not reach the same extents as autonomous static GEDI assays (Figures 2l-2n).

The early genes are seen among mosaic mini-clusters in the upper right corner of the abovementioned SOMs. Differential expressions within the same background (i.e. the same genotype: wild-type versus mutant; or the same stage: a embryonic stem cell-like state versus differentiated cells) remain strikingly evident at each embryonic stage (Figures 2a-2d). We observed more "clouds" in red (i.e. a large number of genes with high transcript abundance) at "embryonic stem cell-like" states versus more "clouds" in blue (i.e. a limited number of genes with low transcript abundance) at a "differentiation-like" stage. Interestingly, the later the embryo stage in *mes-2/E(z)* mutants, the greater the similarity to the "embryonic stem cell-like" state (Figures 2p-2u). Moreover, an inverse relationship in the tendency of genome-wide GEP was also uncovered between *mes-2/E(Z)* mutants and wild-type control *C. elegans* along the process of differentiation (Figures 2s-2u). With a direct comparison of different stages with different genetic backgrounds, i.e. a pseudo-static GEDI assay, however, the mosaic "clouds" in red for high transcript abundance keep descending from a "embryonic stem cell-like" to a "differentiation" state (Figures 2o-2u).

mep-1 or *let-418* mutants are found likely "dedifferentiated" and owning germline-genes reactivated expression profiling, for some unknown reasons, *C. elegans* does not have somatic tumorigenesis, thus we can only consider they have "embryonic stem cell-like" attractors rather than cancer embryonic stem cell-like attractors. Afterwards, we turned to continue with an assay of *Drosophila* L(3)MBT mutants, whose defect causes brain tumours with reactivated germline genes, making it an excellent model for tumorigenesis [32], encoding PcG like proteins among the same family as abovementioned MES-2 /E(Z) in *C. elegans*. Our static GEDI assay revealed that ectopic germline gene expression within defective *Drosophila* L(3)MBT animals can cause cell attractor transitions, and all fly brain tumour cell samples tested with cell transitions are similar within the same genetic background (either the temperature-sensitive strain or normal). However, some variations do exist (Figures 3a-3g). The GEDI assay of allograft of fly *L(3)mbt ts* reveals that more rounds of allograft decrease the dynamics of GEP (figure 3h).

Cancer embryonic stem cell-like attractors: The SOMs of cancer cells likely fall in between those of the "embryonic stem cell-like" state and the "differentiated-like" state

For both *Drosophila l(3)mbt* (Figures 4a and 4b) and *C. elegans let-418/Mi-2* and *mep-1* (Figures 4c and 4d) model organisms, by means of

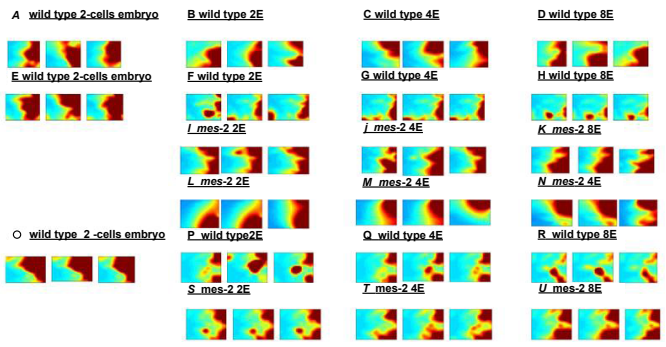

Figure 2: The SOMs along with developmental process from the two-cell embryo to the 8E stage.
The SOMs of the process from the "embryonic stem cell-like" to the "differentiated" state including both wild-type *C. elegans* and mutant animals with a deficiency in PcG/MES-2/E (z).

a-d: The autonomous static SOMs of the developmental process from the "embryonic stem cell-like" to the "differentiated" state in wild-type *C. elegans*
e-h: The dynamic SOMs of the developmental process from the "embryonic stem cell-like" to the "differentiated" state in wild-type *C. elegans*
i-k: The dynamic SOMs of the developmental process from the "embryonic stem cell-like" to the "differentiated" state in *mes-2/E(z)* mutants
l-n: The autonomous static SOMs of the developmental process from the "embryonic stem cell-like" to the "differentiated" state in *mes-2/E(z)* mutant.
o-r: The pseudo-dynamic SOMs of the developmental process from the "embryonic stem cell-like" to the "differentiated" state in wild-type *C. elegans*
s-u: The pseudo-dynamic SOMs of the developmental process from the "embryonic stem cell-like" to the "differentiated" state in *mes-2/E(z)* mutants

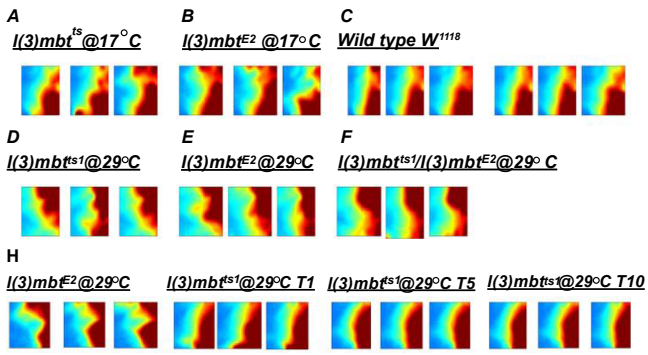

Figure 3: The SOMs of the cancer "embryonic stem cell-like" and the "differentiated" state in *Drosophila*.
The SOMs of cancer "embryonic stem cell-like" pattern could be seen in association with a deficiency of *Drosophila* L(3)MBT, a substoichiometric component of the dREAM/MMB complex; defective animals have *mbt* tumors. 17°C, permissive temperature; 29°C, restrictive temperature for L(3)MBT

a-c: The SOMs of three different genotypes of wild-type *Drosophila* in autonomous static GEDI assays
d-f: The SOMs of three different genotype of *l(3)mbt* -defective *Drosophila* in autonomous static GEDI assays
h: The SOMs of allografts (1 round , 5 rounds, 10 rounds) of *l(3)mbt* -defective *Drosophila* in pseudo-dynamic GEDI assays

genotype-comparing GEDI assays, we found that the SOMs of "cancer-like" cells (i.e. genome-wide distribution of over-expressed and under-expressed genes) are different from those of their autonomous static GEDI assays (Figures 1-5) and the "differentiated-like" state (Figures 1-5). Some mosaics of the ectopic expression of germ-line genes or early genes are evident but have "shrunk" in mutants (Figure 4, marked with dash red circles). Furthermore, the comparison of three or more genotypes changes more dramatically from the comparison of two genotypes (Figures 4a–4d). However, both the static and genotype-comparing GEDI assays of the three genotypes have the same tendency and keep clearly distinct patterns between the mutant and the wild-type controls in both model organisms.

Cell-cycle and cell-division -associated "embryonic stem cell-like" cancer attractors alongside deficiency of its regulatory restraints

Tumour cells can proliferate indefinitely, key factors involved in cell division are essential for the cellular restriction and expected to be targeted in cancer therapy [33]. Whether and how the cell attractors can be smoothly inherited through cell division is essential for the understanding of maintenance of the normal and healthy cellular state. Thus, the key regulator genes in the evolutionally-conserved machinery of cell division and the cellular reprogramming are of interest to investigate. In general, the CRS can act as regulatory constraints of cell fate restriction. Preventing ectopic germline gene expression in somatic cells requires transcriptional repression by cell -cycle key regulator retino blastoma protein (Rb) and its associated CRS including Mi-2/NuRD, dREAM/MMB. Prompted by over-expression of germline gene expressions in defective *Drosophila* L(3)MBT animals causing transitions to cancer cell attractors, our study extended to mammalian cells with a focus on cell cycle systems. Unique SOMs for both "embryonic stem cell-like" and "differentiation-like states" have been identified with autonomous GEDI assays and they appear to be conserved in different model organisms (*C. elegans, Drosophila* and mouse) (Figure 5). In fact, L(3)MBT functions in a complex with the cell cycle-related retinoblastoma protein (Rb) functions in humans [32,34]. Strikingly, the GEP of the *Trp53* knock-out mouse embryonic fibroblast (MEF) cells has its unique SOMs (Figure 5i). It could embrace the plasticity in that its unique SOMs is somehow like that of 2E wild-type embryo stage, (Figure 2b, so phenotypically similar to its developmental plasticity). This kind of plasticity may hypothetically enhance cancer susceptibility in human. Interestingly, the similarity is high for SOMs of deficiency (and normal controls) of components of the Mi-2/NuRD complex as well as its functionally-in tandem CRS in all model organisms. Systematic assays include comparing the SOMs of shRNA against *Rb* as "embryonic stem cell-like" cancer attractors (Figures 5j and 5k). In addition, the SOMs of double shRNA against *Rb* plus either *p107* or *p130* (Figures 5l–5o) showed that the shRNA scrambled controls have "differentiation-like" state SOMs. It was known that the embryo state largely remains unchanged with shRNAs in knock-down LSD1, one special component of Mi-2/NuRD complex that has a reversible dynamic function in gene repression and activation. The SOMs are "embryonic stem cell-like"

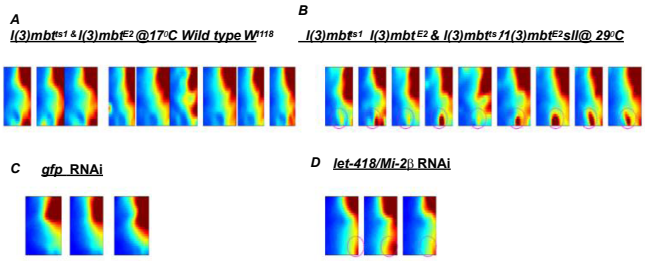

Figure 4: The SOMs of a direct comparison of wild-type and mutant animals.

a-b: The SOMs of a direct comparison of wild-type *Drosophila* and *l(3)mbt* mutant animals in genotype-comparing GEDI assays
c-d: The SOMs of a direct comparison of RNAi against control GFP and *let-418/Mi-2β* in *C .elegans* L1 larvae in genotype-comparing GEDI assays
The circles in red signify the subset of early genes

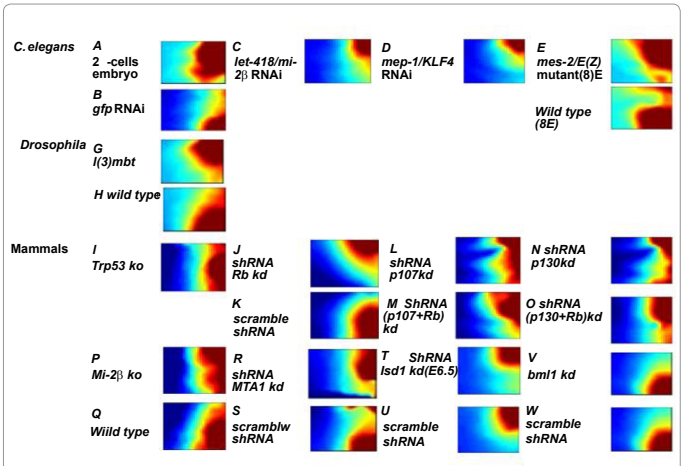

Figure 5: The SOMs of the Mi-2/NuRD/dREAM/PcG super-group in three different model organisms.
The distinct of SOMs is evident for the "embryonic stem cell-like "and "differentiation-like "states. All samples were subjected to autonomous static GEDI assays.

a-f: *C. elegans*
g-h: *Drosophila*
i-w: Mouse

for both LSD1 shRNA (alongside the scrambled shRNA) (Figures 5t and 5u). We examined a knock-down self-renewal PcG-like protein BMI1 with myeloma cells (Figures 5v and 5w). Their SOMs are similar to the "differentiation" state (alongside the scrambled shRNA). This is not unexpected in that BMI is required for self-renewal and but not for differentiation. For a systematic view, we summarize the SOMs for *C. elegans* two-cell embryos (Figure 5a), *GFP* RNAi larvae (Figure 5b), *let-418/Mi-2β* RNAi stage 1 larvae (Figure 5c), *mep-1* RNAi treated stage 1 larvae (Figure 5d), *mes-2/E(Z)* mutants at the 8E stage (Figure 5e) and wild-type animals at the 8E stage (Figure 5f); and then *Drosophila l(3)mbt* mutant (Figure 5g) and its control animals (Figure 5h), the mouse conditional *Mi-2β* knock-out hematopoietic stem cell (HSC) compartment (LSK; Lin−Sca-1+Kit+) along with the control (Figures 5p and 5q), and the mouse embryonic fibroblast (MEF) shRNA knock-down metastasis-associated protein-1 gene (MTA1), another core component of Mi-2/NuRD complex alongside the control (Figures 5r and 5s).

Discussion

This is one systematic study on cancer embryonic stem cell-like attractors with both culture cells and multi-cellular animal models on the dynamics of genome gene expression and the patterns of cancer "embryonic stem cell-like" attractors with deficiency of Mi-2/NuRD and its partners CRS as regulatory restraints of cell-cycle and cell division. Based on genome GEP data and four types of GEDI assays with different model organisms, we observed that the state (i.e. pattern) of the cell attractors of brain tumor cells fall in between the two-cell embryos and the onset of differentiation states. The transition from normal cell attractors to "embryonic stem cell-like" cell attractors can be visualized by the intuitive GEDI map as a simple identification "ensemble marker". We consider that the complete "embryonic stem cell-like" cell attractors are probably close to those of the two-cell state. Importantly, those defective Mi-2/NuRD and its closely functionally related CRS as regulatory restraints of cell cycle cell division may spur tumors by activating both the cell cycle and division and early developmental pathways associated with programming for multipotency, thus potentially are among the driving force of cancer initiation and progression. Of certain, further challenge is to glean more data by using mammalian tumor models or cancerous specimens along with detailed single-cell GEPs for every components as this study in each same cell or animal systems so as to prove the hypothesis thoroughly. Far from as one perfect but proof of principle, our analysis reveals that it is promising for cancer embryonic stem cell-like attractors to smoothly integrate with cell cycle and cell division. This work may first bring down the abstractness of cancer attractors to actual assays and eventually facilitate a better understanding of epigenetic cellular reprogramming in carcinogenesis.

Further, the variations and dynamics of gene expression in "embryonic stem cell-like" and "progenitor-like" cells (especially for highly abundant gene expression) are higher than "differentiated" cells at a whole multi-cellular animal level. We observed the gene mini-clusters of highly abundant gene expression decreases during the process of differentiation (summarized in cartoon in figure 6a). However, it raises the possibility that the dynamics of GEP under CRS regulation may provide the direct driving force of cell attractor transitions, cellular reprogramming in multi-cellular organisms and possibly the heterogeneities of cancer in diseased organisms without the CRS [31]. Thus, cancer cells could be triggered by cells undergoing abnormal cell attractor transitions and may be clinically reversible with "cell education" [6]. One suitable change in the diseased dynamics to normal could reverse the cancerous state. A recent computational study argues that a collective change of expression of more than 80 genes could facilitate transit of cell attractor [35], so it is not unexpected that the switch from the normal state to cancer attractors may be driven by malfunctions of hundreds of target genes regulated by chromatin regulators. Intriguingly, for *l(3)mbt* mutants, a direct comparison among all samples reveals a cocktail of a limited number of differentially expressing genes that can cause a pattern-shift, i.e. a system failing. Increasing levels of the static dynamics and dosage of germline genes' expression in GEP in the fly *l(3)mbt* brains at restrictive temperature (in comparison with wild type) left them impaired as brain tumor. If one could restore their normal -like ability of differentiation, i.e. decrease the levels of this static dynamics and dosage of germline genes' expression in GEP in the *l(3) mbt* brains at restrictive temperature, the fruit fly brain tumor could expectedly resume normality more or less like wild type counterpart. A very similar soma-to-germline transformation was observed in *C. elegans* Rb homolog LIN-35 mutants [19,32], has led some to propose that the acquisition of germline characteristics by somatic cells might contribute to increased fitness and survival, a mechanism that could contribute to the transformation of mammalian cells [19]. The "clouds" in genotype-comparing GEDI assays showed a pattern change from the static GEDI assay. It is understandable that the majority of the GEP remains constant between mutants and wild-type animals during such a direct comparison. Even so, some mosaics of the ectopic expression of germline genes or early genes are clear (but "shrunk") in mutants for genotype-comparing GEDI assays compared to static GEDI assays. Therefore we conclude that "cancer" state cells may fall in between the "embryonic stem cell-like" and "differentiation-like" states.

This decrease of GEP dynamics and the reduction of plasticity during differentiation can be antagonized by mutation in *mes-2* (Figure 6a). Typically, a mutation in this component of PRC2 complex, i.e. *mes-*

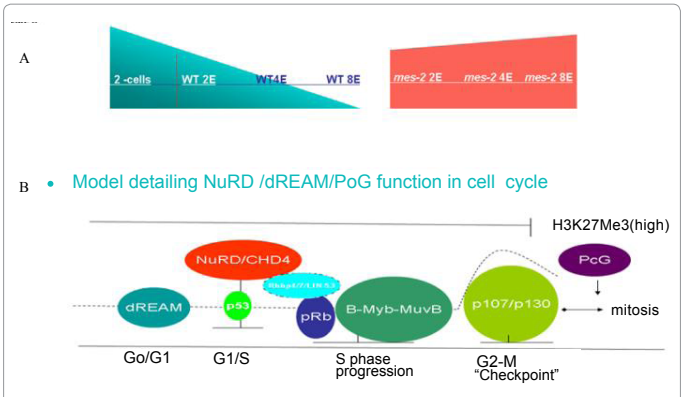

Figure 6: The Mi-2/NuRD complex and its related CRS.
a. The dynamics and plasticity orchestrated by PRC/Mes-2. WT: wild type
b. Scheme of relationship of the mammalian Mi-2/NuRD-Myb-Muv/dREAM and PcG complex.

This simplified model focuses on the sequential action of the mammalian Mi-2/NuRD-Myb-Muv/dREAM and PcG complex in cell division, though it is likely to be context-dependent. Each individual component may have other unique functions. Components of the Mi-2/NuRD complex, p53, dREAM, myb, and pRb are involved in mitotic exit, promoting differentiation. Particularly, pRb is essential for cell cycle exit and the differentiation of multi-/pluri-/bi-potent cells and is also uniquely required to maintain arrest in them. pRb effects permanent cell cycle exit in part by maintaining trimethylation of histone H3 lysine 27 (H3K27) on cell cycle genes, which require the polycomb complex (PcG) for terminal differentiation
Dashed line: gene expression level, *arrowhead: the activation Dash: the repression*

2 causes "differentiated" cells and can partially "recover" the dynamics of high gene expression in "embryonic stem cell-like" and "progenitor-like" cells at a whole-animal level. Furthermore, *mes-2* mutants have the reverse tendency to the differentiation process of wild-type cells. It is notable that the static SOMs, particularly in 8E *mes-2* mutants, can reach the wild-type 2E plastic stage or even the early stage state. With a direct comparison by means of pseudo-dynamic assays, the late-stage mutant has a stronger "embryonic stem cell-like" state, which could hypothetically suggest it could be easier for reversibility for cancer therapy when the same principle could be applied. The PcG in *C. elegans* (i.e. *MES-2/E(Z)*) is a homolog responsible for the repressive mark histone 3 lysine 27 methylation (H3K27me3) and the heterochromatin-related histone 3 lysine 9 trimethylation (H3K9me3), which are critical in the cell cycle and in carcinogenesis [31,36]. Such findings have been partially confirmed in mammalian model or human cancer [1,37,38]. CG-CT antigens are normally expressed restrictedly to adult testicular germ cells, and are aberrantly re-activated in some cancers, especially, more often expressed in advanced stage cases. Consequently, chromatin remodeling, cell cycle and cell division are tightly coordinated by the Mi-2/NuRD complex and its functionally-related complexes for differentiation and any dys-regulation of this coordination may lead to transition of cell attractors and the carcinogenesis. Put an aggregate, those findings suggest: for the differentiation cancer therapy, especially chromatin remodeling epigenetic therapy, the earlier the cell stage, the better the effects because they are more "normal-like". Besides, whether and how the cell attractors can be smoothly inherited through cell division is essential for maintenance of the normal and healthy cellular state. Our study demonstrated that the Mi-2/NuRD complex and the related CRS-linked "embryonic stem cell-like" cancer attractors can make inroads into cell cycle and cell division (Figure 6b). Strikingly, it shows all key regulators have similar SOMs (Figure 5) in the core of the evolutionally conserved machinery of cell division. Hence we may consider it as the cell cycle and cell division-related pattern of "embryonic stem cell-like" cancer attractors, and those elements are at the core of some conservative regulatory constraints. Therefore, the cell cycle and cell division system, through the Mi-2/NuRD complex and the related CRS-involved "embryonic stem cell-like" cancer attractors may have a direct link to reprogramming. However, another possibility is that the antecedent for cell division before modern multi-cellular cells may use other avenues as well to accomplish the regulatory restraints, such as microRNAs. Previous studies show that LET418/Mi-2, Rb, along side their synMuv B pathway has a role in somatic misexpression of germline P granules and enhanced RNA interference [8,39]. The fruit fly brain tumors cells deficient in *l(3)mbt* "reanimate" multiple germ cell small RNA (such as micro-RNAs and piRNAs) characteristics. Many unknown mechanisms remain to be explored in the future. For instance, cell division is discrete and how it smoothly integrates with overall continuous overall trajectory of cell attractors with different cell generations and cell differentiation. What is currently encouraging in cancer research is that accumulated evidence is emerging for the feasibility of the bidirectional reprogramming of cancer cells. Previously Dr.Yamanaka [40] has applied the original concept of epigenetics from Dr. Waddington [3] to explain elite and stochastic models for induced pluripotent stem cell generation. We have even hypothesized that iPS is among the exceptional cancer type [41]. Some recent studies show that the reprogramming of cancer cells generated "embryonic stem cell-like" cells [42,43], and the therapeutic epigenetic reprogramming transformed cancer cells to somehow normal-like cells [44], indeed some cancers can be epigenetically induced to improve their "immature" differentiation, which has been proven by several recent clinic trials of epigenetic drug treatments [45,46] and some translation research reports [47,48].

In conclusion, the stem-like cancer attractors related to both CRS and regulatory restraints of cell cycle and cell division likely underlies some germline genes-reactivated tumors at system level and it opens new thoughts of cancer therapy: we could need find out how therapeutically convert it to "normal-like" state.

Competing Interests

The author declares that he has no competing interests.

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