

Original Article

CMD kinetics and regenerative medicine

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Abstract: The author's theory of the cell memory disc (CMD) offers a radical and holistic picture of the cell from both functional and structural perspectives. Despite all of the attention that has been focused on different regenerative strategies, several serious CMD-based obstacles still remain that make current cell therapies inherently unethical, harmful, and largely ineffective from a clinical viewpoint. Accordingly, unless there is a real breakthrough in finding an alternative or complementary approach to overcome these barriers, all of the discussion regarding cell-based therapies may be fruitless. Hence, this paper focuses on the issue of CMD kinetics in an attempt to provide a fresh perspective on regenerative medicine.

Keywords: CMD theory, cell reprogramming, iPS cells, nuclear transfer, cell engraftment

Introduction

The cell memory disc (CMD), as a dynamic, multi-layered system of information storage, develops gradually over a cell's lifetime and determines all cellular behaviors [1]. In general terms, information fluidity within the CMD is thought to consist of a cycle of three overlapping phases, the robustness phase (R-phase), the fragility phase (F-phase), and the mimicry phase (Mi-phase), based on overall cellular behaviors and temporal properties. Cell robustness refers to the cell's tendency to preserve its basal state both morphologically and functionally. Due to cell robustness, all or at least some layers of the parental CMD remain active after the conversion from a stem cell to a differentiated cell and vice versa. In this sense, it has been extensively reported that stem cell-derived differentiated cells express donor-derived genetic memory [2]. In contrast, developmental memory in the CMDs of differentiated cells has the capacity to dramatically decrease the reprogramming efficiency of these cells. This phenomenon is most likely why stem cells reprogram more easily than differentiated cells [3-8]. The cell is a system with hysteresis, that is, the cell depends not only on its past niche but also on its current environment [9]. Hence, in addition to being robust, the cells must be fragile, which is the inability of CMDs to resist aberrant or forced memorization. This is a

behavior by which cells adapt to new conditions. Beyond the duality of robustness and fragility, a cell also has some degree of ability to mimic the morphology and behavior of other cells [10-16] (**Figure 1A**). If we accept that time is circular, as some physicists believe, and that the CMD is fluid with the ability to navigate both upstream and downstream, then it seems plausible that a cell is both the cell it once was and the fates it can be. Comparatively speaking, the upstream and downstream pathways of cell fate specification should occur over long and short periods of time, respectively. However, the short pathway, in the place of true cell specification, may result in a mimicry phenotype. If Einstein-Rosen time bridges and the theories of the cellular universe and the holographic triad [17, 18] are correct and generalizable, then it is tempting to speculate that the downstream pathway is a journey through a microscopic physiological wormhole (**Figure 1B**).

As can be deduced from the following sections, the fluidity of information likely enables the CMD to have steganographic capability, which means that while the CMD cycle is at a particular phase, it recessively or dominantly overlaps with other phases at two-phase regions (TPRs). Although not necessarily to the same extent, at any given TPR, the cell can simultaneously show behaviors of two phases of the CMD cycle if the hidden phase is reactivated.

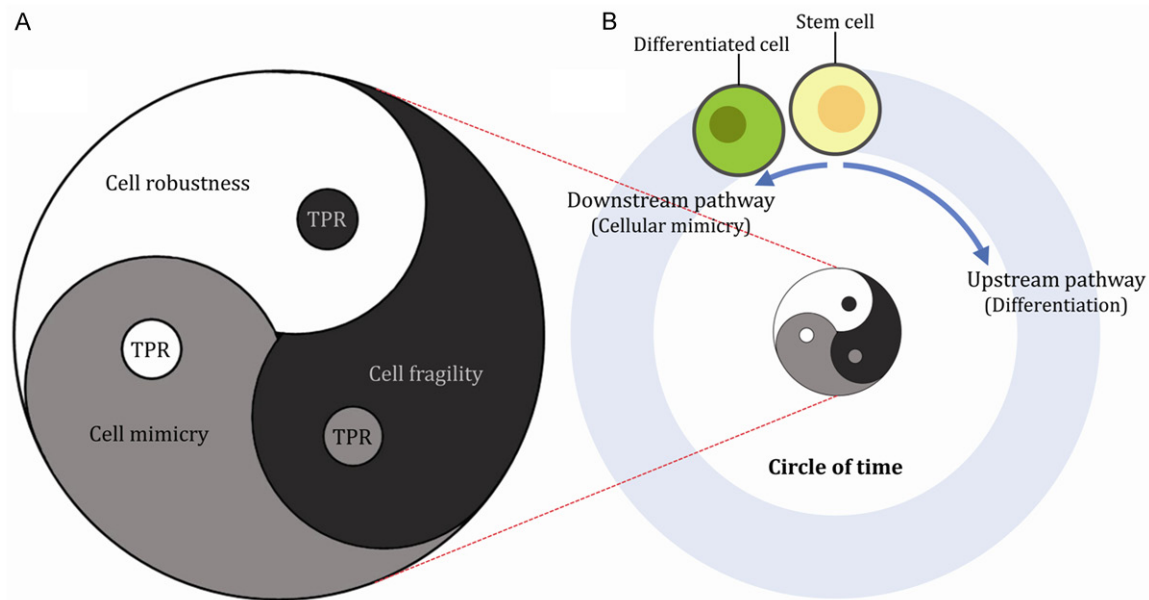


Figure 1. Schematic diagram showing the hypothetical pattern of CMD kinetics. A. The three phases of robustness, fragility, and mimicry constitute the CMD cycle. At two-phase regions (TPRs), the CMD has the capacity to simultaneously exhibit the behaviors of two different phases. B. The information fluidity and circularity of time enables the CMD to navigate the cell both upstream and downstream, during which the cell differentiates and shows mimicry behaviors, respectively. In contrast to the upstream pathway, the downstream flow may occur over a short period of time through a microscopic wormhole.

Steganographically, the CMD might also be able to temporarily exhibit a cell type in the form of another cell type during its Mi-phase (**Figure 1A**).

CMD kinetics of iPS cells

Normally, a large portion of holistic cell memory is formed during cell development and differentiation, which is able to prevent the emergence of any detectable plasticity of R-phase cells caused by CMD fluidity or at least reduce its efficiency. This preventive role has been supported by many studies from the early 1980s until now. For example, one of the first reports is the work of Reyer et al., who indicated that iris epithelial cells from the eye of adult *Notophthalmus viridescens* generate lens tissue when transplanted onto an amputated limb [19]. Nearly 13 years later, Kim and colleagues found that axolotl limb blastemal cells transplanted into the eye give rise to limb structures [20]. Recent evidence also suggests that during the regeneration of missing axolotl limbs, cells near the wound somehow retain memory of their tissue origin [21]. Thus, it would not be surprising if reprogrammed cells also retain a memory of their tissue origin. In agreement

with this, Hanna et al. reported that they could treat a sickle cell anemia mouse model using iPS cells derived from autologous skin cells [22]. However, the initial evidence suggested that the reprogrammed hematopoietic stem cell-like cells used in this experiment did not fully restore all mature blood-cell lineages [23], likely because these reprogrammed cells were not equivalent to the naturally occurring, long-term, repopulating hematopoietic stem cells [24]. In fact, as explained later, perhaps due to more full layers in the skin cells' CMDs, the generated iPS cells had shorter life spans than hematopoietic stem cells (**Figure 2**). Moreover, some aspects of skin memory likely remained active in the iPS cells and accounted for the limitations in their differentiation capacity. Studies by Polo et al. and Barrero and Izpisua Belmonte also revealed that iPS cells retain a transcriptional memory of their cell type of origin that endures through multiple passages and manifests as an altered differentiation capacity and differential gene expression [25, 26].

Hypothetically, if we ignore the persistent layers of parental cell-specific memories, then natural complications of iPS cell generation

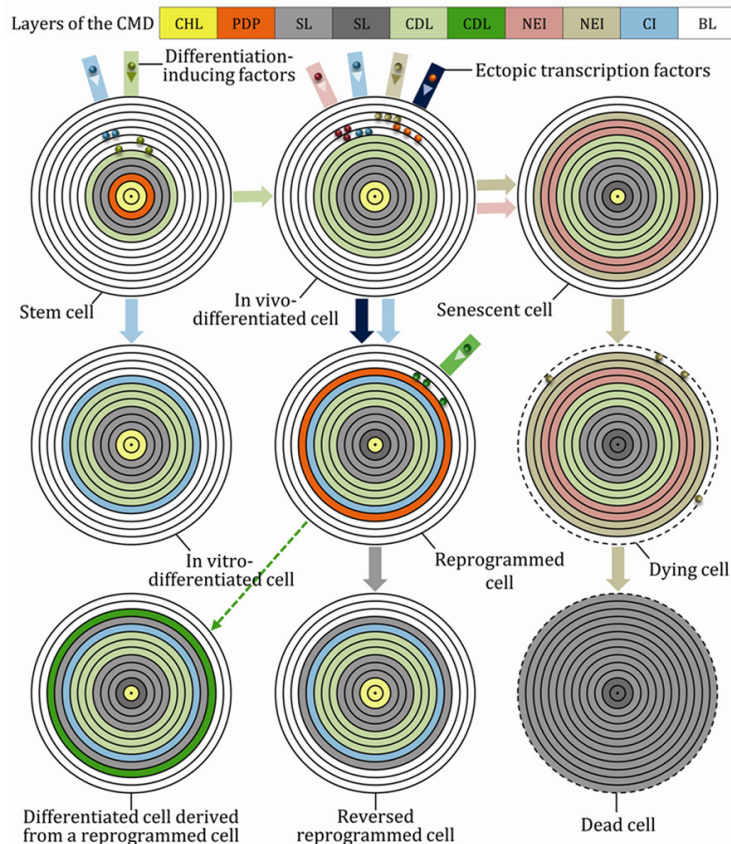


Figure 2. Schematic diagram showing the hypothetical kinetics of CMD layers during cell differentiation, cell reprogramming, and cell aging. Normally, each stem cell differentiates and then undergoes cell death after all of its blank CMD layers have been filled by the entry of controlled information over the cell's lifespan. The active layers of proliferation and differentiation potential (PDP) in the CMDs of stem cells will be gradually silenced as cell differentiation proceeds. Conversely, a differentiated cell can be reprogrammed to a stem cell-like state when a thin layer of PDP is generated with the help of exogenous transcription factors. However, as illustrated in the CMD of a reversed reprogrammed cell, due to a significant volume of differentiation and culture-originated information (CI), the exogenously induced stemness capacity is temporary and thus will be silenced in a majority of transfected cells. A thick layer of PDP may facilitate a more prolonged reprogramming of a very small percentage of the target cells, although at least some layers of the parental CMD remain active in the CMDs of these reprogrammed cells and their derivatives. The presence of the CI layers is a potential concern regarding in vitro-derived cells. CHL: cellular health-related layer, SL: silent layer, CDL: cell differentiation-related layer, NEI: normal exogenous information, CI: culture-originated information, BL: blank layer.

techniques may not be a small issue that can be easily overlooked.

To date, most iPS cells have been made using viral vectors [23, 27-29], and it has been shown that iPS cells have the capacity to carry multiple copies of provirus [30]. On the other hand, for somatic cells to be reprogrammed into iPS cells, the CMD cycle most likely needs to be in

F-phase; however, F-phases of different CMDs may be at recessive or dominant TPRs with varying degrees of activity. Therefore, various levels of exogenous memory that are constructed from proviral copies enter target CMDs and thereby add significant variability to the CMDs of individual infected cells. Given this issue, various negative side effects in the behavior of target cells may be encountered. By looking at previous findings [31-33] from the perspective of CMD kinetics (Figure 2), we can suppose that the overexpression of transcription factors at the recessive TPR creates a small layer of ectopic pluripotent capability in the CMDs of the target cells. As a type of cellular stress, this forced expression can partially silence the cellular health-related layers (CHLs) within the CMD through DNA damage that in turn leads to the activation of the p53 pathway, which acts as a barrier to reprogramming. Hence, owing to cell robustness, the new layer of pluripotency will become silent, and the cell reassumes the CMD of its previous healthy state, albeit with lower blank layers. Conversely, entry of more proviral copies at the dominant TPR may result in more cellular stress that can damage p53 and therefore facilitate cellular reprogramming. However, due to a large layer of pluripotent capacity, such iPS cells may present tumor-like features. To

reduce this safety concern, alternative, virus-free protocols have been developed for iPS cell generation [34-36]. Nevertheless, reprogramming efficiency using these techniques is substantially lower (~0.001%) compared with viral methods (~0.01%) [23, 27, 28]. To increase reprogramming efficiency, additional novel virus-free strategies have been developed [37], but the reprogramming efficiency of these pro-

protocols is still low (~1%). Even this low percentage of iPS cells may not be generated at all; rather, it might have arisen from a data misinterpretation. Indeed, CMD fluidity can enable a somatic cell to closely resemble a pluripotent cell during a physiological or pathological Mi-phase. Physiologically, a rare subpopulation of adult stem cells with characteristics of pluripotent stem cells has been found within cell populations isolated from multiple tissues [2, 38, 39]. Pathologically, the presence of apparently pluripotent cells may be due to cellular stresses or aberrant processes that lead to cellular look-alikes [10, 12, 15, 16]. Such heterogeneity within stem cells, which can be considered a cause of both their extensive plasticity and the related controversies [40-42], may have been overlooked in many commonly used research methods.

The determination of proper reprogramming in human cells is very difficult, but even if the creation of real iPS cells is achievable, the natural deposition of unwanted information in the CMD's blank layers during cell culture is still concerning. In this regard, recent studies have shown that human iPS cells accumulate chromosomal, subchromosomal, and single-base pair changes over time [43-45], some of which are the same as those described in human embryonal carcinoma cells [46]. The deposition of foreign layers in the CMDs of reprogrammed cells may be, at least in part, due to the high propensity of cultured cells to adapt to in vitro conditions during the F-phase of the CMD cycle. However, these undesirable layers may cause aberrant gene expression [25, 47-50] and genomic aberrations [43-46, 51]. Moreover, these layers can dramatically influence the cells' shape, epigenome, and biological properties [10, 15, 52, 53]. Each of these issues renders the clinical use of iPS cells ethically untenable.

CMD kinetics and nuclear transfer

The unphysiological nature of nuclear transfer to an enucleated oocyte produces a hybrid CMD that harbors memory layers of both the donor nucleus and the egg cytoplasm. Moreover, in vitro conditions, donor cell developmental memory, and various cell manipulations significantly change the architecture of the resultant CMD. If abnormal CMDs are produced, the development of nuclear-transfer

embryos can be delayed, incomplete, and faulty, both qualitatively and quantitatively.

Qualitatively, in frog embryos produced by the nuclear transfer of muscle cells, Ng and Gurdon found that muscle cell-derived nuclei sometimes continue to strongly express lineage-specific differentiation genes in non-muscle lineages [54]. In the cloned embryos, memory of the differentiated status of the parental somatic cells frequently leads to faulty activation of key embryonic genes at the blastocyst stage [55, 56]. The presence of these stubborn layers may also explain a finding of Bauersachs et al., who observed different responses of the endometrium to cloned versus fertilized blastocysts [57]. Possibly due to donor cell memory, the CMDs of stem cells produced by nuclear transfer have fewer blank layers than those of cells derived from a fertilized zygote; this difference could presumably result in cloned animals with a reduced lifespan [58]. The premature death of cloned animals may be preceded by serious developmental and genetic problems [59, 60], overweightness with age, and tumor [60-66]. Accordingly, and in line with previous notions [61], it is tempting to speculate that the type, number, and activity level of the developmental layers within the nuclear portion of R-phase donor CMDs contribute to the distinct problems and abnormalities in clones.

On the other hand, the subtle information gaps within the oocyte's CMD and natural traces associated with the cloning process may also result in a wide range of defects, such as fetal and placental overgrowth [5, 8, 61, 62, 67], liver and kidney defects, bacterial infections, and respiratory distress, which are usually observed in clones [68] and are not specific to clones derived from adult cells because ES cell-derived clones exhibit the same defects [5]. Hypothetically, because cell maturation is a gradual process that fills the blank layers of the CMD precisely and completely, it is likely that forced maturation of oocytes by various superovulation protocols leaves subtle information gaps in their CMDs. Thus, despite the apparent maturation of oocytes, the majority of these cells lack sufficient information to sustain normal embryo development following nuclear transfer or in vitro fertilization. Moreover, as oocytes must be forcefully activated again following nuclear transfer, a zygote is a much better recipient because it is properly fertilized and

not artificially activated [58]. The forced activation of oocytes may leave stress footprints within the oocyte's CMD that can contribute to subsequent developmental anomalies.

Quantitatively, the more filled a donor CMD, the lower its nuclear transfer success rate because donor cells with more-filled CMDs require more time to precisely replicate all of the filled layers within the nuclear portion of their CMDs. Therefore, regarding the time schedule of the zygote, the donor nucleus cannot complete the duplication of its memory layers in time for the first nuclear cleavage of the recipient zygote. As a result, most cloned embryos cannot survive. This may explain why the nuclei from differentiated or adult cells exhibit a lower percentage of nuclear transfer success relative to nuclei from larval or embryonic cells [7, 8, 62, 69, 70]. Generally, less than 1% of all nuclear transfers from adult or differentiated cells generate apparently normal offspring [59]. However, because of the CMD's Mi-phase, this small population is likely to represent G0 stem cells mimicking the phenotype of differentiated cells. These outwardly differentiated cells must have fewer filled layers than truly differentiated cells, thus enabling the donor cell nuclei to complete the duplication of their filled layers in time. Moreover, because the CMD at G0 phase of the cell cycle is at its smallest size and hence has the fewest information layers, donor nuclei arrested in G0 stage have a greater chance of successful cloning [71, 72]. On the other hand, if an enucleated oocyte arrested at the second metaphase of meiosis is used as a recipient, it will be a better recipient than an enucleated zygote [58] because more time is allowed for the donor nucleus to replicate all of its information layers within the egg cytoplasm. However, as mentioned previously, the need to force-activate nucleus-transferred oocytes is the next concern.

Generally, the efficiency of nuclear transfer is too low to detect all of the possible negative consequences of the cloning process. Additionally, the abnormalities of live clones may be so subtle that they cannot be detected using standard methods [8] and are only revealed later in life [64, 65], which suggests that correct reprogramming of a nucleus is extremely rare.

It is conceivable that during therapeutic cloning, cells undergo more memorization com-

pared with reproductive cloning because, in contrast to reproductive cloning, therapeutic cloning requires the explantation of cloned blastocysts into culture to extract and proliferate or to differentiate ES cells for therapeutic purposes. This extra manipulation step may allow time for additional memorization of unwanted types in the CMDs of F-phase stem cells and their derivatives. In this respect, it has been demonstrated that stem cells accumulate ectopic genetic memories as they multiply in culture. However, in some circumstances, the cells can be manipulated to remove well characterized and unwanted genetic memories [73, 74]. Unfortunately, as noted by Sandhaus [75], the tools for manipulating the genome also tend to leave traces of unwanted memories within the cell. Of greater concern is the possibility that a memory engram can be oncogenic and lead to neoplasia, particularly the very subtle engrams in the regions of genes known to be involved in cell-cycle control and cancer. In fact, in these regions of the genome, events such as point mutations, deletions, or amplifications are prone to occur and the CMD can memorize them. As long as we do not know whether the activation of these barely detectable changes has any functional significance, it would likely be unwise to use such cells clinically. Along with these concerns, problems pertaining to widely unknown aberrant memorizations still remain. Although there is no evidence to support this claim, the absence of evidence is not evidence of absence. Perhaps this is why attempts to create normal human cell lines via SCNT have not yet succeeded.

CMD kinetics following cell engraftment

The regenerative behavior of stem cells following transplantation depends on the phase of the CMD cycle at which the cells are transplanted. Because stem cells at the Mi-phase of the CMD cycle look like differentiated cells, they may not be identified using routine methods of stem cell isolation; thus, R- and F-phase stem cells have a greater chance of being isolated. Hypothetically, the in vivo behavior of endogenous stem cells is controlled not only by the primary layers of their CMDs during R-phase, in which the cells proliferate, but also by exogenous information originating from their niches during F-phase, which is when the cells differentiate. If this notion is true, then the regenerative behavior of transplanted stem or repro-

grammed cells that have in vitro-derived layers in their CMDs and are in the pathological niches of the recipient tissue will most likely be more complicated and possibly uncontrollable in a clinical setting. Although the nervous system regulates stem cell trafficking and governs the local relationship between stem cells and their microenvironments [76], the presence of foreign layers within the CMDs of manipulated donor cells may be capable of removing transplanted cells from nervous system control or may cause them to respond aberrantly. Moreover, in addition to immunologic incompatibility, these unwanted layers may have immunogenic potential. Therefore, cell rejection is not only a frequent complication of heterologous and homologous cell transplantations but also a source of concern in autologous grafts of F-phase cells. Supporting this hypothesis are the findings of Zhao et al., who showed that even if transplanted cells are derived from the recipient's own iPS cells, they can still be immunogenic [77]. To prevent host-versus-graft disease, immunosuppressive agents are commonly prescribed to transplant recipients, a treatment that causes serious side effects. Furthermore, because of the high costs associated with cell production, safety checks, and immunosuppressive drugs, this personalized form of therapy would likely be extraordinarily expensive in practice.

Relative to the low engraftment probability of F-phase stem cells, R-phase cells have a greater chance of engrafting in a syngenic recipient. Following the transplantation of R-phase cells, in addition to cell proliferation and the production of extracellular or memory microvesicles [78, 79], cell fusion is one of the dominant processes by which grafted cells engage in tissue regeneration [80-82]. Stem cells behave in the former way if the transplanted cells continue to remain in the CMD's R-phase. Normally, to prevent exhaustion [83], these proliferative R-phase stem cells need to enter Mi-phase and thereby form teratomas that contain various differentiated cell types. However, due to CMD fluidity, these differentiated cells may in fact be various types of Mi-phase stem cells. If not, why would derivatives of more than one germ layer develop in a shared encapsulated environment?

The latter path, cell fusion, is adopted if donor stem cells enter F-phase. Based on quantum

physics, it can be deduced that cell fusion is a spontaneous cellular reaction by which F-phase cells attempt to promote their CMDs to more equilibrated states. In theory, when all of the CMD's layers are filled with new information, either by physiological or pathological processes, the cell ages and progresses to a state of maximum entropy or energy dispersal. This is a point beyond which extra entropy leads to cell death because the CMD's space is limited by the horizon of the plasma membrane (**Figure 2**). Cell fusion results in hybrids that harbor the CMDs of both parental cells. This pathophysiological phenomenon acts as a double-edged sword in cancer progression. Pathologically, the fusion of proliferative cells with normal migratory cells results in metastatic aneuploid hybrids [84]. Therefore, just like a wolf in sheep's clothing, CMD kinetics can make cell fusion a hidden enemy. Moreover, during the fusion of two young cells, the resulting hybrid CMD may acquire more blank layers than held by either parental cell. As a result, the complete filling of a hybrid CMD with exogenous information can take more time than for each parental CMD. This, in parallel with the literature [85], means that hybrids must be more drug- and death-resistant than their parental cells. However, physiologically, the majority of hybrids (~99%) will die or become quiescent because, theoretically, most fusion processes are able to completely fill up the CMDs of their resulting hybrid cells. In this regard, in addition to the greater chance of adult somatic cells fusing with donor stem cells compared with younger cells, the larger surface of the resulting hybrid provides more gates for the entry of information into its blank layers, thus paving the way for hybrids towards cell death. Generally, it is assumed that the number of blank layers and the function of filled tracts in the CMDs of the parental cells, as well as the size of cell-cell contact surface, are three main factors that determine the behavior and fate of hybrids.

Because Mi-phase is the resting phase of stem cells, it is expected that during the regeneration of recipient tissue, the CMDs of transplanted stem cells do not enter Mi-phase before the completion of regeneration.

The transplantation of differentiated cells can overcome some problems of stem cell therapy. However, these cells, due to the greater num-

ber of full layers of memorized information in their CMDs, have a significantly lower chance of precisely duplicating all of their full layers than undifferentiated cells. Consequently, while undifferentiated cells can proliferate, terminally differentiated cells cannot. However, proliferative differentiated cells, such as insulin-secreting β cells in the adult pancreas [86], might represent stem cells at a dominant TPR of the CMD's Mi-phase (**Figure 1**).

In addition to inability of differentiated cells to expand, the number of cells that survive transplantation will be significantly reduced by the presence of unwanted in vitro-derived layers in their CMDs (**Figure 2**). The transplanted cells that remain viable may also exhibit aberrant behavior. These CMD-based issues are similar to those being addressed in the context of cytotherapy for several diseases. The cases in point are the replacement of cells lost in Parkinson's disease and juvenile diabetes [53].

Conclusion and perspectives

In summary, there is one fundamental question to ask ourselves. If undesirable CMD kinetics is a major impediment to successful cell-based therapy, then is there any possible solution to this problem? To address this question, the author proposes a *holographic theory* that states "*the cell memory is both the obstacle and the solution to successful regenerative medicine*". Accordingly, the therapeutic dimension of cell memory must be deciphered if we expect to make significant progress in the future. In this regard, the author has taken the first step by publishing a theoretical paper entitled "Cell Memory-based Therapy", which has appeared recently in the Journal of Cellular and Molecular Medicine [87].

Disclosure of conflict of interest

None.

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