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Cancer Stem Cells: Foe or Reprogrammable Cells for Efficient Cancer Therapy?

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Abstract

Embryonic development and carcinogenesis share many molecular pathways and regulatory molecules. While the induction of a pluripotent state involves a significant oncogenic risk, as in induced pluripotent stem cells (iPSCs), the embryonic environment *in vivo* has been shown to suppress tumor development. In this review, we discuss the subtle equilibrium between the nanotopography (niche) of the hosting tissue resident stem cells and their biological dynamics, including the transformation in cancer stem cells. We review consistent findings indicating the potential for modulating the biology of human cancer stem cells by the aid of naturally occurring or synthetic molecules, including developmental stage *zebrafish* embryo extracts, hyaluronan, butyric acid (BA) and retinoic acid (RA), hyaluronan mixed esters of BA and RA, melatonin, vitamin D3, and endorphin peptides. Within this context, we dissect the multifaceted mechanisms orchestrated by endorphinergic systems, including paracrine cell-to-cell communication, as well as the establishment of autocrine and intracrine (intracellular) peptide actions driving transcriptional responses and self-sustaining loops that behave as long-lived signals imparting features characteristic of differentiation, growth regulation and cell memory.

Based upon the remarkable action of electromagnetic fields and mechanical vibration on (stem) cell signaling, differentiation, and senescence, we also consider the potential for using these physical energies as a tool to afford a fine tuning of cancer stem cell fate.

On the whole, we forecast future deployment of the physical and/or chemical approaches described herein aiming at reprogramming, rather than destroying cancer stem cells, eventually placing cancer therapy within the context of Regenerative Medicine.

Keywords

Stem cells, Cancer stem cells, Carcinogenesis, Metastasis, Nanotopography, Reprogramming, Natural molecules, Synthetic molecules, Mechanical vibrations, Electromagnetic fields

From Stem Cell Biology, New Paradigms in Carcinogenesis

There is growing evidence that embryonic development and carcinogenesis are closely related, as it can be inferred from the fact that these processes share many molecular pathways and regulatory molecules [1-7], and from the high oncogenic risk associated with the acquirement of an embryonic-like state, as it has been shown in human induced pluripotent stem cells (iPSCs) [8, 9].

Malignant transformation emerges as a dynamic process resulting from the accumulation of multiple genetic and epigenetic alterations that give rise

to a complex network of pathological molecular signaling pathways.

Within the context of tumor growth and metastasis, nano-scale remodeling of tissue environment plays a key role, involving a multifaceted interplay between the local *nanotopography* (niche) and its embedded (stem) cells [10-13]. Some distinctive features of tumors, now recognized by the scientific community, include: self-sufficiency with respect to growth signals (autocrine stimulation and *intracrine* actions, linked to intracellular receptor/ligand patterning), insensitivity to signals that prevent cell growth, a virtually unlimited replicative potential, the ability to evade apoptosis, the support of angiogenesis, immune evasion, tissue invasion, the ability to give rise to metastases. Each of these abilities comes from the breakdown of a delicate physiological balance made by the *collaboration* of multiple mechanisms of signal transduction and the solidarity of the tumor microenvironment.

The theory of cancer stem cells predicts that a small number of cells within the tumor, the cancer stem cells, are resistant to conventional chemotherapy and radiotherapy [14-17]. It is believed that cancer stem cells play a crucial role in the maintenance of tumor growth and initiation of metastatic process [15-16]. Chemotherapy and radiation therapy mainly work on actively proliferating tumor cells, the "cancer transient amplifying cells". In contrast, cancer stem cells proliferate slowly and are not affected by chemotherapy and radiotherapy [15]. For this reason, conventional therapeutic strategies may fail to maintain a prolonged control of the tumor process.

In recent years, therapies have been introduced that are intended to target specific molecular pathways. Many "biological drugs" based upon a strong rationale related to the understanding of well-defined pathological mechanisms are now available for clinical oncologists [18, 19]. Promising or even excellent results have been obtained in some types of tumors. However, used individually or in combination with a more conventional treatment, such approaches so far have not substantially improved the prognosis of most types of advanced-stage tumors [18, 19].

It is now clear that alternative approaches and new therapeutic paradigms should be considered, based upon the pathophysiological understanding of the biology of cancer, with particular reference to the nature of cancer stem cells.

Intriguingly, evidence obtained from the study of the interactions between tumor cells and embryonic tissues indicate that tumor growth is reduced or even suppressed by the embryonic microenvironment *in vivo* [20, 21]. In particular, administration of carcinogens during organogenesis causes embryonic malformations, without leading to the formation of tumors in the offspring; on the contrary, when the organogenesis is complete, the administration of carcinogens increases the frequency of tumors in the offspring [22-24]. In this regard, it has been shown that the proliferation curves of human tumor cell lines can be slowed down following the exposure to extracts of *zebrafish* embryos collected during the phases of cell differentiation. On the contrary, there is no significant anti-proliferative effect in the presence of extracts solely yielded from the initial duplicative phase of *zebrafish* embryogenesis [25-29].

On the whole, taking into account these findings, the *reprogramming* of cancer stem cells may ensue as a new way to fight cancer. The term *reprogramming* was initially introduced to identify the transformation of a normal adult somatic cell into an embryonic-like stem cell [30, 31].

A new era with unprecedented therapeutic implications may emerge in case the concept of cellular reprogramming may be extended to the cancer stem cells with the attempt to develop any intervention capable to act at the genetic and epigenetic levels to resume their differentiation abilities towards a normal phenotype. In principle, these interventions should not only focus directly on cancer stem cells, but they should also take into deep consideration the crucial role of the embryonic microenvironment in tumor *reprogramming*.

In this commentary review, we consider the possibility of conceiving the tumorigenic process as a deviation from a normal evolutionary process, which may be susceptible for control and reversibility by the aid of regulatory agents (chemical and/or physical stimuli) of cell differentiation. We discuss a number of findings that may form the underpinning for future attempts for cancer stem cell reprogramming in the presence of natural or synthetic molecules, or as a result of exposure to physical energy, such as electromagnetic fields or mechanical vibrations.

Tuning Human Cancer Stem Cell Dynamics with Chemical Agents: The Role of Natural and Synthetic Molecules

Hyaluronic, Butyric and Retinoic Acids

It is now evident that both naturally occurring and synthetic molecules or compounds can deeply affect stem cell biology. Among these agents, hyaluronic acid (HA), butyric acid (BA) and retinoic acid (RA) have long and consistently been shown to modulate growth and differentiation even at the stem cell level (see below for detailed references). A combination of HA, BA, and RA has been shown to increase the survival and differentiation potential in human adipose tissue-derived mesenchymal stem cells (ADhMSCs) [32]. To this end, *ex vivo* stem cell preconditioning with a mixture of these molecules followed by stem cell co-transplantation with beta-pancreatic islets in syngeneic diabetic rats also resulted in the optimization of islet engraftment and survival, and normalization of glycemic control [32]. BA and RA have also been grafted within a synthetic molecule in the form of HA mixed esters (HBR) [33]. As a result, HBR has been shown to remarkably enhance the differentiation potential of stem cells, affording a high throughput of cardiogenesis in both mouse embryonic stem cells (mESCs) and human adult mesenchymal stem cells (hMSCs) of different origin, including the bone marrow (BMhMSCs), the dental pulp (DPhMSCs) and fetal membrane of term placenta (FMhMSCs) [33-35]. HBR, owing to the HA moiety, uses the CD44 hyaluronan receptor, present in both mESCs and hMSCs, to obtain a specific internalization of BA and RA followed by hydrolysis of HBR itself, operated by ubiquitous intracellular esterases [33]. These mechanisms result into a controlled and timely intracellular release of each hyaluronan grafted moiety (BA, RA and HA

itself) with consistent sequelae of intracellular events [33, 35]. In particular, HA is known to act as a docking place for intracellular hyaluronan binding proteins (hyaladerins), which include relevant protein kinases and tissue-restricted transcription factors [36-43]. Most of these interactions involve molecular motors and occur at the level of cytoskeletal and nucleoskeletal elements, or at the LINC (Linker of Nucleoskeleton and Cytoskeleton) interface, forming a highly dynamic mechanocoupling network for the transduction of low-magnitude mechanical signals into complex roadmaps for the control of cell growth and differentiation [44]. Among the other HBR constituents, BA is able to create an epigenetic context leading to chromatin relaxation and remodeling, capable of increasing the interaction with transcription factors of the family of "Zinc Fingers" and "Homeodomains", involved in differentiation processes [45-48]. Retinoic acid is able to give a *direction* to the differentiation processes, emphasizing a remarkable cardiovascular commitment [49-52]. In fact, depending upon the intracellular concentration, retinoic acid can produce either neurogenesis or cardiogenesis [49-52]. The fine tuning of the degree of substitution of RA within HBR, obtained through the development of the process of synthesis and esterification, has allowed us to obtain a mixed ester releasing intracellular nanomolar concentrations of RA, which mostly results into cardiogenesis [33-35], with neurogenesis prevailing at micromolar concentrations [52]. Following the treatment with HBR of the different populations of hMSCs we were able to enhance by several orders of magnitude the expression of genes and proteins involved in cardiogenesis, such as GATA4, NKX-2.5 and different isoforms of Smad proteins [34]. The treated stem cells consistently expressed markers for terminal cardiac differentiation, including alpha-myosin heavy chain, alpha-sarcomeric actinin and troponin I (Tn-I). Nuclear run-off experiments, performed in isolated nuclei, have shown that the effect of HBR occurred at the transcriptional level [33]. Preconditioning with HBR of hMSCs *ex vivo* has also greatly increased the ability of these cells to repair infarcted hearts in both small (rats) and large (pig) animal models of post-infarct heart failure [35, 53]. In subsequent studies, we have shown that the intramyocardial injection of HBR alone was even able to induce a substantial repair of the infarcted myocardial tissue, by acting on endogenous mechanisms of tissue regeneration and recruitment/activation of endogenous stem cells, without having to resort to stem cell transplantation [54].

Of note, the exposure of both mESCs or hMSCs to HBR or a mixture of HA, BA, and RA resulted in the overexpression of the prodynorphin gene [33-34], which was shown to act as a master regulator of cardiogenesis [55, 56]. To this end, dynorphin B, a biologically active end-product of the prodynorphin gene was also found to promote the terminal differentiation of embryonal carcinoma (EC) cells into spontaneously beating cardiac myocytes [57]. EC cells are the stem cells of teratocarcinomas, and the malignant counterparts of embryonic stem (ES) cells derived from the inner cell mass of blastocyst-stage embryos, whether human or mouse [58]. Our observation [57] provides evidence for the extreme flexibility in the potential for fate direction in a cancer stem cell population. Whether a combinatorial treatment with HA, BA, and RA or with HBR or endorphin related peptides may be able to resume the differentiating potential of human cancer stem cells remains to be established and it is the subject of our ongoing investigations.

Melatonin

This molecule controls many events that can impact the molecular dynamics in cancer stem cells by: (i) modulating their circadian rhythms of gene and protein expression [59], (ii) inducing autophagy and then a cytotoxic effect on "stem cells capable of starting glioma", a subpopulation of cancer stem cells in the context of malignant glioblastomas, responsible for development and tumor progression, drug resistance and relapse [60], (iii) increasing the anti-cancer activity of other natural signaling molecules such as fisetin (a vegetable bioflavonoid) [61], (iv) inducing methylation of specific promoters (ABCG2 / BCRP promoter), which is configured as a new mechanism to bypass the multiple drug resistance (multidrug resistance) in brain cancer stem cells [62], and (v) reducing proliferation and neoplastic transformation in a receptor-dependent fashion [63].

Vitamin D3

The active form of vitamin D, 1,25-$(OH)_2D_3$, has been associated with the control of metabolism, cell growth, differentiation, and it has been shown to induce anti-proliferative effects, apoptosis, and adaptive/innate immune responses, in addition to its function to control bone integrity and calcium homeostasis [64-66]. Moreover, 1,25-$(OH)_2D_3$ modulates the expression of circadian genes in stem cells derived from adipose tissue (ADSCs), causing the synchronization of BMAL1 and PER2 genes [67], indicating an essential role of vitamin D3 in the control of molecular clocks, which in turn are essential in positioning cellular homeostasis along the subtle line that separates normal or pathologic/neoplastic growth. 1,25-$(OH)_2D_3$ cooperates with BRCA1 playing an essential role in the acetylation of the promoter of p21WAF1 and inhibition of cancer cells and cancer stem cells of the breast [68]. Current knowledge on the powerful anti-cancer activity of vitamin D3 is completed by the investigation of its ability to induce apoptosis in subpopulations of gastric cancer cells [69], to modulate the expression of pluripotency genes in cancer testicular germ cells *in vitro* and *in vivo* [70] and to suppress the expression of telomerase and the growth of human tumors through the microRNA-498 [71].

Reprogramming Human Cancer Stem Cells with Physical Energies

Electromagnetic fields

Extremely – low frequency pulsed electromagnetic fields (ELF-MF) of 50 HZ, 0.8mTesla (rms), are able to orchestrate stem cell commitment towards one of the most complex embryogenetic outcomes, inducing cardiogenesis in embryonic stem cells [72]. ELF-MF are also able to modulate a cardiogenic program throughout the adulthood, as shown by the ability to increase the expression of genes needed for cardiogenesis and the maintenance of a myocardial phenotype in adult ventricular cardiomyocytes [73].

We have recently shown that asymmetrically conveyed electromagnetic fields (ACEF) of 2.4 GHz optimize the expression of pluripotency in mouse ES cells, inducing myocardial, neuronal and skeletal muscle differentiation [74]. Similar results are obtained upon exposure to ACEF of ADhMSCs [75]. This effect is the result of a fine modulation (initial increase and subsequent transcriptional inhibition) of the expression of stemness related genes [75]. Exposure to ACEF was able to induce a biphasic effect, i.e. overexpression followed by transcriptional inhibition of Sox2, Nanog, Oct3/4, Klf4 and c-myc in human skin fibroblasts, affording for the first time a direct high-yield reprogramming of adult non-stem somatic cells into myocardial, neural and skeletal muscle cells (about 15-20% for each phenotypic commitment) [76].

For the first time, through the exposure to ACEF we were able to reverse the process of stem cell senescence in human adult stem cells (ADhMSCs) subjected to prolonged (30 to 90 days) *in vitro* expansion [77, 78]. This effect resulted from and was associated with (i) the activation of a telomerase dependent pathway, linked to the re-expression of TERT, the gene coding for the catalytic core of telomerase with subsequent increase in telomere length [77], (ii) the induction of a telomerase independent pathway associated with the activation of Bmi-1 and the transient increase in the expression of pluripotency genes, such as Nanog, Sox2 and Oct4 [77], and (iii) the resumption of multilineage differentiation potential, as shown by recovery of high throughput of differentiation along vasculogenic, osteogenic, and adipogenic fates [78].

On the whole, these findings suggest that electromagnetic fields may have a role not only in the specification but also in the persistence of a complex cellular identity. The ability of electromagnetic fields to drive efficient cardiogenesis in both embryonic and adult stem cells and to reprogram even human skin fibroblasts into myocardial-like cells poses intriguing trans-disciplinary musing. In fact, the heart has the lowest risk for primary malignant transformation, which may very rarely develop in the form of cardiac sarcomas [79-81]. Cardiogenesis is the first morphogenetic event in different animal species, including humans. The risk for tumorigenesis throughout embryo development is also very rare [5-7]. The canonical view speculating that primary cardiac malignant tumors are so rare since cardiac cells divide very rarely appears to be too simplistic. An alternative although non-mutually exclusive hypothesis may consider the heart as a tumor suppressor organ, capable of secreting a large network of growth regulatory and differentiating peptides that may potentially limit the onset and progression of a local cancer. In this regard, the attainment of cardiogenesis in the presence of either chemical agents or physical stimulation encompasses the transcription and protein expression of endorphin peptides [33]. These molecules, besides their role in cardiogenesis [55-57], have long been shown to act as negative regulators for the development and spreading of different types of cancer [82-87].

In isolated (stem) cell nuclei endorphin peptides have been shown to bind and activate nuclear receptors and signaling leading to the transcription of their own coding gene (self-sustaining loop), as well as the transcription of the cardiogenic genes GATA4 and Nkx-2.5 [88, 56]. These findings suggest that a consistent part of the action of these growth factors on stem cell dynamics may have occurred intracellularly (*intracrine action*) [89, 90]. Cell plasma membrane has long been considered an insuperable barrier for hydrosoluble peptides. The discovery that regulatory peptides and transcription factors can be exchanged among cells being packaged inside exosomes, acting in an *intracrine* fashion, discloses novel paradigms in cell-to-cell communication and adds further relevance for *intracrine* regulation of cell biology [90, 91]. We cannot exclude that cardiogenesis, within its morphogenetic role, may act as a paracrine/intracrine process that contributes to make the developing embryo remarkably refractory to cancerogenic risks. Whether exposure of human cancer stem cells to electromagnetic fields may resume the ability to differentiate along a cardiogenic lineage and other differentiation patterns remain to be elucidated. Addressing this issue will require thorough investigation *in vitro* and *in vivo* in different animal models for cancerogenesis.

Life as a Symphony of Oscillatory Rhythms: The Discovery that the Cells Emit Sounds

All life is manifested in a world of vibrations: the rhythm is essential in any form of life. Our life contains a seeming infinity of rhythms, with vibrations now recordable at atomic and molecular levels, and within biochemical reactions [92-94]. The correlations between rhythms and life do not emerge only at the macro level and maximum systems. Very recent studies show that biorhythms emerge and self sustain at the cellular and subcellular level [92-94]. Therefore, such rhythms are not generated by, but rather they are coordinated through the central nervous system. At single cell level, molecules engaged into structural and functional plans exhibit intrinsic oscillatory rhythms that tend to progress towards phase synchronization which may set the basis for biomolecular recognition, leading to complex integrated functions that extend from single cell to the level of multicellular systems, tissues, and organs [92-94]. It is now evident that also the activity of our genes is expressed through rhythmic oscillatory patterns [92-94]. The transcription of genes into the various mRNAs, which are translated into proteins essential for cellular life, is not a "discrete" (variable for long intervals of time above or below a baseline) phenomenon over time. On the contrary, the mRNA levels rapidly oscillate with time, even in minutes and hours [92-94]. The connectedness of complex cellular functions resulting from gene activity arises from the synchronization of multiple oscillatory rhythms through which one or more groups of genes become expressed. The conformation of DNA itself, a molecule about two meters long, varies over time within the three-dimensional space of the nucleus (only a few thousandths of a millimeter in diameter!). These conformational variations follow oscillatory rhythms that mainly pertain to the non-coding part of the DNA, which accounts for about 99% of this molecule [92-94]. Until recently this non-coding portion was referred to as "junk DNA". Since little we know that this is indeed an architectural DNA, and that the timely change of this architecture within the nuclear 3D space follows rhythmic, oscillatory courses that progress

to synchronization phenomena. Such synchronization is now considered a prerequisite for the *concerted* action, a *symphony* of multiple different genes placed at long distances in the genome.

A turning point in our studies on the interactions between cells and physical energies came from the discovery that the cells not only "feel" physical energies (electromagnetic fields, mechanical vibrations), but even produce acoustic vibrations themselves. To this end, we have demonstrated and patented for the first time the ability of cells to express "vibrational" (nanomechanical) signatures of their health and differentiating potential [95]. These are extremely low-amplitude vibrations that can be recorded by the aid of Atomic Force Microscope (AFM). These vibrations arise from the integration of the various oscillatory rhythms described above, from the so-called nanomechanical properties of subcellular structures and cell membranes [95, 96]. The AFM is a scanning microscope provided with a "probe" that measures a local property, such as topography, mechanical properties, thermal and electrical, optical absorption or magnetism, thanks to the positioning of the tip of the probe in atomic contact with the cell surface. The small probe-sample interface allows measurements on a very small area. Since the AFM can provide images of biological samples with sub-nanometer resolution in their natural aqueous environment, such a technique has the potential for the development of a revolutionary mode of characterization of living cells. Using the AFM, it is possible to observe such cells under physiological conditions, by carrying out the detection and the application of small forces with high sensitivity. "Sonocytology" is the term that has been introduced to identify a novel area of inquiry based upon the fact that in intact cells, after an accurate process of amplification, given the frequency range of nanomechanical motions recorded by AFM, the vibrations could be transformed into audible sounds, providing a thorough assessment of mechanistic cellular dynamics [95, 96].

Overall, the analysis of vibrational signatures in normal and cancer stem cells may reveal novel cues on the way these cells organize their fate. Consonant with such perspectives there are compelling data showing that (i) tumours display unique mechanical properties, being considerably stiffer than normal tissue and that (ii) the mechanical microenvironment may cause malignant transformation [97]. Hence, the application of localized forces, the use of localized probes, nanopatterned substrates or substrates designed to apply localized forces, may eventually become a strategy to enhance or direct cellular differentiation in cancer stem cells.

Cancer Stem Cell Reprogramming

Embryonic stem cells share key features with cancer cells, including (i) proliferation and expression of embryonic proteins (AFP carrier, or ABC), (ii) common signals and molecular pathways (i.e. beta-catenin within the context of TCF/WNT signals, Notch, Hedgehog and BMP), (iii) anaerobic metabolism. Moreover, the epithelial-mesenchymal transition (EMT), a normal cellular process that regulates embryogenesis and wound healing, is exploited during tumor progression to generate a metastatic cellular phenotype and can also be viewed as a reactivation of an embryonic program [98-100].

Cancer stem cells are arrested at different stages of their differentiation process in which the proliferation is still active, namely, before the terminal differentiation. This *maturation stop* can be the result of genetic and/or epigenetic alterations. As in normal somatic or stem cells, chromatin remodeling plays an essential role in the onset, progression and terminal specification of fate decisions. We may assume that in cancer stem cells the trajectory of multifaceted cross-talk between the genetic/epigenetic environment and cell signaling networks may undergo a deviation from the road map for terminal differentiation. Such hypothesis is supported by decisive experiments performed by Biava and Coworkers [1, 25-29] showing that factors extracted from *zebrafish* embryos at different stages of embryonic development (embryonic stage-dependent factors) are able to induce differentiation and/or apoptosis of human cancer stem cells, bypassing the mutations that are at the origin of the malignant tumor.

Cancer stem cells represent most of the chemoresistant cells of tumors. Cancer stem cells have been identified in many types of solid tumors, such as glioblastoma multiforme, breast cancer, lung cancer, prostate cancer, ovarian cancer, liver cancer, stomach cancer, colon cancer, pancreatic cancer and squamous cell carcinoma of the head and neck. The presence of cancer stem cells is characteristic of many hematological malignancies. The ability of cancer cells to develop metastatic features depends on the acquisition of motility, matrix digestion, and invasive characteristics. EMT is necessary to form metastases and for this reason the studies focused on arresting EMT are essential in modern oncology. While EMT is a physiological process during embryogenesis and acute repair mechanisms, uncontrolled EMT associated with cells that already suffer from a malignant transformation leads to the appearance of cancer stem cells capable to spread and circulate through the blood or lymphatic vessels. Many highly metastatic tumors (e.g. pancreatic cancer) already include dysregulated pathways involved with the progression of EMT, including RAS and TGFβ dependent signaling, as well as inhibition of other tumor suppressors [101]. A common feature of this process is the down-regulation in the expression of E-cadherin, the main protein of epithelial adhesion, or cytokeratins, occludin and other adhesion markers (i.e. claudins) closely related to the tight junctions of the epithelial cells [102]. The central role of this downregulation is based upon the activation of transcriptional regulators belonging to Snail, ZEB and bHLH families. In this frame, epigenetic factors involved in methylation and activation of the Snail promoter also appear to play a significant role [102]. On the other hand, despite the expression of pathways diverging from terminal differentiation, cancer stem cells still retain mesenchymal markers (N-cadherin, vimentin, fibronectin) and produce matrix metalloproteinases (MMPs) [102]. In normal stem cells, MMPs take an active part in shaping the microenvironment of stem cell niche. In specific circumstances, MMPs become essential in the reconstitution of stem cell niches within permissive microenvironments surviving within

damaged tissues, as it may be inferred by the ability of MMP9 to cleave and mobilize Kit ligand, which enables bone marrow repopulating cells to translocate to permissive topographies and reconstitute the niche after irradiation [103]. Taking into account the other side of the coin, having the subtle alchemy of matrix digestion and remodeling in the hands of cancer stem cells may open a way for their own dissemination and may also give the tumor mass access to the blood or lymph flow to reach new sites.

With such available armamentarium, cancer stem cells may easily be considered as the major enemy to defeat in the battle against cancer. Nevertheless, they share remarkable similarities with crucial embryonic pathways and still retain several features from MSCs. Both somatic and adult stem cells can now be considered as reprogrammable entities capable to "sense" both physical energies (i.e. electromagnetic fields and mechanical vibration) and synthetic (i.e. HBR) or natural (i.e. embryonic stage-dependent factors, endorphins, vitamins) chemistry, being transformed into cell types in which these cells would never otherwise appear, including myocardial, neural, and skeletal muscle cells. These observations indicate that the molecular mechanisms underlying normal stem cell differentiation and embryo development do not quit after birth but are still in part operating and remodeled throughout the adult life to maintain the self-identity of and the interplay between tissues and organs. Supporting this view, the transcription factor GATA4 has been shown to act as a critical regulator of both embryonic and postnatal heart development and morphogenic maintenance due to a fine tuning of its structural/regulatory domains [104]. Whereas the N-terminal domain of GATA4 is required for inducing cardiogenesis and for promoting postnatal cardiomyocyte survival, distinct residues and domains therein are necessary to mediate these effects [104]. Cardiogenic activity of GATA4 requires a 24-amino-acid (aa) region (aa 129 to 152) which is needed for transcriptional synergy and physical interaction with BAF60c. The same region is not essential for induction of endoderm or blood cell markers by GATA4, suggesting that it acts as a cell-type-specific transcriptional activation domain. On the other hand, a serine residue at position 105, which is a known target for mitogen-activated protein kinase (MAPK) phosphorylation, is necessary for GATA4-dependent cardiac myocyte survival and hypertrophy but is entirely dispensable for GATA4-induced cardiogenesis [104]. An intriguing example of morphogenetic flexibility is also provided by the existence of reverse pathways of transformation, from the postnatal back to an embryonic-like stage retaining the memory and the ability for re-differentiating backward to the same original phenotype. A vivid example of such flexibility is shown by the ability of post-natal cardiomyocytes to generate iPSCs with enhanced capacity toward cardiomyogenic re-differentiation [105]. Similarly, adult neurogenesis has been shown to occur throughout life from adult neural precursors in restricted brain regions in mammals [106].

Hence, a kind of memory/projection of the embryonic patterning may be conceived as a relevant background in tissue resident stem cells in the adulthood for the execution of self-healing and "learning" (acquirement of new knowledge) tasks. In this frame, cancer may be considered as a degenerative disease occurring in any organ and cancer stem cells may be viewed as tissue resident stem cells deviating from the normal potential to afford self-healing duties and the maintenance of tissue organ identity.

Our understanding of stem and even more cancer stem cell biology is still far from being at an advanced stage. Further dissection of cancer stem cell features will require a trans-disciplinary effort from multiple disciplines in biomedical and physical sciences, in the hope to generate a major paradigm shift in oncology, i.e. considering the cause of metastatic dissemination as the subject for a novel chapter in cell reprogramming: making cancer stem cells capable to enter differentiating paths. Resuming the potential for multilineage commitment and differentiation would also have the crucial implication that, as in normal stem cell commitment, apoptosis will occur in a consistent proportion of the cells that do not meet the requirements necessary to achieve an advanced commitment or a terminal differentiation.

Future Perspectives

Due to the extremely *fluid* scenario in stem and cancer stem cell biology, the present review deliberately avoids to end-up with some conclusion.

We prefer to look at the cancer stem cell story as a work-in-progress that may hold future perspectives. Among them is the potential for efficient reprogramming of cancer stem cells towards normal stem cells. Whether cancer stem cells may be able to (re)gain this ability remains to be established and it is the subject for numerous ongoing and encouraging studies, even in human subjects. In our view, the ultimate goal should not be to destroy cancer stem cells, but to differentiate and reprogram them: this goal can only be achieved by providing the cell with all the required reprogramming factors or physical energies, which requires further efforts resulting from the convergence of multiple disciplines. If successful, these efforts may ensue into a major paradigm shift: placing the cancer therapy within the context of Regenerative Medicine.

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